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Potential processes leading to winter reddening of young Douglas-fir *Pseudotsuga menziesii* Mirb. Franco. in Europe

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Abstract

Key message Winter reddening of young Douglas-fir (*Pseudotsuga menziesii* Mirb. Franco), triggered by large thermal fluctuations in late winter, is a critical problem for European forestry. A literature review identified certain climatic conditions that are characteristic of 'reddening' years, including warm daily temperatures, high daily temperature amplitude, low relative humidity, moderate wind speeds, as well as the occurrence of freeze-thaw cycles with cold night temperatures.

By describing the triggering environmental and stand factors, we propose three hypotheses for the physiological processes leading to winter reddening, namely (i) hydraulic failure due to winter drought stress, (ii) photo-oxidative stress in shade-acclimated trees, and (iii) early cold deacclimation during warm periods.

i) Low soil temperature, by reducing root water uptake, combined with anticyclonic conditions, by increasing water losses, can induce hydraulic failure in the xylem. Hydraulic failure may be further accelerated by night frosts.

ii) Winter reddening can occur when low temperature and high irradiance coincide, disrupting photostasis. Overwhelming of winter photo-protection may lead to photodamage and subsequent reddening.

iii) Warm periods, by inducing cold deacclimation, make trees susceptible to frost damage.

Finally, the three processes may interact under atypical anticyclonic conditions in late winter (e.g. cold or dry soils, warm days, high irradiance and/or freezing nights). Indeed, trees under water stress would develop a higher sensitivity to freezing night and photooxidative stress. We therefore proposed mitigation actions to avoid exposing trees to stressful conditions based on e.g. stand characteristics, understorey vegetation and planting.

Keywords Cold deacclimation, Freezing, Hydraulic failure, Photo-oxidative stress, *Pseudotsuga menziesii*

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1 Introduction

Forest productivity and tree survival are significantly affected by biotic and abiotic disturbances. Summer drought stress which is often combined with hot spells, can lead to leaf browning and/or shedding, compromising long-term tree survival (Allen et al. 2010). In conifers, severe and prolonged water deficits in summer can also lead to partial or complete crown browning (Drénou and Rosa 2014). However, a change in needle colour, from green to red, has also been observed outside the summer season i.e. during late winter - early spring in Montana



forests (Hedgcock 1912). During spring 2012, 250,000 ha of forest in Northwestern Ontario were affected by needle browning, of which 190,000 ha were severely affected, i.e., 25% to 100% of the trees were damaged (Man et al. 2013). In France, the occurrence of winter needle browning in mountainous areas has increased dramatically in recent decades and now occurs almost every 3 years, according to the French department for the forest health (DSF, pers. comm.).

Winter needle browning has been observed in many conifer species such as balsam fir *Abies balsamea* (L.) Mill. and black spruce *Picea mariana* (Mill.) BSP (Cayford et al. 1959; Man et al. 2013, 2009), white spruce *Picea glauca* (Moench) Voss (Cayford et al. 1959; Man et al. 2013), jack pine *Pinus banksiana* Lamb. (Man et al. 2013), Sitka spruce *Picea sitchensis* (Bong.) Carr. (Redfern et al. 1987), Douglas-fir *Pseudotsuga menziesii* (Mirb.) Franco (Hedgcock 1912; Malmqvist et al. 2018; McMinn and Funk 1970; Nicolescu 2020), Silver fir *Abies alba* Mill. (Robakowski et al. 2021) and locations (Japan, North America, Sweden, Norway, France, Belgium, Switzerland). Different phenomena, occurring in late winter/early spring, could lead to winter browning: red belt (Bella and Navrati 1987; Henson 1952), winter burn (Maple 1975), winter freezing damages (Man et al. 2013), winter reddening (Hughes 2011), frost damages (Christersson and Fircks 1990), late spring frost (Man et al. 2013), winter drought (Hart et al. 2017), frost-drought (Tranquillini 1982) or winter desiccation (Christersson and Fircks 1990; Sakai 1970). Needle discolouration is not a highly specific symptom i.e. orange or red needles do not necessarily indicate serious and irreversible damage leading to tree death. Needle discolouration may be a temporary visual symptom which may disappear with increasing air temperature or resumption of growth (Steyn et al. 2002).

