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# Seasonal and altitudinal variation of ericoid shrub freezing resistance in temperate bogs

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**Abstract**

Changes in freezing resistance and its mechanisms (i.e., avoidance by supercooling or tolerance of ice formation) were studied in two deciduous (*Vaccinium myrtillus*, *V. uliginosum*) and two evergreen (*V. vitis-idaea*, *Oxycoccus palustris*) ericoid shrubs at two elevations (470 m and 1070 m a.s.l.) throughout the growing season. The temperature causing 50% damage (i.e. Lt50) and nucleation temperature at which ice was formed in the tissues (i.e. exotherm) were measured. The aim was to investigate whether the freezing resistance varied during the growing season and whether the changes were determined by elevation. Freezing resistance declined from the spring to the summer (late May to August) and then it increased toward the end of the season. In half of the cases the resistance remained comparable between the summer (August) and the early autumn (September). The majority of plants were tolerant during most of the season and avoidance occurred only in early or late May. There was no distinct elevational difference in plant freezing resistance between the localities, since the plants from higher elevation were more resistant only in September. Hence, the greatest risk of frost injury was found during the early weeks of the growing season when the ericoid shrubs develop new leaves.

*Keywords:* freezing avoidance, freezing tolerance, *Oxycoccus palustris*, *Vaccinium myrtillus*, *Vaccinium uliginosum*, *Vaccinium vitis-idaea*

## Introduction

Resistance to subzero temperatures can be accomplished by two distinct mechanisms in most temperate plants, i.e., by the avoidance of ice nucleation and the tolerance of extracellular ice (Levitt 1980, Sakai & Larcher 1987). The ice formation inside protoplasts is lethal for plants employing both mechanisms. The avoidance mechanism prevents ice formation inside plant body so that living cells are protected from damage. If an avoidant plant is exposed to freezing temperatures exceeding avoidance capacity, ice nucleation occurs in both, apoplast and protoplasts. The avoidance involves the so-called transient supercooling by which water in the apoplast remains in a liquid state below 0°C, i.e., it is supercooled and no ice is formed inside the tissue. However, the supercooled state has distinct limits as regards the subzero temperature and duration (Levit 1980, Sakai & Larcher 1987, Beck 1994). When nucleation inside the tissues is inevitable due to, e.g., long-term exposures to severe frosts, the only possible strategy to survive is the freezing tolerance mechanism which involves ice nucleation in extracellular spaces. With ice crystals formed outside the cells, water is transferred from the protoplast which modifies the freezing point but, on the other hand, results in cell dehydration. Thus the freezing tolerance is closely related to drought resistance.

Ericoid shrubs represent a distinct growth form at high latitudes and elevations, such as boreal and subalpine forests and peat-bogs. They form extensive polycormons due to their high ability of vegetative propagation by horizontal rhizomes. Some species maintain their leaves during winter and are able to retain acquired nutrients in extremely poor habitats (Ritchie 1955, Jacquemart 1997), whereas other species are deciduous. The freezing resistance of ericoid shrubs is generally about –5°C during the growing season (Lindholm 1982, cited in Jacquemart 1996). The growing performance of most of the species is affected by weather, mainly by temperature and precipitation. *Vaccinium uliginosum* seems to be quite indifferent of low temperature but is very dependent on soil humidity (Jacquemart 1996). Water supply also strongly determines the performance of *Oxycoccus palustris* (Jacquemart 1997).

The ability of temperate plants to withstand freezing temperature varies during the year. Winter hardiness is obtained during autumn through the process of acclimation but it is usually easily lost in spring (Pagter & Arora 2013). Whereas leaves of *Vaccinium vitis idaea* may not be damaged by temperature as low as –70°C in the winter, plants may be affected by only mild frosts about –4°C in the summer (Sakai & Larcher 1987). Temperate plants generally rely on the avoidance by supercooling to survive accidental, short-term summer frosts. Nevertheless, high-alpine and arctic species, especially, may retain a limited tolerance of the extracellular ice even during the growing season (Sakai & Larcher 1987, Robberecht & Junttila 1992, Junttila & Robberecht 1993, Taschler & Neuner 2004).

