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Autumn cold acclimation of shoots does not explain the northern distribution limit of three southern exotic tree species in Finland

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ABSTRACT

Frost hardiness (FH) is one of the key traits that limits the distribution of tree species in the north. Different species and ecotypes respond differently to the drivers of frost hardening and may therefore have different survival capacities, especially in their northern distribution range. Several southern tree species such as pedunculate oak (*Quercus robur* L.), Norway maple (*Acer platanoides* L.), and mountain elm (*Ulmus glabra* Hudson) have their natural northern distribution limit in Southern Finland. Here, we examined whether insufficient frost hardening in the autumn limits the northwards distribution of these three species. To that end, we monitored phenological events and frost hardening in the autumn in these exotic species in Central Finland, further north from the natural distribution range of the three exotic species, and for the sake of comparison, in the native silver birch (*Betula pendula* Roth.). In all species, a timely growth cessation was observed. The water content of the stem decreased, and its dry mass content increased rapidly in August before the onset of frost hardening, after which no major changes were observed in these two traits. In the early phase of hardening, there were differences in leaf colouring between the four species. The values of the electrical impedance parameters of the stem changed during frost hardening, with a clear turning point after the first night frosts at the end of September. Depending on the species and hardening phase, the high temperature exotherm (HTE) of the stem was observed between -3 °C and -10 °C, but HTE did not indicate differences between the species in frost hardening. In all four species, frost hardening started at the beginning of September and proceeded first at equal rates, but the hardening was more rapid later in birch than in the other species. The damaging temperature range assessed by the controlled freezing tests became wider with increasing FH. The occurrence rate of the low temperature exotherm (LTE) varied between the species, and the LTE was observed at around -35 °C in the last sampling at the beginning of December (no LTE was observed in birch). We conclude that the hardening of all four species took place in a timely manner so that in each, the frost hardiness was sufficient to survive the autumn frosts at the experimental site in Central Finland. Further studies are needed to examine whether the exotic three species also tolerate temporarily occurring extreme winter conditions such as temperatures below -35 °C, and the high short-term temperature variations in winter and especially in spring.

1. Introduction

Low temperatures are one of the most important factors that limit tree species' distribution to the north in the boreal zone. The acclimation and adaptation of long-living trees to prevailing climate conditions depend on the timing of phenological events, as well as on the timing of frost hardening and dehardening in the autumn and spring respectively, and maximum frost hardiness in the winter (Sakai and Larcher, 1987; Bannister and Neuner, 2001; Hänninen, 2016).

An increase of night length and a gradual decrease of temperature in late summer lead to the cessation of growth and the onset of cell wall lignification, both of which are necessary for the timely initiation of cold acclimation. Different species and ecotypes respond differently to the changes in these drivers. Native ecotypes are typically well adapted to the local variability in environmental conditions. However, southern species and genotypes may respond to environmental cues more slowly

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than northern species and genotypes (Repo et al., 2000a; Nilsson, 2001), and southern ones may therefore be predisposed to frost damage if moved northwards. This may limit their growth and cultivation in northern latitudes. In addition, the maximum wintertime frost hardiness (FH_{max}), must be sufficient to survive the lowest winter temperatures. Previous studies have shown that an annual minimum temperature isotherm of -40 °C sets the limit to the distribution of several coniferous and deciduous tree species (George et al., 1974; Gusta et al., 1983; Bannister and Neuner, 2001).

Pedunculate oak, Norway maple and mountain elm are considered exotic tree species in Finland, because their natural northern distribution limit is in southernmost Finland, below the latitude of 61 °N (Hämet-Ahti et al., 1992). Single scattered trees of these species may grow at higher latitudes in central Finland, and some oaks even close to the Arctic Circle. Oak and maple are known to have a deep-supercooling ability of xylem ray parenchyma (George et al., 1974, 1982). Ice nucleation of deep-supercooled cells is indicated by a low temperature exotherm (LTE) that results in xylem death. The occurrence of the LTE is typical of species with a ring-porous xylem structure. The temperature of the LTE is considered to set a limit to frost hardiness and subsequently to the geographical distribution of the species, even though in some species, the killing temperature was found to be lower than the LTE (George et al., 1974). In North America, species with a small LTE are native to the northern United States and southern Canada, and the species with a large LTE are typically among the least frost-hardy species (George et al., 1974). On the other hand, there are also very hardy species, with their distribution ranges extending into northern Canada and Alaska that have no observable LTE. In a previous study, oak seedlings grown in central Finland beyond their natural distribution started their frost hardening at the beginning of September. According to the LTE, they reached a frost hardiness of −36 °C at the beginning of December, and this may have not been their potential FH_{max} (Repo et al., 2008). Similar distribution maps to those published for tree species growing in North America, i.e. maps showing whether the species has an LTE or not (George et al., 1974), are unavailable for Europe. Contrary to the three exotic species, silver birch has a wide south–north and west-east distribution not only in Finland but in Europe too, with different ecotypes adapted to the high seasonal variation in climate conditions in different eco-climatological and geographical zones (Hämet-Ahti et al., 1992; Vakkari, 2009; Possen et al., 2015). Similar to the northernmost broadleaved species in the North America, silver birch is not expected to have an LTE (George et al., 1974).

It has been predicted that climate warming will change the conditions in boreal areas (IPCC, 2018) such that frost hardiness during overwintering may not limit the growth and survival of southern exotic tree species at higher latitudes, contrary to the situation in the current climate. This may alter the species composition of boreal forests and the biodiversity of forest ecosystems. Although temperatures may rise, one key driver of cold acclimation, photoperiod, does not change. However, there is little knowledge of the responses of different exotic southern broadleaved tree species to changes in the photoperiod and temperature at their northern distribution range in Fenno-Scandinavia in the late summer and autumn.