Depending on the type of damage, three patterns of needle browning can be distinguished: red belt, spring frost damage and winter reddening. In Scandinavia and western North America, red belt affects different tree cohorts, from seedlings to mature trees. The damage appears as a narrow red band at given elevations along slopes (Hedgcock 1912; Jalkanen 1990; Venn 1993). The red belt is mainly caused by Chinook winds, which are characterised by warm, dry air in conjunction with limited water uptake. Mature stands of Douglas-fir, lodgepole pine *Pinus contorta* Douglas and ponderosa pine *Pinus ponderosa* Douglas are mainly affected. In 1909, 16,000 hectares of trees died in Montana (Hedgcock 1912). Spring frost damage, or false spring, refers to direct frost injury to elongating buds, stems, newly emerging shoots and foliage that occurs after bud break (Reich 1990; Chamberlain et al. 2019). False springs

happen after plants have been exposed to mild temperatures in late winter. Mild temperatures, by accelerating the release of ecodormancy, make buds highly vulnerable to freezing temperatures (Charrier et al. 2015a, 2018a). Frost damage is mainly observed on annual shoots of Douglas-fir, which lose turgor and turn brown when the temperature rises after freezing events (Malmqvist et al. 2018). Another pattern of winter injury includes winter burn, winter browning, winter reddening, winter drought and desiccation stress, which cause needle colour change before budbreak. Such damage has been observed in North America on jack pine, balsam fir, white spruce and black spruce (Man et al. 2013; Ontario 190,000 ha with severe damage i.e., more than 50% of damaged trees), Scandinavia and central Europe (eg. on Douglas-fir and Scots pine). Such a damage pattern will be referred to as winter reddening in this paper.

In recent decades, winter reddening has been observed mainly in young stands, resulting in significant mortality and the need for replanting in severely affected stands. This raises critical issues for forest managers in their replanting strategy. To identify the current knowledge gaps, we aimed to characterize the risk of winter reddening through the different risk components: hazard, exposure and vulnerability (Bréda and Peiffer 2014). Although some abiotic and individual factors have been identified, the aetiology of winter physiological reddening is still not clearly understood, preventing foresters from developing mitigation measures.

Douglas-fir, due to its wide adaptability to planting sites, rapid growth, and high wood yield, has been extensively planted throughout Europe, particularly in France, where plantations increased from 330,000 hectares in 1993 to 415,000 hectares in 2021 (Nicolescu et al. 2023). Over the last few decades, the number of young Douglas-fir plantations has increased, leading to an increase in observations of winter reddening in France (DSF, pers. comm.), Belgium (Walloon observatory of forest health) and Switzerland (Swiss Federal Research Institute, WSL), among others. Therefore, this opinion piece will mainly focus on the winter reddening phenomenon in Douglas-fir. We will first describe the symptoms and triggering environmental and stand factors. We will then propose three hypotheses detailing the physiological processes that can lead to winter reddening.

2 Symptoms of winter reddening

Winter reddening is a gradual process during which the colour of needles changes from glaucous green to orange and ultimately to red (Fig. 1; Baubet and Goudet 2015; Solberg 1993; Venn 1993). Red discolouration is visible in early spring before budbreak (Cayford et al. 1959; Malmqvist et al. 2018; Man et al. 2013; McMinn and



Fig. 1 Different stages of a Douglas-fir winter reddening (Livradois-Forez in 2022). The colour of needles changes from glaucous green to orange and finally to red

Funk 1970). Young needle cohorts (1 or 2 years old for an average lifespan of 3 years) are affected earlier than older ones (Venn 1993; Redfern et al. 1987; Solberg 1993). The reddening process begins at the tip of the needle and extends to the base and along the branch eventually reaching the trunk. In extremely severe cases, the buds dry out and the bark necroses (Bella and Navrati 1987; Sakai 1970; Solberg 1993).