Ericoid shrubs resume growth almost immediately after snow melts in the spring (Taulavuori et al. 2002) and new growth is accompanied by a rapid loss of the freezing tolerance. Therefore, developing tissues face the risk of being damaged by a sudden frost in early spring. Such a damage due to spring freezing episodes was observed in non-mature leaves of the deciduous species *V. myrtillus*, whereas the evergreen species, such as *V. vitis-idaea*, survived without apparent harms (Kučerová, unpubl. results). Nevertheless, the plants are able to recover even from pronounced damages, e.g., in *V. myrtillus* 70% damage of new shoots caused by spring or summer frost was restored from dormant buds of older stems within three years (Tolvanen 1997).

It can be hypothesized that, in order to prevent early spring damage due to freezing, plants may either retain the extracellular ice tolerance or postpone the development of new, freezing-sensitive leaves. We presumed that ericoid shrubs use different resistance mechanisms to survive freezing in different parts of the year. We tested this hypothesis by studying seasonal changes in the freezing resistance of two deciduous and two evergreen ericoid shrubs from peat-bogs. Since the resistance to freezing is known to vary with the geographical origin and elevation of the species (Levitt 1980, Sakai & Larcher 1987, Körner 2003), we examined populations from two different elevations. In particular we hypothesized that: (i) the deciduous species will be avoidant whereas the evergreen species will be tolerant, (ii) the freezing tolerant species will switch to avoidance mechanism during the peak of growing season, and (iii) the freezing resistance of a given species increases with elevation as the air temperature decreases with higher elevations.

## Material and methods

### Study sites and species

Four species of ericoid (sub)shrubs were selected for the study, two evergreen species, *Vaccinium vitis-idaea* and *Oxycoccus palustris*, and two deciduous species, *V. myrtillus* and *V. uliginosum*. Ecologically, *O. palustris* and *V. uliginosum* represent typical peat bog plants (Jacquemart 1996, 1997), whereas the other two species prefer disturbed or degraded parts

**Table 1.** The sampling dates with indication of the sampled species (codes: V = *Vaccinium vitis-idaea*, O = *Oxycoccus palustris*, M = *V. myrtillus*, U = *V. uliginosum*).

| Sampling date | Apr 14 | May 5 | May 26 | Aug 5 | Sep 19 | Oct 9 | Oct 23 | Dec 17 |
|---|---|---|---|---|---|---|---|---|
| **Jezerní Slať** | none | V, O | V, O, M | all | all | V, O, M | V, O | V, O |
| **Červené Blato** | V, O | all | all | all | all | V, O, M | V, O | V, O |

of the peat bogs (Ritchie 1955, 1956). All four species are rather abundant and occur more or less together at the study sites.

Study sites were chosen with reference to their different elevation. The Červené Blato mire (~470 m a.s.l., 48°51'N, 14°48'E) is a valley bog with *Pinus rotundata* being the dominant woody species in drier part of the locality. The Jezerní Slať mire (~1070 m a.s.l., 49°2'N, 13°34'E) is a typical ombrotrophic raised bog with *Pinus* × *pseudopumilio* growing along the margins. The lowest minimal air temperatures in the Bohemian Forest are regularly recorded in this locality during radiation frosts also in summer (Jůza et al. 2011). Both localities are protected areas although they were affected by human activities in the past.

### Temperature measurements at the study sites

Temperature measurements were carried out using field microclimate weather stations placed at the study sites nearby the sampled populations during the growing season of 2009. Both ground surface temperature and air temperature at 40 cm height above ground were measured with shielded needle thermocouples (Cu-Co type; supplied by EMS Brno, Czech Republic) and recorded with dataloggers every 10 minutes.