We tested the hypothesis that insufficient frost hardening in the autumn restricts the northwards distribution of oak, maple, and elm. To that end, we observed frost hardiness and some related phenological and physiological traits in tree seedlings in Central Finland, well above the northern distribution limit of the exotic southern species in Finland. For the sake of comparison, silver birch seedlings were included in our study. The results for oak have been published previously (Repo et al., 2008), but to facilitate the comparisons, oak is included in the present study. We hypothesised that phenological events in the late summer and frost hardening in the autumn would be delayed in the exotic tree species compared with the native silver birch.

2. Materials and methods

2.1. Plant material

The material consisted of second-year seedlings of pedunculate oak (*Quercus robur* L.) and mountain elm (*Ulmus glabra* Hudson), and first-year seedlings of Norway maple (*Acer platanoides* L.) and silver birch (*Betula pendula* Roth.). The oaks originated from Malmi, Helsinki, Finland (60°10’ N, 24°58’ E, 17 m asl), the elms from Tuusula, Finland (60°24’ N, 25°01’ E, 48 m asl), the maples from Punkaharju, Finland (61°45’ N, 29°23’ E, 76 m asl), and silver birches from Central Finland (seed orchard no 379, Saarijärvi, Finland; 62°42’ N, 25°15′ E). There were approximately 200 seedlings of each species in the experiment. After germination, the oak seedlings were transplanted to styrofoam trays (Takopot TA-913, cell volume 0.58 dm^3, 22 cells in each tray) at the beginning of June 1996. The elm seedlings were raised in the nursery bed for their first growing season. At the beginning of the second growing season, they were transplanted to the containers (Ecopot PS-1008, cell volume 0.453 dm^3, 33 cells in each tray) on 27 May 1997. The maple seeds were sown in trays in the autumn of 1996 and then moved outside to overwinter. The next spring, at the beginning of May, the trays were moved to a plastic greenhouse to germinate, and transplanted to the containers (Ecopot PS-1008, cell volume 0.453 dm^3, 33 cells in each tray) on 23 May 1997. Birch seeds were sown on 2 May 1997 and transplanted on 25 May to the containers (Plantek PL-25, cell volume 0.38 dm^3, 22 cells in each tray).

The initial growth of the seedlings took place in the seedling nursery of Luke (Natural Resources Institute Finland), Suonenjoki field station (62°39’ N, 27°03 E, 130 m asl), first in the greenhouse and then outside in the nursery field. Second-year seedlings were kept in the nursery field for their first winter. In the first growing season, the oak and elm seedlings were fertilised once in the greenhouse and twice outside with Kekkilä 9 and Kekkilä 5 fertilisers (10–15 g m^{-2}) (Kekkilä Co., Tuusula, Finland) respectively, and in the second growing season once with Kekkilä 5 fertiliser (15 g m^{-2}) (Kekkilä Co.). The fertilisation of maple and birch took place once with Kekkilä 9 (10 gm^{-2}). On 21 July 1997, the seedlings were transported to the botanical garden of the University of Eastern Finland (Joensuu campus, 62°36’ N, 29°43’ E, 81 m asl) and raised outside until the beginning of December. The measurement of different traits started during the growing season at the beginning of August and continued at intervals during the cold acclimation until the end of the study. Daily minimum air temperatures (at a height of 2 m) for the study period and for the time period between 1980 and 2020 were obtained from the nearest weather station of the Finnish Meteorological Institute at Joensuu airport (62°66’ N, 29°64’ E, 112 m asl).

2.2. Shoot growth

Shoot length was measured with a ruler once per week between 22 July and 12 September 1997 for 16 different seedlings each time. The diameter of the new stem was measured between 8 August and 1 December at two- to four-week intervals for 18 different seedlings of each species and time with a calliper gauge (Vernier calliper, accuracy ±0.01 mm). The cessation of shoot elongation and stem diameter growth was defined as the date when no additional increment in the length or diameter, respectively, was observed between two subsequent measurements.

2.3. Changes in leaf colour

The reflectance spectra of the leaves (2 leaves of each of 9 seedlings) were measured at one-week intervals between 29 August and 2 October, using a spectrophotometer (CM-2002, Minolta, Japan). The measurements took place at spots (diameter 8 mm) between the leaf veins across a range of wavelengths between 400 and 700 nm at 10 nm intervals. A noticeable change in the reflectance spectra with leaf colouration was observed at high wavelengths. Reflectance at the absorption maximum of chlorophyll *a* at a wavelength of 660 nm was therefore selected as a measure of change in leaf colour.

2.4. Water and dry mass content of stem

The water content (WC) and dry mass content (DMC) of stem sections (n = 18 seedlings) were measured six times at 2–3-week intervals between 8 August and 1 December. A 10-millimetre section was cut from the middle of stem, its fresh mass (FM) was measured, and it was dried for 2 days at 60 °C. Its' dry mass (DM) was then measured. The WC and DMC were calculated as:

$$\mathrm{WC} = \frac{FM - DM}{DM} \quad (1)$$

$$\mathrm{DMC} = \frac{DM}{FM} \quad (2)$$

2.5. Electrical impedance spectroscopy (EIS) of stem

In EIS, the electric current of different frequencies is driven to the study object. The current- carrying capacity of different cell compartments depends on the electrolyte balance between the apoplast and symplast, which changes, e.g. with cold acclimation and as a result of freezing damage in cell membranes (Repo et al., 1994, 2000b). As a result, parameters of the model describing the measured organ change. To monitor the changes in the EIS parameters of the stem during cold acclimation, a 15-mm section was cut in the middle of the shoot of 18 seedlings at each six sampling times. The cut ends of the stem section were placed in contact with the Ag/AgCl electrodes (RC1, WPI Ltd., Sarasota, FL, USA) (Repo and Zhang, 1993; Repo et al., 1994) with the electrode paste. Impedance spectra were measured at 42 frequencies between 80 Hz and 1 MHz using a HP4284A LCR meter (Agilent Technologies, Palo Alto, CA, USA). The input voltage of the sine signal was 100 mV (rms). According to the spectra, the stem was described with a single-DCE model (distributed model) (Repo et al., 1994, 2008). The model's parameters were estimated using the complex nonlinear least squares (CNLS) curve fitting program LEVM v.6.0 (J.R. Macdonald, Department of Physics and Astronomy, University of North Carolina, Chapel Hill, USA), resulting in the values of the extracellular (R_e) and intracellular resistance (R_i), relaxation time and its distribution coefficient. The resistances were normalised with respect to the cross-sectional area of the stem (A) and the length of the sample (l), resulting in values of the corresponding specific resistance (r_e and r_i respectively; unit Ωm) as:

$$r_x = \frac{A}{l} \times R_x \quad (5)$$

2.6. Differential thermal analysis

A differential thermal analysis (DTA) of stem sections was carried out on six occasions between August and November for nine seedlings at each sampling time. Eight-millimetre samples were dissected in the middle of the current-year shoot and exposed to controlled freezing tests. The initial temperature was 5 °C, and the cooling rate to the target temperature of -45 °C was 5 $^{\circ}\mathrm{Ch}^{-1}$. The DTA was measured with a custom-designed device, and the HTE and LTE were assessed from the curves (Repo et al., 2008). Some of the tests indicated a small exotherm between the HTE and LTE, which was noted as an intermediate exotherm (ITE). The temperature difference between the sample and the reference junctions, and the temperature of the sample were measured using iron-constantan thermocouples (diameter 0.2 mm), which were connected to the recorder (Yokogawa LR 4110, Japan). The initiation of the exothermic peaks was noted from the curves.

2.7. Assessment of frost hardiness

The frost hardiness (FH) of stem sections was assessed four times between the beginning of September and the beginning of December by controlled freezing tests (four ARC 300/−55 + 20 chambers, Arctest, Finland; one GCC-30 chamber, Carbolite, UK with a Taylor-Whatron XL-180 liquid N2 tank). In each test, there were five freezing temperatures, ranging from -3 °C to -120 °C, and the control was +5 °C. The freezing temperatures were changed with the hardening to cover the critical temperature range for damage. There were 36 seedlings of each species in each test, i.e. six seedlings for each test temperature. The initial and final temperature in the test was 10 °C, the rate of cooling and warming was 5 $^{\circ}\mathrm{Ch}^{-1}$, and the duration of the minimum temperature was 4 h. Before running the test, shoots were dissected at the root collar, placed in plastic bags (6 shoots in each bag), and divided between the test temperatures. After the test, frost damage to the stem sections was assessed by the relative electrolyte leakage method (REL) and electrical impedance (EIS).

For REL, four 10 mm long sections were dissected in the middle of each shoot. The sections were cut longitudinally in two halves, rinsed with distilled water, and divided into test tubes (12 halves per tube, 4 tubes per temperature). Fifteen millilitres of distilled water were added to the tubes and shaken for 24 h at 125 rpm, before the measurement of the first electrical conductivity (C1). The samples were heat-killed at 92 °C for 1 h and shaken for 24 h, before the measurement of the second conductivity (C2). The relative electrolyte leakage was obtained as:

$$REL = \frac{C1}{C2} \times 100 \quad (3)$$

Frost hardiness was estimated by a non-linear regression as the inflection point (C) of the logistic sigmoid function fitted to the REL data (Eq. 4) (SPSS 25.0, IBM Co., New York, USA):

$$y = \frac{A}{1 + e^{B(C-x)}} + D, \quad (4)$$

where y is REL, and x is the exposure temperature. The parameters A and D define the asymptotes of the sigmoid curve, and the parameter B is the slope of the curve at the inflection point C. To visualise the damaging temperature ranges in the freezing tests, the first derivate of the fitted function (Eq. 4) was calculated for each species and sampling time as:

$$\frac{dy}{dx} = \frac{A \times B \times e^{B(C-x)}}{\left[1 + e^{B(C-x)}\right]^2} \quad (5)$$

For the EIS of the stem, a 15-mm section was cut in the middle of the shoot of six seedlings for each test temperature and the control (5 °C). The impedance spectra were measured and analysed as described above. Specific extracellular resistance (r_e) is a measure of the leaching of symplastic electrolytes to the apoplastic space as a result of cell membrane injuries. It was therefore used to assess the frost hardiness of stems. To that end, REL was replaced by r_e as the variable y in Eq. 4, and the logistic sigmoid function was then fitted to the r_e data. As with REL, the frost hardiness estimate was obtained as the inflection point C of the fitted function.

2.8. Statistical analyses

The differences between the species in the leaf reflectance and EIS of the stem were analysed for each sampling time using t-tests with the step-down Holm-Bonferroni multiple comparison method (Holm, 1979) using an R script (R version 3.3.2, R Foundation for Statistical Computing, Vienna, Austria). The differences between species and sampling times in the water and dry mass content of the stem, and the stem's high temperature exotherm were tested using univariate analysis with Bonferroni multiple comparisons (IBM SPSS 26.0, IBM Co., New York, USA).

