Artificial deacclimation response of *Vaccinium myrtillus* in mid-winter

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Artificial deacclimation was provided for the bilberry (*Vaccinium myrtillus*) in Oulu, northern Finland (65°N) in mid-winter 1995. The aim was to study the susceptibility of the bilberry to deacclimate in constant temperatures elevated just above the realistic levels (+5 and +10 °C). Frost resistance (LT_{50}) of the bilberry stem and bud phenology were followed. The results so far indicate that the bilberry is susceptible to complete deacclimation in mid-winter. However, this deacclimation requires unrealistic temperature elevations or unrealistically prolonged periods of scarcely realistic temperatures.

Key words: deacclimation, ecology, frost resistance, temperature, *Vaccinium myrtillus*

Introduction

Deacclimation is a shift from dormant to an active growth state in perennial woody plants. A phase between these two states is quiescence, in which growth competence has been already achieved by completed chilling requirement (Hänninen 1995 and references therein). Molecular activity occurs during this phase (Lindfors & Kupila-Ahvenniemi 1988) although growth is arrested as long as temperature is unfavourable (e.g. Fuchigami *et al*. 1982). Onset of growth (bud break) is temporally associated with the rapid end of dehardening process during which frost resistance is practically lost.

Current atmospheric carbon dioxide concentration of around 370 ppm is expected to be doubled by the end of 2100 (e.g. Murray 1995). The globally averaged surface temperature is projected to increase by 1.4–5.8 °C over the period 1990 to 2100 (Houghton *et al*. 2001). A climatic warming in winter by as much as 4–7 °C for the boreal zone and even 12 °C for the arctic is predicted while the expected in-

crease in temperature in southernmost latitudes is smaller (e.g. Bach 1988, Maxwell 1992, Houghton *et al.* 2001). Temperate episodes in winter are possible, however, in the north irrespective of climatic warming. In Oulu, northern Finland (65°N), +9.3 °C is the recorded maximum temperature in January since the 1960s, and +4.9 °C is the maximum daily mean temperature within the same period. Thus, we wanted to test whether or not such elevated temperatures experienced for realistic periods of time could initiate premature dehardening and bud break, and hence increase the incidence of frost injuries.

Constant temperature environments were established in phytotrons to deacclimate the bilberry (*Vaccinium myrtillus*), a characteristic dwarf shrub in European boreal and subarctic forests. According to Havas (1971), the bilberry is susceptible to early deacclimation, and our long-term studies in the field also showed that only 2–3 °C rise in mean temperatures accelerated its dehardening (K. Taulavuori *et al.* 1997). An extensive dieback, probably caused by premature dehardening, is also reported in other studies (Ögren 1996). The present work investigates the effect of high winter temperatures on deacclimation of the bilberry in mid-winter. The main questions were: (1) Does the bilberry deacclimate completely in mid-winter as a result of ecologically realistic temperatures? (2) Does the deacclimation at such temperatures occur in a realistic time span?

Material and methods

Patches containing mainly the bilberry (*Vaccinium myrtillus*) and some cowberry (*V. vitisidaea*; only the bilberry was studied) were excavated in the spring 1994 from forest sites in Oulu, northern Finland (65°N, 25°E). They were planted in plastic boxes of size $40 \times 30 \times 20$ cm containing sand, peat and raw forest humus (1:1:1) as a substrate. A fine shadow system of fibre cloth was built above them to prevent extreme radiation. The patches were watered daily with deionized water during summer months (June–August) and fertilized once a month ac-

cording to Ingestad (1973). The shadow system was removed in September to allow natural snow cover to accumulate.

Nine plant boxes were dug out from beneath the snow cover (approx. 40 cm) on 20 January 1995. The boxes were kept overnight in a greenhouse at +10 °C in order to stabilize and thaw. The experiment was started by inserting three boxes ($n = 3$) into each of the three climate chambers, whose temperatures were kept at +5, +10 and +20 °C. The plants experienced a short day (8 h) photoperiod, 180 µmol m^{-2} s^{-1} light intensity (PAR) (Osram 250 W HQI-T 250 W/D lamps) and 60% humidity. The plants were watered twice a week with deionized water. Analysis of the material was started three days after bringing the plants indoors, since the material was assumed to be in a dormant and frost-hardy state, and the scanty material restricted the number of harvests. Analysis continued at weekly intervals for three weeks, making four sampling dates (i.e. when the plants had been indoors 3, 10, 17 and 24 days) in all to analyse frost resistance (LT_{50}) and bud phenology. The experiment was terminated after the fourth sampling by cutting all the aerial shoots exactly at the soil surface. Further capability to produce compensatory growth was followed, and is reported elsewhere (Tolvanen & Taulavuori 1998).