### Material sampling

Entire plants with root balls and soil were collected on the same day at both localities. The sampling dates were chosen to cover the entire growing season in 2009, intervals between the sampling dates varied from three weeks (at the beginning and the end of the growing season, when we supposed more pronounced changes in the freezing resistance) to six weeks (during the peak of the growing season) (Table 1). The last two samplings were carried out when snow was already present on both localities. Plants were transported to the Institute of Botany in Třeboň where they were put in a cultivation basin supplied with fresh water. The laboratory measurements were carried out during the following day.

### Freezing resistance measurement

Six to eight fully developed, undamaged leaves were detached from individuals of each species. At the first sampling date (i.e., in April at Červené Blato and in early May at Jezerní Slať) overwintering leaves were sampled in case of evergreen species. Later on during the season new leaves were used for all four species. A Cu-Co thermocouple (supplied by EMS Brno, Czech Republic) was attached to the leaf surface and the leaves were put immediately into the freezer (a commercial freezer with a minimum temperature of –25°C) for the estimation of the freezing temperature. Leaf temperature was recorded every 6 seconds by 12--channel dataloggers. The leaves were acclimated by keeping the temperature in the freezer between 0° and 1°C for 10 minutes. After that time, the temperature was lowered at a constant rate of 5°C per hour to below –22°C. The temperature in the freezer was regulated by heating and control units. A sudden rise in leaf temperature measured by the thermocouples, i.e., the exotherm, indicated the freezing of water inside the leaf.

The temperature at which 50% damage to the tissue occurred (Lt50) was measured on detached leaves placed in the freezer that were subjected to the same temperature treatment as in the previous experiment, i.e., acclimation at 0–1°C followed by a temperature decrease of 5°C per hour. The leaves were taken out of the freezer at regular intervals (at about 0°C, –4°C, –8°C, –12°C, –16°C, <–22°C). Circular segments of 4 mm in diameter were cut from the leaves of *Vaccinium myrtillus*, *V. uliginosum*, and *V. vitis-idaea* using stainless steel cork borer. The leaves of *Oxycoccus palustris* were cut in half with a scalpel. Each segment or two divided leaves (i.e., four halves coming from two leaves) were put in 10 ml plastic tubes with 5 ml of deionized water. The samples were shaken for one hour and were left to settle for another hour.

The freezing damage was estimated by measuring electrical conductivity of the supernatant (Prášil & Zámečník 1998). The conductivity of the samples is proportional to the electrolyte leakage from damaged cells. To control for the size differences among the leaves/leaf discs, the degree of damage by freezing was standardized by the maximum el. conductivity of the sample, which was obtained after boiling the sample for 15 minutes. The degree of tissue injury $I_t$ was then calculated as:

$$I_t = \frac{R_t - R_0}{R_k - R_0} \qquad \text{(Eqn. 1)}$$

where $R_t$ = standardized electrical conductivity of the sample, $R_0$ = standardized electrical conductivity of the controls (not subjected to freezing), and $R_k$ = standardized electrical conductivity of the sample killed by freezing (i.e., sample exposed to temperature below –22°C for an hour) (Prášil & Zámečník 1998). Electrical conductivity was measured with a Gryf 158 conductometer and a Gryf 356/tD probe. Six replicate samples per species and temperature were measured.

The injury temperature Lt50 was estimated by fitting data ($I_t$ and $T$) to the sigmoidal curve:

$$I_t = I_{min} + \frac{I_{max} - I_{min}}{1 + e^{(4 \times slope \times (T - Lt50))}} \qquad \text{(Eqn. 2)}$$

where $I_{min}$ and $I_{max}$ is minimum and maximum degree of tissue injury, respectively, *slope* is slope of the fitted curve at Lt50, and $T$ is treatment temperature (Janáček & Prášil 1991).

We were not able to estimate Lt50 values for *V. vitis-idaea* in two last sampling dates due to technical limitations (insufficient cooling capacity of the freezer).