3. Results

3.1. Weather conditions

The temperature sum (with a threshold for the daily mean temperature of 5 °C) for the growing season in the field conditions in Joensuu was 1335d.d. (degree days). The first night frost (-0.5 °C) occurred on 3 September. Slightly heavier frosts occurred on 24 and 25 September (-2 °C and -3 °C respectively) (Fig. 1). In October and November, frosts became more common. At its lowest, the air temperature dropped to -17 °C on 30 November. Shortly after the last sampling at the beginning of December, the temperature dropped to -23 °C. The daily minimum temperatures at the beginning and end of September, and at the beginning of October, November, and December, were close to the long-term minimum temperatures at this site. The daylength decreased from 18 h at the beginning of August to 5 h at the beginning of December, being 15 h at the beginning of September and 12 h at the beginning of October.

3.2. Cessation of shoot elongation and diameter growth

Shoot elongation ceased in the first week of August in maple, oak, and elm, and on 24 August, in birch (data not shown). Major diameter growth occurred before 10 August, and it ended in all species before the end of August (data not shown).

3.3. Changes in leaf colour

Leaf reflectance increased in all species with the change of leaf colour in autumn. The change was most noticeable at long wavelengths, as was shown for maple, for example (Fig. 2A). The reflectance (wavelength =660 nm) started to increase after 10 September. There were significant differences between the species in the timing of the increase in the reflectance, which took place first in elm, next in maple, and finally in oak and birch (Fig. 2B).

3.4. Water and dry mass content of stem

There were significant differences in the WC and DMC between the species and sampling times (Fig. 3). In all species, the most remarkable decrease in the WC and increase in the DMC of the stem took place before the beginning of September, with some differences between the species occasionally. Both the WC and DMC levelled off in September, with no major changes during the remaining study period, but with some differences between species at different sampling times occasionally. In the last sampling, the WC of oak was lower, and the DMC higher, than in the other species.

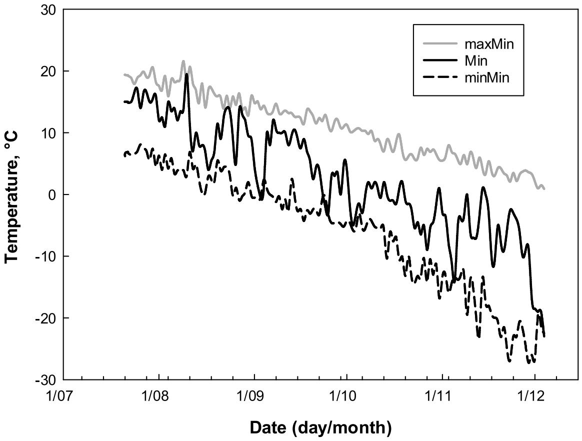

Fig. 1. Daily minimum air temperature (Min) during the study period, and the minimum (minMin) and maximum (maxMin) of the daily minimum temperature records between 1980 and 2020 at the nearest weather station at Joensuu airport.

3.5. Electrical impedance of non-frost-exposed samples

In most of the sampling times, the EIS parameters differed between the species (Fig. 4). The extracellular resistance of the stem was in a range of 10 Ωm to 30 Ωm until the beginning of October. It then significantly increased in oak and maple, but less and more gradually in elm and birch (Fig. 4A). There was a steady increase in the intracellular resistance of the stem in elm from August to December, but the decrease was more stepwise in other species, and the intracellular resistance occasionally even decreased in maple at the end of October (Fig. 4B). The relaxation time decreased from 30 to 100 μs at the beginning of August to approximately 3μs at the beginning of October, and then increased to 10 μs in other species besides elm, where it remained at a level of 2.5 μs (Fig. 4C). Typically, the differences seen in the relaxation time and in the distribution coefficient at the beginning of the hardening disappeared at the beginning of October, and then the parameter values either remained the same or diverged again (Fig. 4C, D). In maple and elm, there was no difference in the relaxation time at the beginning of the frost hardening, but it later differed significantly between these two species. The distribution coefficient of the relaxation time had similar patterns to the relaxation time, i.e. it first decreased until the end of September and then increased in the other species besides elm, where it increased throughout the study period (Fig. 4D).

3.6. Differential thermal analysis

A high temperature exotherm was observed in all species throughout the observation period – typically, between -5 °C and -10 °C. There were significant differences in the HTE between the species and sampling times. In elm, the HTE decreased to above -5 °C at two sampling times in September and October (Fig. 5). There was a slight tendency for the HTE to increase in maple and to decrease in oak during the cold acclimation between mid-September and the beginning of December. Oak and birch behaved similarly, and throughout the study period, there were no differences between these species.

No LTE was observed in birch. In the other species, the occurrence rate of the LTE varied from 40 % to 100 % (Table 1). In the first sampling at the beginning of August, the LTE was observed between -14 °C and -20 °C. It gradually decreased during cold acclimation to between -34 °C and -35.5 °C in the last sampling at the end of November and the beginning of December. Some of the samples of maple and elm had an intermediate exotherm at around -24 °C at the last two sampling times.

3.7. Frost hardiness

At the beginning of September, FH was the lowest in elm (-5 °C to -6 °C) and the highest in oak (-10 °C), with some differences, depending on the assessment method (Fig. 6). Initially, frost hardening proceeded in parallel in all the species. In October, the rate of hardening was fastest in birch, and its frost hardiness reached the highest level of approximately -60 °C at the end of October or at the beginning of December, as assessed by REL and EIS respectively. At the end of October, maple was the least frost-tolerant, its FH being -28 °C and -25 °C, as assessed by REL and EIS respectively. Generally, FH as assessed by the REL method was higher than FH assessed by the EIS method. For example, at the beginning of December, the REL-estimated FH of maple, oak, and elm ranged from -44 °C to -47 °C, whereas at that time the EIS-estimated FH was -30 °C, -35 °C, and -45 °C in maple, oak, and elm respectively. Significant differences were found in FH between the species as assessed by EIS in the last sampling in December. The correlation coefficient for the frost hardiness values estimated by the two methods was 0.69.