At tree level, the upper segment of the crown is usually more damaged than the lower one, resulting in a gradient from red to green from top to bottom (Fig. 2). A common pattern is a multi-coloured crown: red at the top, glaucous green – orange in the middle and green in the lower part of the crown. Branches covered by snow and/or understorey vegetation usually remain green (Hedgcock 1912; MacHattie 1963). During the following growing season, most of the affected whorls in, and above, the red fraction die, although the red needles remain on the branch until the middle of the growing season (Pourtet 1949; McMinn and Funk 1970).

Needle mortality on affected trees can reach 100% (DSF – dataset; Man et al. 2009, 2013; Goudet 2009). Different patterns of reddening (i.e. completely red, red crown and green base, or, less frequently, green crown and red base) are observed in different stands and even within an affected stand. Individuals close to openings in the stand, on edges created by nearby clearcuts, windthrow, or in a relative isolation are more likely to be affected (Cayford et al. 1959; Redfern et al. 1987), although not systematically (Fig. 3).

Winter reddening mainly affects young trees, up to 20 years-old and 8 m tall (Malmqvist et al. 2018; Man et al. 2009, 2013; Nicolescu 2020). When only the upper part of the crown is damaged, trees display a reduced growth, with an unbalanced architecture leading to tree decline. The reduction in growth is proportional to the amount of red needles (Bella and Navrati 1987; McMinn



Fig. 2 Douglas-fir affected by winter reddening (Livradois-Forez in 2022). The Douglas-fir in the foreground is entirely red, while the one in the second foreground is in the process of turning red (red crown and green base)

and Funk 1970; Redfern et al. 1987). In lodgepole pine, the length of terminal buds formed in the following growing season is reduced by 25% after winter reddening (Bella and Navrati 1987). The reduced growth of affected trees delays the time of canopy closure and, in addition to the death of some individuals, reduces the overall density



Fig. 3 Aerial view of a plot of Douglas-fir affected by winter reddening (Livradois-Forez in 2022). The red Douglas-fir are relatively evenly distributed within the plot

of the stand. Remaining trees are therefore more exposed to additional stress events (e.g. high winds, irradiance and/or daily thermal amplitude). The delayed growth of affected trees raises forest management issues due to increased stand heterogeneity. Although affected trees can recover normal physiological function after a winter reddening event, their growth rarely return to pre-stressed levels. Following winter reddening, 60 to 100% of severely damaged trees died within 2 years (Malmqvist et al. 2018). The mortality rate of slightly damaged trees was between 20 and 60% 4 years later. When most of the buds are dead and more than 60% of the needles are red, a steady decline in physiological functions is observed until complete decay. For balsam fir and black spruce, overall survival after six growing seasons was also around 70% of the affected trees (Man et al. 2013).

3 Factors affecting the risk of winter reddening

Based on the observations recorded by the DSF in France between 1989 and 2019 (Frame 1) analysed through a risk management approach, we identified three groups of factors involved in the risk of winter reddening: meteorological hazard, exposure, and tree vulnerability (Kron 2005; Bréda and Peiffer 2014; Charrier et al. 2015a). Hazard is the probability of occurrence of one or more natural disturbances during a given period and over a defined spatial extent (mainly meteorological events such as high daily thermal amplitudes in February). Exposure reflects how the subject, regardless of its level of organisation (e.g. individual tree or stand), experiences the hazard. Several factors are related to exposure such as topography, soil properties, position in the stand, forest management. Finally, vulnerability is the predisposition to be adversely affected by the hazard and therefore depends on its intrinsic characteristics (e.g. age, acclimation state, provenance).

3.1 Meteorological hazard

Winter reddening is observed during late winter and early spring before growth resumption. Two different types of meteorological events were observed before winter reddening events.