**Statistical data analyses**

The difference between the exotherm and Lt50 was tested by means of a t-test. A significantly lower value of Lt50 than that of exotherm indicates freezing tolerance, whereas the absence of a difference would indicate avoidance by supercooling. The Lt50 values of two adjacent sampling dates were compared using a t-test to examine changes in the resistance level. If the difference between previous and following sampling was positive then the resistance increased from the first sampling to the other and *vice-versa*. The statistical packages R and SYSTAT 5.05 were used for the calculations.

**Table 2.** Number of days in each month when air temperature dropped below 0°C and the minimum temperature (T) recorded at the ground level and at 40 cm above ground (n.d. = T not determined in this period).

| **Locality:** | **Červené Blato** | | | | **Jezerní Slať** | | | |
|---|---|---|---|---|---|---|---|---|
| Depth above ground: | 0 cm | | 40 cm | | 0 cm | | 40 cm | |
| **Month** | Days | T (°C) | Days | T (°C) | Days | T (°C) | Days | T (°C) |
| **April** (from Apr 14) | 10 | –3.3 | 10 | –3.7 | n.d. | n.d. | n.d. | n.d. |
| **May** | 4 | –1.9 | 7 | –2.6 | 9 | –4.4 | 9 | –4.4 |
| **June** | 0 | 0.8 | 6 | –2.5 | 10 | –5.6 | 9 | –5.3 |
| **July** | 0 | 8.4 | 0 | 3.7 | 4 | –1.2 | 4 | –1.9 |
| **August** | 0 | 4.9 | 2 | –0.5 | 0 | 0.3 | 3 | –5.1 |
| **September** | 0 | 3.8 | 7 | –1.8 | 8 | –1.0 | 11 | –5.8 |
| **October** | 0 | 1.3 | 13 | –4.9 | 11 | –7.1 | 18 | –18.3 |
| **November** | 2 | –0.4 | 16 | –5.6 | 18 | –7.4 | 23 | –13.1 |
| **December** (till Dec 17) | 4 | –2.4 | 12 | –9.7 | 13 | –3.4 | 16 | –16.8 |

## Results

### Temperature at the study sites

The locality/mire of Jezerní Slať was generally colder than Červené Blato in terms of both, the number of freezing days and the minimum temperature recorded during each month (Table 2), as well as overall number of hours with frost (data not shown). Temperature at the ground level as well as at 40 cm height dropped below 0°C each month at Jezerní Slať except for the ground level in August. Air temperature at the ground level varied in a narrower range than the temperature at 40 cm above ground, as the water-saturated soil during the growing season acts as a thermal buffer. The temperature at the ground level was very stable during the last period of the measurement, i.e., in the late autumn and in the winter, when snowpack accumulated at both localities.

### Changes in freezing resistance during the season

The freezing resistance (i.e. Lt50) decreased significantly from the start of the growing season (i.e., the first sampling date at Červené Blato and the second sampling date at Jezerní Slať) to the end of May (the third sampling date) in the evergreen species at both localities (Fig. 1). The resistance of *Vaccinium vitis-idaea* changed consistently at both localities, i.e., it decreased from the start of the growing season to late May and increased from September to October. However, we failed to obtain the Lt50 values in August and in two last sampling dates. The maximum freezing resistance was observed in April (–19.2±1.2°C) and the lowest resistance in late May (–10.2±0.4°C) in plants from Červené Blato. At Jezerní Slať, the plants reached the highest resistance in early May (–14.0±0.5°C) and the lowest in late May (–10.6±0.3°C).

The freezing resistance decreased from the begining to early May (Červené Blato) or late May (Jezerní Slať) in *Oxycoccus palustris*. The Lt50 values from the following (i.e., third and fourth) sampling dates were not obtained. No difference was found in the resistance between August and September when the plants were least hardy (~ –12.5°C) at Červené Blato. In the plants from that locality, resistance increased from September to achieve the maximum of –17.3±0.9°C in early October but then slowly decreased toward the end of the season. The pattern was different in Jezerní Slať where the Lt50 values increased from September to October and then decreased to December when plants reached the maximum resistance (–17.3±0.5°C). The lowest resistance was observed in late May (–10.1±0.5).