At the time of the first sampling in September, the temperature range

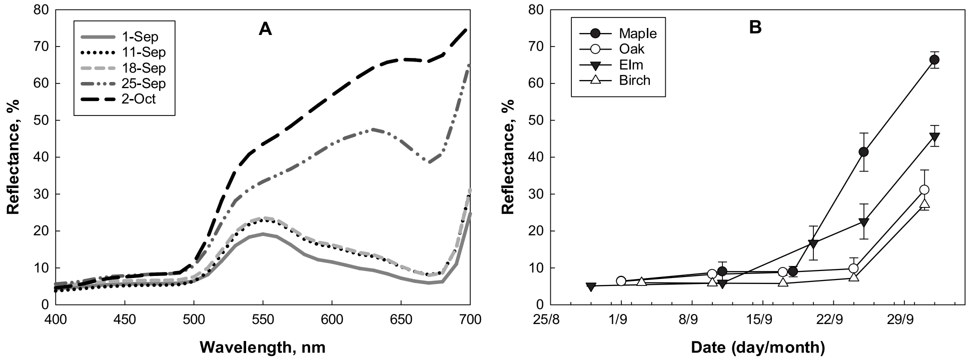

Fig. 2. The mean reflectance spectra (n = 18) of maple leaves (A), and the mean leaf reflectance (± standard error, n = 18) of oak (Repo et al., 2008), maple, elm, and silver birch leaves at a wavelength of 660 nm at different sampling times during autumn colouration in field conditions (B) in Central Finland, north of the natural distribution range of oak, maple, and elm.

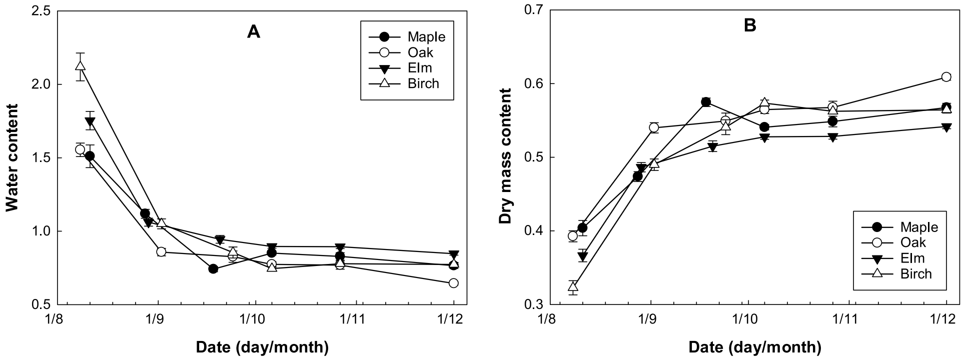

Fig. 3. The mean (± standard error, n = 18) of the water (A) and dry mass (B) content (see Eq. 1 and 2 respectively) of the current-year stem of oak (Repo et al., 2008), maple, elm, and silver birch seedlings at the end of the growing season and during cold acclimation in field conditions in Central Finland, north of the natural distribution range of oak, maple, and elm.

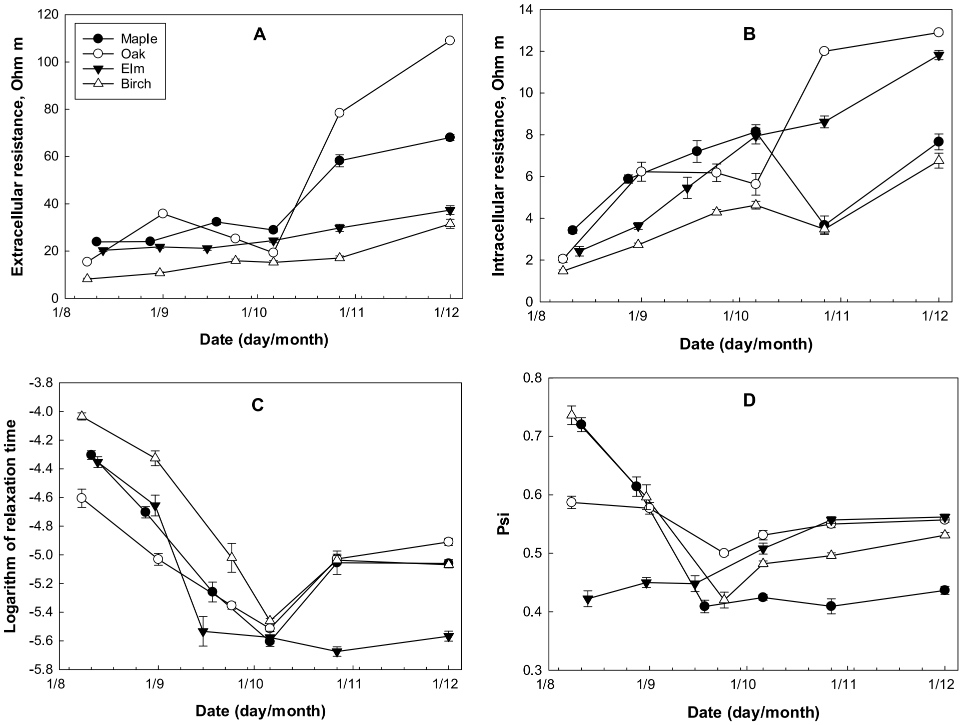

Fig. 4. The electrical impedance parameters, i.e. extracellular resistance (A), intracellular resistance (B), relaxation time (C), and distribution coefficient of relaxation time (D) of the current-year stem of oak (Repo et al., 2008), maple, elm, and silver birch seedlings at the end of the growing season and during cold acclimation in field conditions in Central Finland, north of the natural distribution range of oak, maple, and elm. Bars indicate standard errors (n = 18).