Frost resistance (LT_{50}; 50% lethal temperature) of stem pieces was determined with artificial laboratory tests. The LT_{50} was analysed with the freezing induced relative electrolyte leakage method according to E. Taulavuori *et al.* (1997) and K. Taulavuori *et al.* (1997) with the following exceptions: six shoot apices from one ramet were detached and distributed in one of the six test temperatures (–2, –5, –10, –20, –40 and –70 °C). Since 50% injury did not occur always in response to the lowest test temperature, the LT_{50} was calculated by probit model (SPSS 1992), which estimates the temperature at 50% probability for lethal injury. The sample size was three ($n = 3$): one ramet from each replicate box.

The phenological stage of the buds was assessed by visually scoring the 30 samples from each replicate, and these were classified (K. Taulavuori *et al.* 1997, Tolvanen & Taula-
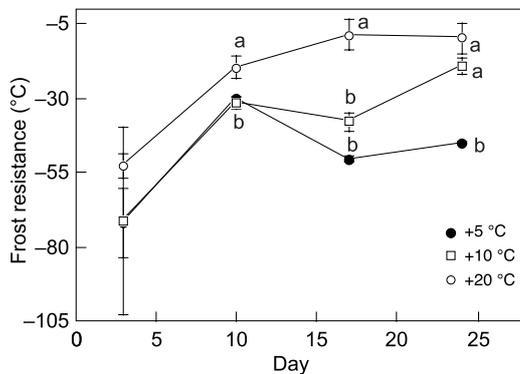
Fig. 1. Frost resistance (LT_{50}) of bilberry stems during artificial deacclimation (mean ± SE, $n = 3$). Different letters indicate differences at $P < 0.05$.

vuori 1998) as: (1) unopen bud scales, (2) open bud scales, (3) visible leaves and (4) elongated internodes (i.e. new shoot). The frequency (%) of each class was noted.

Statistical analyses included two-way and one-way ANOVA prior to post-comparison by Sheffe's test (SPSS 1992).

Results

Dehardening, i.e. decrease in frost resistance, occurred most rapidly in the plants kept at +20 °C. Their LT_{50} was around –53 °C at the beginning of the experiment, but attained –19 °C within ten days and about –9 °C within seventeen days (Fig. 1). Plants exposed to +5 and +10 °C dehardened slightly within ten days to a level of around –30 °C. After that the plants kept at +10 °C dehardened to a level of around –20 °C by the end of the experiment, while those kept at +5 °C even slightly rehardened. Statistics (two-way ANOVA) also showed significant effects of the sampling date ($P < 0.001$, $F = 10.6$, df = 3) and treatment ($P < 0.01$, $F = 7.1$, df = 2) on frost resistance. The one-way ANOVA results also indicated differences between the treatments: $P < 0.05$, $F = 8.6$, df = 2 (day 10); $P < 0.001$, $F = 38.1$, df = 2 (day 17); $P < 0.001$, $F = 27.8$, df = 2 (day 24).

Bud phenology also indicates the progression of deacclimation in response to varied temperatures (Fig. 2). The plants kept at +20 °C showed a
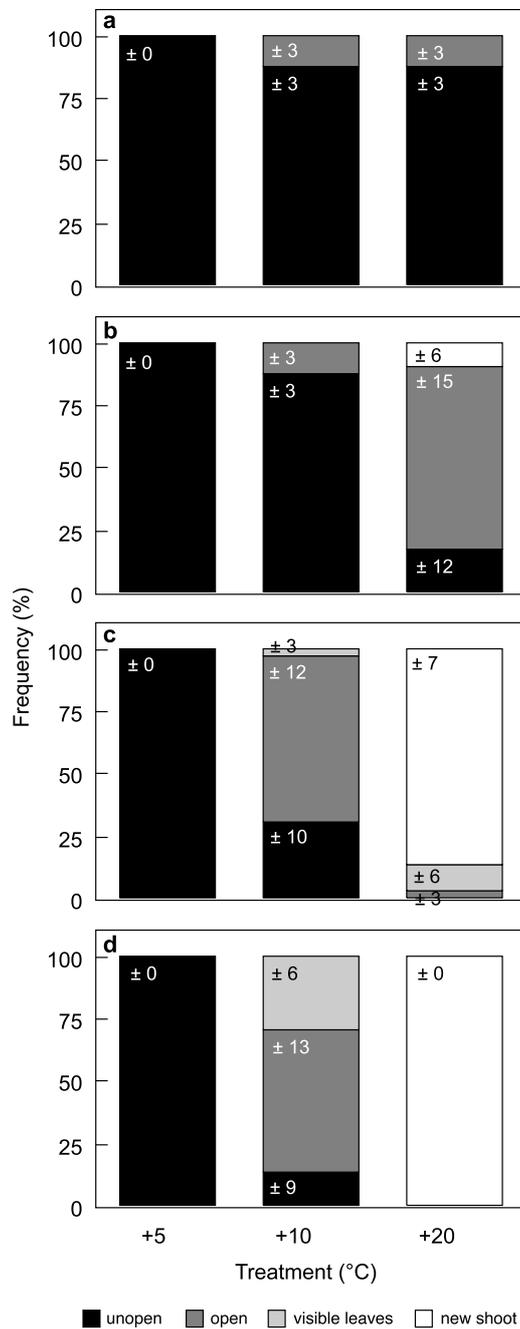