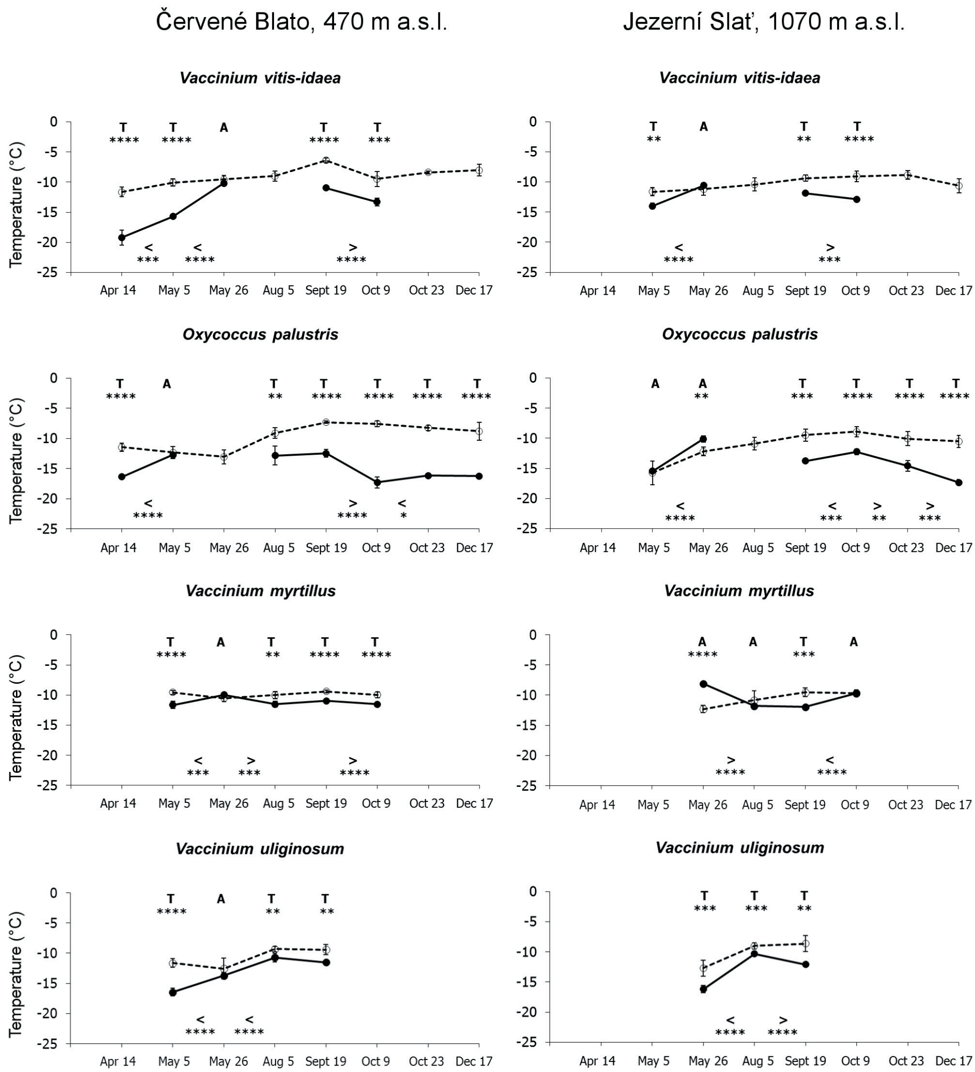


**Fig. 1.** The course of Lt50 (solid circles, full lines) and exotherm (open circles, dashed lines) for each species and locality (means with 95% confidence intervals). The signs in the bottom of each panel compare the Lt50 values of following sampling dates, i.e., the increasing (<) or the decreasing (>) resistance level during a particular period. A letter on the top of each graph refers to the resistance mechanism, i.e., avoidance and tolerance: if the Lt50 value is lower than the exotherm level, the plants is considered to be tolerant (T); if there is no difference between the two values or the Lt50 is higher than the exotherm, then the plant is considered to be avoidant (A); missing Lt50 values indicate that they were not obtained (evergreen species) or plants were not sampled (deciduous species in the early spring and in the late autumn). Significance levels: $p<0.0001$ ****, $p<0.001$ ***, $p<0.01$**, $p<0.05$ *.