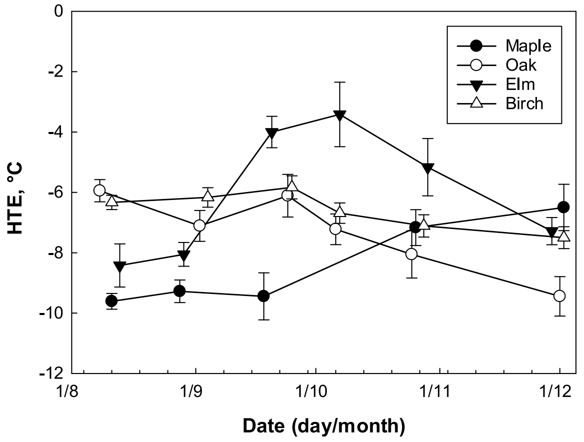

Fig. 5. The mean (± standard error, n = 9) high temperature exotherm (HTE) in the stems of oak (Repo et al., 2008), maple, elm, and silver birch at the end of the growing season and during cold acclimation in field conditions in Central Finland, north of the natural distribution range of oak, maple, and elm.

Table 1
The means and occurrence rates (in parentheses the proportion of samples showing the exotherm of the total number of the tested samples, n = 9) of the low temperature (LTE) and intermediate temperature (ITE) exotherms in stems of oak (Repo et al., 2008), maple, and elm at different sampling times during cold acclimation in field conditions in Central Finland, north of the natural distribution range of oak, maple, and elm. If only one exotherm was observed after the HTE, it was classified as an LTE.

Date	Maple		Oak	Elm	
	ITE, °C	LTE, °C	LTE, °C	ITE, °C	LTE, °C
08–13 Aug		−19.8 (6/9)	−14.4 (7/9)		−19.2 (9/9)
28 Aug–02 Sept		−16.9 (7/9)	−22.9 (6/9)		−19.5 (8/9)
18–24 Sept		−20.3 (9/9)	−21.2 (8/9)		−20.6 (6/9)
06–07 Oct			−28.6 (4/9)		
25–29 Oct		−22.2 (6/9)	−29.2 (6/9)	−24.3 (4/9)	−34.6 (4/9)
29 Nov–02 Dec	−23.8 (6/9)	−34.1 (9/9)	−35.5 (2/9)		−34.3 (6/9)

from no damage to full damage was narrower (ca. 10 °C) in elm than in the other species (ca. 20 °C), as indicated by the derivative curves of REL (Fig. 7A). With cold acclimation, the range became wider in all species – as wide as 50 °C at the beginning of October (Fig. 7B). At the end of October, the REL derivative curve obtained for birch deviated from those obtained for the other species, because it was wider, and its peak was at a much lower temperature than in the curves for other species (Fig. 7C). The derivate curves at the beginning of December indicate that the cellular injuries took place within a wider temperature range in maple and oak than in the other two species, even though the peak of maple and oak was at approximately the same temperature as in the elm curve (Fig. 7D).

The relationship of the LTE to the peak of the derivate of the REL curve changed during frost hardening (Fig. 7). At the beginning of the cold acclimation, the LTE occurred at lower temperatures (at around -20 °C) than the peak of the REL curve (Fig. 7A). At the end of October, the LTE took place around the peak of the derivate curve, i.e. depending on the species, at a higher or lower temperature than the peak (Fig. 7C). At the beginning of December, the LTE was observed at higher temperatures than the peak of the derivate curve in those samples where it was detected (Fig. 7D).

4. Discussion

Contrary to our hypothesis, we found no delay in the phenological events and physiological changes related to the initial autumn hardening in the three studied exotic species compared with the native birch. Growth cessation, a decrease in the water content of the stem, an increase in its dry mass content, and changes in leaf colouration all took place similarly in the four studied species. Nor was any delay in the initiation of frost hardening found in any of the species. Based on these findings, we can conclude that the exotic species are not predisposed to frost damage in the initial phase of cold acclimation. However, in accordance with our hypothesis, in the late autumn, hardening proceeded at a higher rate in the native birch than in the exotic species.

Shoot elongation ceased even earlier in the exotic species than in the native birch, and in a timely manner in all species, compared with the occurrence of the first autumn frosts. Stem diameter growth also ceased in August in all species, well before the first frosts. Growth cessation is considered a prerequisite for proper cold acclimation, and it typically takes place later in southern than in northern ecotypes due to their different responses to increasing night length and the decreasing temperature (Koski and Sievänen, 1985). In species with a free growth pattern, such as silver birch, growth cessation takes place as a result of the effect of increased night length, or the joint effect of night length and temperature (Koski and Sievänen, 1985). Since the oak, elm, and maple seedlings were of more southern origins than the birch seedlings, they were expected to stop growing later than the birch seedlings, but no such differences were observed in this study.