In some cases, a period of anticyclonic weather is sufficient to induce damages in symptomatic years (years in which winter reddening is observed). Anticyclonic events are mainly characterized by a period of warm daytime and low nighttime temperature, large thermal amplitude, strong wind, and high irradiance (Pourtet 1949; Henson 1952; Baubet and Goudet 2015; Malmqvist et al. 2018). The occurrence of warm and dry weather in late winter usually occurs when the soil is still cold or even frozen (Cayford et al. 1959). The concomitance of frozen soil and warm temperatures for several hours, i.e. up to 17 °C on southern, sun-exposed, slopes, induces winter damages (Sakai 1970). In April–May, high light irradiance (*ca.* 2 000 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ PPF) combined with freezing temperatures induced photodamage in *Abies mariessi* Mast. (Yamazaki et al. 2007).

Winter reddening damage can also be observed when a mild period is preceded and followed by a cold period with negative temperatures and several freeze-thaw cycles (Man et al. 2009, 2013; Malmqvist et al. 2018; Redfern et al. 1987). Two weeks of warm daytime temperature (20 to 25 °C) followed by a period of normal daytime temperature with freezing nights (down to -22 °C) induced severe damages on conifers (Redfern et al. 1987; Man et al. 2013). Winter damages have been also observed when the temperature in January was warmer than usual (2.9 to 4.7°C above normal) and colder in February (minimum temperature of -30°C; Kamp and Worral 1990). Thus, in late winter, relatively warm and dry conditions, combined or not with a cold spell, are predisposing factors for winter reddening.

3.2 Exposure

Meteorological factors are not the only drivers of winter reddening, as there is a high degree of variability between and within stands. Indeed, site-related factors are crucial, as winter reddening has been observed several times on the same plot (Venn 1993). In France, winter reddening is mainly observed in hills and low mountain (500–900 m a.s.l.; Fig. 4A), corresponding to about 70% of the Douglas-fir stands. Damage was more frequent on the northern than on the southern slopes in France (Fig. 4B). In winter, temperatures on southern slopes are typically higher than those on northern slopes. This is because southern slopes receive approximately eight times more daily irradiance than northern slopes (Lhotellier 2005). Additionally, the snow cover on southern slopes melts earlier than on flat lands or northern slopes and the soil temperature can quickly return to positive values (Morén and Perttu 1994).

Stands located on ridges, upper slopes and flat areas are more affected by winter reddening whereas almost no winter reddening is observed on lower slopes and in basins (Fig. 4C). Cold temperature does not seem to be a direct factor, as northern slopes do not systematically suffer from winter reddening, and lower slopes and basins can be exposed to colder temperatures than ridges in mid-winter during thermal inversions. Exposure to strong winds on ridges and upper slopes may be a more important factor than temperature on southern slopes (Morén and Perttu 1994). Indeed, trees that experience winter reddening on southern slopes are more often located on ridges and upper slopes whereas on northern slopes they are more frequently in lower slopes and flat lands.

At stand level, trees on the edge display larger damage than those located in the centre of the stand. Trees on the edges are exposed to higher irradiance, wind and temperature fluctuations than those in the core of the stand (Hiratsuka and Zalasky 1993; Stoutjesdijk and

Barkman 2015). Recent clear-cuts in the stand exacerbate the intensity of winter reddening, by exposing shade-acclimated trees to higher exposure to light, wind and temperature extremes (Sagar and Waterhouse 2010). The presence of perennial understorey plants reduces frost damage (Aussenac 2000; Petritan et al. 2011). Perennial understorey plants (e.g. brooms, shrubs) also mitigate damage by reducing light intensity, buffering nighttime temperatures, reducing the number of freeze-thaw cycles and limiting warm daytime temperatures. The temperature difference between a clear-cut and a dense stand of Norway spruce seedlings was positive for the maximum and mean temperature (+4.5 °C and +0.7 °C, respectively) and negative for the minimum temperature (-2.1 °C) at 0.4 m above the ground (Langvall 2010). Thus, clearcutting increases the maximum temperature and decreases the minimum temperature encountered by the trees, exposing them to larger daily thermal variations. Exposure to greater variations in meteorological parameters appears to be an aggravating factor for winter reddening.