**Table 3.** Difference in Lt50 values between localities. Positive values indicate higher resistance at Červené Blato, negative values means higher resistance at Jezerní Slať. Significance levels: p<0.0001 ****, p<0.001 ***, p<0.01 **, p<0.05 *; x indicates missing data for Jezerní Slať where foliage in the deciduous species developed in later date; n.d. indicates missing Lt50 values.

| Sampling date | *V. vitis-idaea* | *O. palustris* | *V. myrtillus* | *V. uliginosum* |
|---|---|---|---|---|
| **Apr 14** | x | x | x | x |
| **May 5** | 1.75 *** | –2.75 **** | x | x |
| **May 26** | 0.06 | n.d. | 1.85 **** | –2.46 **** |
| **Aug 5** | n.d. | n.d. | –0.27 | 0.40 |
| **Sept 19** | –0.91 **** | –1.30 ** | –1.00 **** | –0.57 **** |
| **Oct 9** | –0.39 | 5.05 **** | 1.87 **** | x |
| **Oct 23** | n.d. | 1.60 ** | x | x |
| **Dec17** | n.d. | –1.01 ** | x | x |

The freezing resistance of *Vaccinium myrtillus* decreased from early to late May at Červené Blato. It then increased to August and remained constant until September at both localities. Later, the resistance increased in plants from Červené Blato toward October in contrast to a decrease observed in the plants from Jezerní Slať. The highest and lowest resistance throughout the growing season were measured in early and late May (Lt50 = –11.6±0.6°C and –10±0.3°C, respectively) for plants from Červené Blato and in September (Lt50 = –12±0.3°C) and late May (–8.1±0.2°C) for plants from Jezerní Slať. The freezing resistance in *Vaccinium uliginosum* decreased from the beginning of measurement to August at both localities. Then, the resistance remained unchanged from August to September in the plants from Červené Blato, but increased in the plants from Jezerní Slať. Plants from both sites achieved the maximum resistance in May (–16.4±0.7°C at Červené Blato, –16.2±0.6°C at Jezerní Slať) and were least resistant in August (–10.7±0.7°C at Červené Blato and –10.3±0.2°C at Jezerní Slať).

**Variation in freezing resistance mechanisms**

Plants were found to be freezing tolerant (i.e., displayed lower Lt50 than exotherm) most of the time, although there are some exceptions from this general pattern (Fig. 1). *Vaccinium uliginosum* from Jezerní Slať was tolerant during all sampling dates. *Vaccinium vitis-idaea* from both localities and *V. myrtillus* and *V. uliginosum* from Červené Blato were tolerant except late May when the plants were avoiding freezing by supercooling (or the Lt50 value was not obtained). *Oxycoccus palustris* from both localities was avoidant in early May and the plants from Jezerní Slať were also avoidant in late May, whereas the plants were tolerant during the remaining parts of the growing season. In contrast, *Vaccinium myrtillus* from Jezerní Slať mostly avoided freezing and was found tolerant only in September. To summarize the observed pattern, all plants except *V. myrtillus* from Jezerní Slať were tolerant from September to the end of the measurement, they were tolerant also at the beginning of the season, and avoidance occurred mainly in late May.

There was no clear pattern of freezing resistance variation between the plants from the two localities throughout the growing season (Table 3). All species from the high-elevation locality at Jezerní Slať were more freezing resistant than the plants from Červené Blato in September. Deciduous species did not differ between the localities in August.