The autumn colouration of leaves started during the latter half of September, first in elm and maple, and finally, in oak and birch. At the time of the onset of autumn colouration, the frost hardiness of the stem

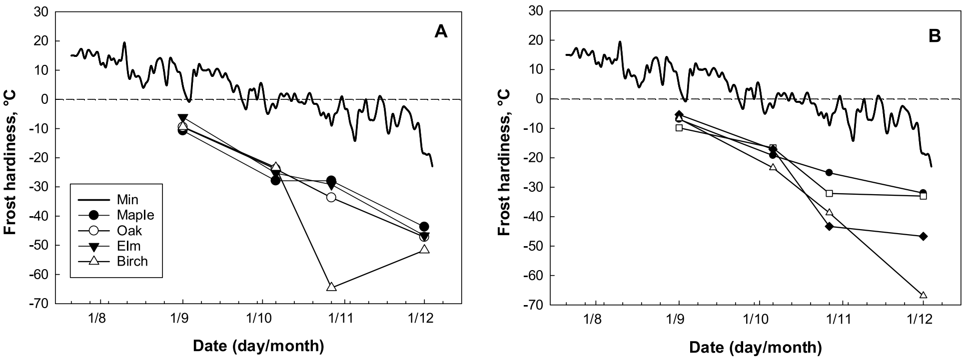

Fig. 6. Frost hardiness of the stem of oak (Repo et al., 2008), maple, elm, and silver birch seedlings during cold acclimation in field conditions in Central Finland, north of the natural distribution range of oak, maple, and elm, as assessed by the relative electrolyte leakage (A) and the electrical impedance (B) methods. The daily minimum air temperature during the study period is indicated.

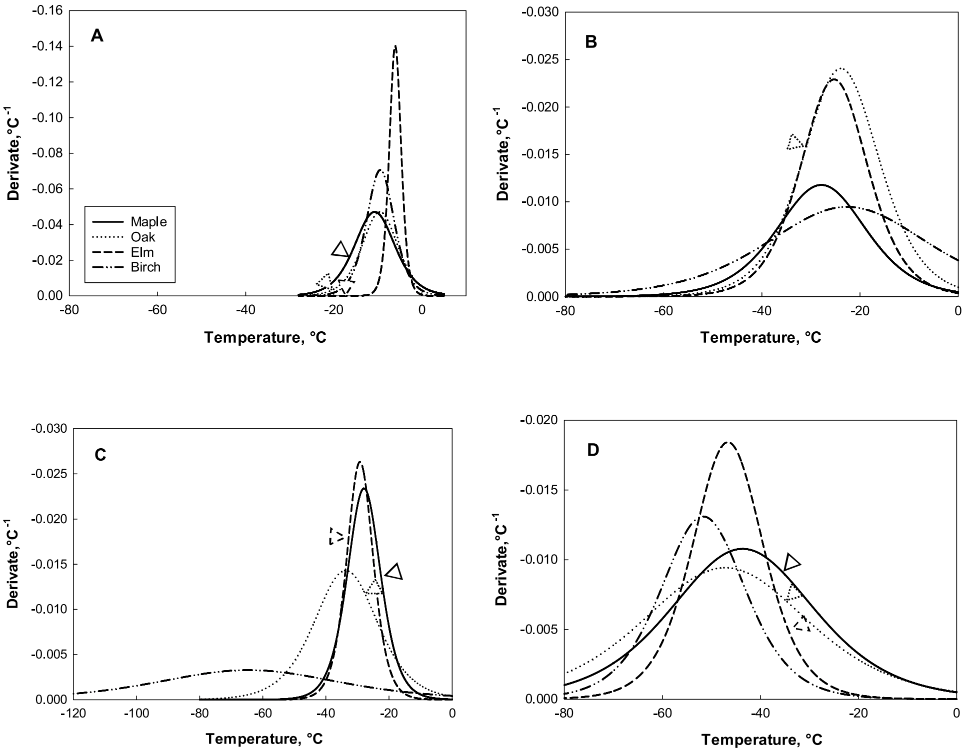

Fig. 7. The damaging temperature range in oak, maple, elm, and birch seedlings during frost hardening in field conditions in Central Finland, north of the natural distribution range of oak, maple, and elm. The damaging temperature range was assessed with the derivate (Eq. 5) of the sigmoidal function (Eq. 4) fitted by nonlinear regression to the relative electrolyte leakage data of the stems at four sampling times, at the beginning of September (A), at the beginning of October (B), at the end of October (C), and at the beginning of December (D). The tip of the triangle indicates the mean of the low temperature exotherm at the derivate curve for the corresponding species.

started to increase too, but no differences were observed between the species yet. It is common knowledge that autumn colouration is an indication that trees are preparing for the winter. However, there are no previous studies of broadleaved species that examine how the timing of leaf colouration is linked to the frost hardiness of the stem. Previously, the autumn colouration of needles of first-year Scots pine seedlings of different origins that were growing close to the location of the experimental seedlings of the present study took place earlier in northern than in southern origins (Toivonen et al., 1991). However, the timing of needle colouration was not significantly related to frost hardiness. We can conclude that despite some coincidence, autumn colouration is not a good indicator of the frost hardiness of the stem in broadleaved trees.

In accordance with the previous study, stem water content decreased and dry mass content increased most prominently already in August before the start of frost hardening (Luoranen et al., 2004). Changes in water and dry mass content have been considered indicators of frost hardiness, and in tree seedling nurseries, for example, dry mass content is used to monitor cold hardening before the seedlings are removed to freezer storage (Hultén, 1980; Calmé et al., 1995). Our results for the stems of different broadleaved species indicate that decreased water content and increased dry mass content are prerequisites for frost hardening, but they are not proper indicators of frost hardiness.