Fig. 2. Development of bud phenology during artificial deacclimation: (**a**) 3 days, (**b**) 10 days, (**c**) 17 days and (**d**) 24 days after bringing the plants indoors. The bars indicate mean frequencies (%) of three boxes ($n = 3$), the standard errors (± SE) are written in the bars.

clear deacclimation response within ten days (Fig. 2b) and 100% of them displayed new growth at the end of experiment (Fig. 2d). The response of the plants kept at +10 °C was much slower, as they showed visible leaves but no new shoots at the end of the experiment (Fig. 2d). However, all the bilberry plants kept at +5 °C remained dormant throughout the experiment.

Summarising, the rate of dehardening and the development of bud phenology both showed that the bilberry is able to deacclimate completely in mid-winter. However, this requires ecologically unrealistic temperatures (+20 °C), or marginally realistic temperatures (+10 °C) prolonged to an unrealistic span of time: the lower the temperature the longer exposure is required for complete deacclimation. The temperature of +5 °C was not sufficient to initiate any deacclimation during the period studied (24 days).

Discussion

The treatments +5 and +10 °C in the present study represent approximately the warmer side of the realistic temperatures in January in Oulu. Plants kept at +5 °C showed practically no dehardening response during the entire 24 days in either experiment (Fig. 1). The plants kept at +10 °C dehardened almost to the same extent as the plants kept at +20 °C. However, the former plants required a considerable period (24 days) to attain this stage (Fig. 1). In addition, it should be emphasized that they were relatively frost-tolerant still on day 17.

Bud phenology (Fig. 2) supports the above findings: buds of the plants kept at +5 °C remained at unopen stage throughout the study period, and the buds which experienced +10 °C required more than 10 days to show any change in phenology. Given these findings and the fact that +4.9 °C is the maximum daily mean in January recorded in Oulu during the last almost 40 years, the deacclimation susceptibility (evaluated from frost resistance and bud phenology) of the bilberry during this time of year could be interpreted to have no ecological significance.

Our earlier work showed that a mean temperature rise of only 2–3 °C accelerates markedly the dehardening of the bilberry compared to ambient control (K. Taulavuori *et al.* 1997). However, we used constant temperatures in the present investigation, since the aim of this work was to investigate the range of temperatures, which would promote premature dehardening, and how long these are required to induce physiological response. In nature, fluctuating temperatures may promote budburst (Hänninen 1990, Myking 1997). Also a great number of freeze-thaw cycles during winter may have been a reason for an extensive dieback in the bilberry (Ögren 1996). This is attributed to respiratory loss of cryoprotective sugars (Ögren 1996, 1997, Ögren *et al.* 1997).

Respiration is less affected than photosynthesis by freezing temperatures. The latter should be negligible when freeze-thaw cycles are frequent (Ögren 1996). In this study the bilberry plants were brought indoors in mid-winter and treated with above-zero temperatures without any freeze-thaw cycles. The photosynthesis of the bilberry thus may have provided sufficient carbon balance at +5 °C under short day conditions, while at +20 °C and partly at +10 °C the photosynthesis may not have compensated for accelerating respiration. Therefore, the results obtained here both on frost resistance (Fig. 1) and bud phenology (Fig. 2) are in accordance with respiratory loss of sugars accompanied with premature dehardening (Ögren 1996, 1997, Ögren *et al.* 1997). In addition, once deacclimation proceeds the respiration increase of the bilberry is cumulative (Havas & Mäenpää 1972, Ögren 1996).

In conclusion, frequent freeze-thaw cycles apparently promote premature dehardening of the bilberry (Ögren 1996). However, when the temperature remains above zero for exceptionally long periods during short day conditions in mid-winter, deacclimation proceeds as a function of increasing temperature. This study showed that (1) the bilberry is able to deacclimate completely in mid-winter by elevated temperatures, which, however, are far from ecologically realistic, or (2) requires an unrealistically prolonged exposure. As for future prospects, additional research is needed to clarify the relationship between deacclimation process and freeze thaw-cycles, especially in controlled environments (chambers, field heating).

Acknowledgements

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