## DISCUSSION

Changes in the freezing resistance of ericoid shrubs were studied both during the growing season (BEERLING et al. 2001) and winter (TAULAVUORI et al. 1997). However, most of the research was carried out on plants from the (sub)Arctic. Our study described changes in the freezing resistance in plants inhabiting temperate ombrotrophic peat bogs, where they experience considerably longer growing season than plants in the Arctic. All examined species demonstrated changes in both, the degree of resistance and its mechanism. At the beginning of the growing season, the resistance generally declined in all species. The decline in resistance continued to late May, or to August. Between August and September, the resistance level increased or remained comparable. From the late summer towards the end of the growing season, the resistance increased in most of the species. These observations are in agreement with the general patterns seasonal changes observed in temperate plants (SAKAI & LARCHER 1987).

Exceptions from the above outlined pattern were *O. palustris* and *V. myrtillus* from Jezerní Slať. The resistance of *O. palustris* declined from September to October, although cold hardening would be expected to occur during this period. The cold acclimation process may have been postponed since the resistance of the plants increased after the October sampling date. The resistance increased in *V. myrtillus* from May to August, remained constant to September, and later declined. In May, which was the first sampling date of that species at Jezerní Slať, the newly developed leaves were apparently very sensitive to frost. This sensitivity decreased later in the spring and summer as the leaves matured. The leaves of *V. myrtillus* at Jezerní Slať already showed signs of senescence (by the moderate degree of leaf yellowing), which may explain the partial loss of the freezing resistance during the last sampling date.

The freezing resistance of *V. myrtillus* in August (–11.5±0.5°C at Červené Blato and –11.7±0.6°C at Jezerní Slať) are distinctly lower than the resistance of about –7°C observed in plants from the Central Alps at elevation >2100 m at the end of July (MARTIN et al. 2010) and also from *in situ* measurement (~ –5°C) in plants from the Alps at 2600 m (TASCHLER & NEUNER 2004). On the other hand, stems of *V. myrtillus* from northern Finland were much more resistant (~ –30°C in April, ~ –60°C in October, ~ –70°C in December) than our respective observations for leaves, although the May resistance values were only marginally different (TAULAVUORI et al. 1997).

The late May freezing resistance values of *V. vitis-idaea* (–10.2±0.4°C at Červené Blato, –10.6±0.3°C at Jezerní Slať) were lower than the summer damaging temperature of ~ –5.5°C in the Alps at 2600 m (TASCHLER & NEUNER 2004). On the other hand, the temperature of –5°C did not cause any damage (Lt0) in leaves of *V. vitis-idaea* from ~2000 m (SAKAI & LARCHER 1989). The range of reported values could be due to different methods used between the studies, i.e., measurements of detached and undetached leaves. In comparison to plants from northern Finland (TAULAVUORI et al. 2001), the resistance values obtained in our study were comparable during September but higher during October.

*Vaccinium uliginosum* was sampled only four times at Červené Blato and three times at Jezerní Slať. Its resistance was relatively high even in newly developed leaves (~ –16°C in early May at Červené Blato and late May at Jezerní Slať). This rather high resistance may reflect adaptations of this species to growth at high latitudes and elevations (JACQUEMART 1996). The species generally avoids growing under closed canopy and usually occurs on exposed, windward sites (SAKAI & LARCHER 1987), which means poor protection with thin layer of snow cover in winter. The resistance on the last sampling (September) did not reach the spring level, although it was higher than in the summer. The Lt50 value obtained in August (~ –10°C on both localities) was lower than Lt50 = –4°C for plants at ~ 2000 m in summer (Sakai & Larcher 1987).

In general, *V. myrtillus* leaves exhibited the lowest overall resistance level among all tested species at both localities. Intriguingly this is in contrast with findings of Palacio et al. (2015) where the bud freezing resistance in May was lower for *V. uliginosum*, higher for *V. myrtillus,* and the highest for *V. vitis-idaea* from similar elevation. According to our results, *V. uliginosum* leaves were of a comparable or even higher resistance level as those of *V. vitis idaea* at the both localities. However, the bud and the leaf resistance may not exhibit similar trends.