In all species, the EIS parameters of the stem indicated a clear turning point at the end of September and the beginning of October. The extracellular resistance of oak and maple, the relaxation time of all four species, and the distribution coefficient of the relaxation time of oak, maple, and birch all suggest that there were changes in the electrolyte balance of stem cells at that time. Previously, the EIS parameters were found to change with the cold acclimation of Scots pine (*Pinus sylvestris* L.) and willow (*Salix viminalis*) (Repo et al., 1994, 1997; Hietala et al., 1998). The changes in the EIS parameters observed in birch in the present study showed very similar patterns to those in the previous study (Luoranen et al., 2004). In willows growing at the same geographical location as the seedlings of this study, the change in some of the parameters took place at approximately the same time as in this study (Repo et al., 1997). In our study, the turning point was preceded by night frosts, with the minimum air temperature dropping to -3 °C at the nearest weather station. The temperature may have been even lower at the ground surface on the experimental site, thus inducing ice nucleation in the stem and causing freeze-desiccation. It is possible that extracellular freezing took place on those nights, which then changed the electrolyte balance in the cells. It is known that due to vapor pressure difference, there is drift of symplastic water to apoplastic ice, which then leads to intracellular desiccation (Sakai and Larcher, 1987; Arora, 2018). This launched changes in the cellular processes, e.g. the dilution of apoplastic sap, concentration of the symplasm and altered cell membrane properties, which were manifested in the EIS parameters and also hastened the frost hardening (Repo et al., 1994, 2000b; Mancuso and Rinaldelli, 1996).

Apoplastic freezing took place at around -7.5 °C in oak, maple, and birch and between -3 °C and -4 °C in elm, with some changes during the frost hardening. The ice nucleation temperatures of the stems were not measured in field conditions but only in the laboratory tests. The measured HTE`s are in the same range as observed in other laboratory studies (e.g. Kaku and Iwaya, 1978; Rajashekar and Burke, 1978; Ketchie and Kammerech, 1987; Räisänen et al., 2006). However, these may be overestimates compared to the ice nucleation temperature in field conditions, due to the higher rate of cooling and/or the lack of Ice Nucleation Active Substances in the lab tests (e.g. Wisniewski et al., 2014; Arora, 2018). All laboratory tests were carried out in a similar way. Therefore, comparisons between species and sampling times are relevant, and may also reflect the differences in field conditions.

Frost hardiness, as determined by the inflection point of the test temperature response curve, started to increase in all species at the beginning of September. At that time, the margin between the measured FH and the minimum air temperature was at its smallest (approximately 5 °C, Fig. 1). Differences in FH between the species started to appear in the latter part of hardening, especially when frost hardiness was determined by the EIS method. As expected, birch as the native species became more frost-hardy than the other species. According to the inflection point of the temperature response curve, FH, as assessed by EIS, was less than -35 °C for maple and oak at the beginning of December.

The measurements of the LTE by DTA indicated that the FH_{max} values of all exotic species were around -35 °C, which agrees with the results obtained by EIS. These were probably not the potential values of FH_{max} for the species of the present study, however. In another study with different Finnish oak provenances, the LTE was observed somewhat below -40 °C in midwinter (unpublished data), suggesting that the seedlings in our study had not yet reached FH_{max} at the beginning of December.

The occurrence rate of LTE varied depending on the species and the time of sampling. A possible explanation is that in some of the samples there was a small number of, or none at all of deep-cooled cells in stems, or they were dehydrated effectively by apoplastic freezing, and therefore, no LTE was observed (Gusta et al., 1983; Wu et al., 2019). In some of the samples of maple and elm, an additional exotherm located between HTE and LTE was observed. It was called the intermediate exotherm in the previous study with apical buds and a piece of stem of Norway spruce (Räisänen et al., 2006). Multiple exotherms at low temperatures between HTE and LTE have been observed in other species too, for example, in apple (*Malus domestica* Borkh.) (Ketchie and Kammerech, 1987) and different *Pyrus* species (Rajashekar and Burke, 1978). In apple, ITEs varied with the time of year and they were connected to the ice nucleation in pith cells and xylem tissues (Ketchie and Kammerech, 1987). In black currant buds (*Ribes nigrum* L.), they were considered as multiple LTEs, and were assumed to originate from some isolated ice nucleation events (see e.g. Takeda et al., 1993).

The temperature response curve of REL changed remarkably during frost hardening, as shown by the derivate of the sigmoid function fitted to the measured data. A similar phenomenon has also been observed in previous studies for different species and organs (Sutinen et al., 1992; Leinonen, 1996; Luoranen et al., 2004). A single FH value therefore gives a very limited idea of how plants and plant organs respond to freezing temperatures. In the stems, the cellular injuries gradually increase with the dropping of the exposure temperature, whereupon electrolytes leak from the symplast to the apoplast. The electrolyte leakage, as measured in the REL method, is an integrated measure of damage in different tissues, i.e. in the phloem, cambium, and xylem. It is not an ON/OFF phenomenon, similar to the LTE, which originates from the xylem ray parenchyma cells in species with a ring-porous xylem structure (George and Burke, 1977; Hong and Sucoff, 1980; Ashworth et al., 1988). When using REL or EIS, one is measuring a different phenomenon than when measuring the LTE by exotherm analysis (Rajashekar and Burke, 1978; Ketchie and Kammerech, 1987). This explains why the relationship between the LTE and the inflection point of the REL curve changed with frost hardening in the present study.

5. Conclusions

Oak, maple, and elm grow at their northern distribution limit in Southern Finland. According to our results, they stop their growth and start their cold hardening in a timely manner, well before the occurrence of autumn frosts and simultaneously with the native silver birch. The differences between the species appeared only at the end of the study period in December, when the maximum frost hardiness may have not yet been reached, but some more hardening may have occurred because of low midwinter temperatures. We conclude that the hardening phase is not critical for the distribution of oak, maple, and elm to the north from their current natural distribution range.

Data availability statement

The data of this study are available from the corresponding author (TR) on request.

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Author statement

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Author contributions

Conceptualisation (TR, HH), Methodology (TR), Data curation (TR, HH), Writing – Original draft preparation (TR, DW, HH), Visualisation (TR, DW), Writing – Reviewing and Editing (TR, DW, HH).

Declaration of Competing Interest

The authors declare no conflict of interest.

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