We failed to obtain the Lt50 values in the evergreen species in the summer (i.e., late May and August). This may have been due to the unsuitable range of 4 °C temperature steps which was used to assess the freezing injury. Since unhardened leaves of *V. vitis-idaea* may be 100% damaged at very mild temperature about –2.1°C (Levitt 1980), our sampling may have missed the point at which injury developed in the tissues, which resulted in failure to fit the logistic model to data and estimate the Lt50 values. On the contrary, in two last sampling dates, the lowest exposure temperature in our sampling was probably insufficient to cause high injury, which would enable logistic fitting. In fact, resistance in stems of *V. myrtillus* may be below –50°C in autumn (Taulavuori et al. 1997), whereas minimum reached in our setting was about –29°C.

In general, the plants were tolerant at the beginning and at the end of the growing season and avoidant in the late spring and/or in the summer. *Vaccinium uliginosum* from Jezerní Slať was tolerant during the entire season. This supports our initial hypothesis that plants become tolerant toward the end of the summer and are avoidant during the full growth. We also presumed that newly developed leaves of the deciduous species would be avoidant in the spring. On the other hand, the leaves of the evergreen species can retain some degree of tolerance from the previous winter. These ideas correspond with the situation in *V. vitis-idaea* from both localities, *O. palustris* from Červené Blato, and *V. myrtillus* from Jezerní Slať. In contrast, *O. palustris* from Jezerní Slať was already avoidant in early May, *V. myrtillus* from Červené Blato, and *V. uliginosum* from both localities were tolerant in early spring.

Two interesting results were obtained when Lt50 exceeded exotherm temperatures in late May in *O. palustris* and *V. myrtillus* from Jezerní Slať. These situations are marked as avoidance in Fig.1 but the question stands what the real biological meaning of these data is. They suggest that leaves are capable to sustain subzero temperatures but at some point they are damaged by those temeratures without ice nucleation taking place. As these events were found in new leaves, we propose that they are not adapted to freezing temperatures occurring in spring.

Evergreen species showed consistent patterns of resistance variability during the season with the highest resistance at the beginning or end of the season and lowest resistance in late spring (and probably also in the summer). On the contrary, the deciduous species do not form a consistent resistance pattern. In terms of freezing resistance, *V. uliginosum* shows more similarity to the evergreen species than to *V. myrtillus*. We failed to confirm the expected pattern of increasing resistance with elevation. The Lt50 values were not distinctly lower in the high-elevation locality at Jezerní Slať, although Jezerní Slať represents one of the most severe localities in terms of minimum air temperature in the Czech Republic. Apparently, the freezing resistance of ericoid shrubs is determined not only by the elevation itself but also other factors influencing the temperature of surface of vegetation, such as snow cover. For example in northern Finland, the resistance of *V. myrtillus* and *V. vitis-idaea* was lower in snow-covered than in exposed plants (Taulavuori et al. 1997, 2011). However, high-elevation plants of *V. vitis-idaea* exposed directly to frost were more susceptible to frost than plants from a lower elevation protected with a thick snow cover in a reciprocal transplantation experiment (Saarinen & Lundell 2010).

During the peak of growing season, all four species were more freezing resistant than the measured minimum air temperature. The resistance of the plants was comparable to the minimal air temperature measured in 40 cm above ground at Jezerní Slať in late autumn and in winter during which period, however, the plants were covered by a protective layer of snow. The most critical period thus appears to be the spring (early and/or late May) during which most species of the ericoid shrubs are avoidant and sudden and significant drops of air temperature below zero might be damaging to the newly developing leaves.

## Conclusions

We found that, in general, freezing resistance level changes during the growing season being lowest in summer and reaching the highest values in early spring or in winter. All the species were tolerant for the most part of the season, avoidance mechanisms were detected only in spring or summer. No systematic effect of locality, i.e. of elevation, on resistance level was found although the temperatures occurring at Jezerní Slať were substantially lower than those at Červené Blato. Apparently, early weeks of the growing season, when the ericoid shrubs develop new leaves, pose the greatest risk of frost injury.

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