3.3 Vulnerability

Factors related to tree exposure explain a significant part of the observed reddening, but at the individual level some factors related to the intrinsic vulnerability of the tree are also important. Winter reddening was mainly observed on young trees, up to 20 years old (Fig. 5). At the juvenile stage, trees are usually exposed to harsher conditions than crowns of mature trees. Colder leaf temperature than the surrounding air is generally observed in early morning (Johnson et al. 2011). Such a leaf temperature depression is likely to promote ice formation even when air temperatures are above freezing. Additionally, young trees with a reduced root system explore a limited volume of soil, which limits the amount of water they can access. The shallow distribution of the root system renders it more exposed to freezing temperature. Frost

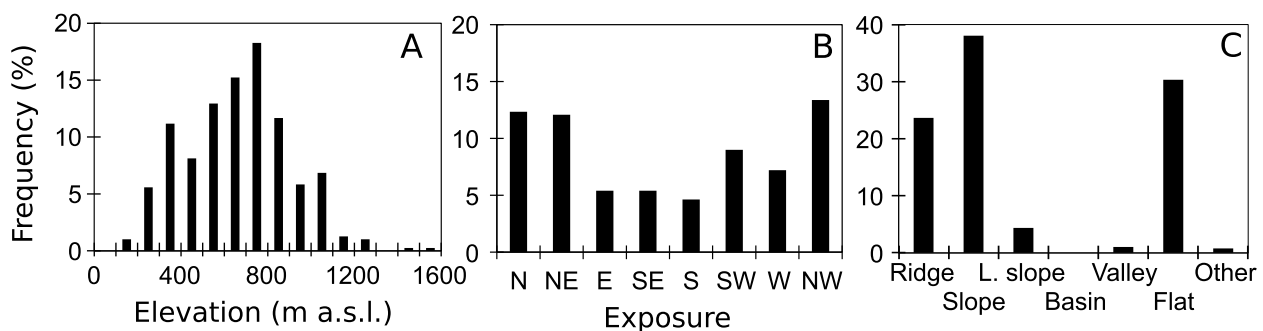


Fig. 4 Distribution of reddening observations according to several factors. **A** Frequency of winter reddening depending on elevation. **B** Frequency of winter reddening depending on azimuth – N: North, E: East, S: South, W: West - **C** Frequency of winter reddening depending on the topography of the Douglas stand with L.slope: Low slope. Based on DSF data

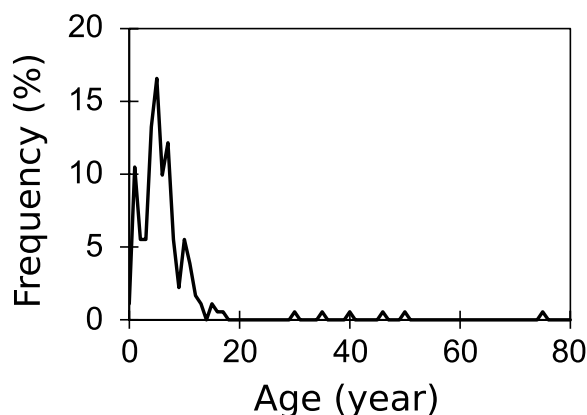


Fig. 5 Distribution of reddening observations according to the age of Douglas-fir stands – Based on DSF data

damage to the root system leads to reduced growth and yellowing of needles in the following growing season by affecting water supply and mineral nutrition (Jalkanen 1990; Cox and Zhu 2003). Freezing injury to Douglas-fir seedling roots may be triggered by delayed autumn cold acclimation (Malmqvist et al. 2018).

Instances where all Douglas-fir trees in a stand are affected are rare (refer to the previous section on symptoms for additional information). This implies that not all young trees are equally sensitive to winter reddening. In 2014, in 41 stands exhibiting winter reddening, 75% of symptomatic trees showed root system abnormalities: taproot malformation or superficial distribution of fine roots (Baubet and Goudet 2015). By reducing water and nutrient uptake, root system anomalies weaken trees, making them vulnerable to winter damage. As trees grow, the canopy closes and the variability of microclimatic parameters such as irradiance, wind and temperature are reduced. Finally, it is unclear whether the age of the tree corresponds to the vulnerability or the exposure component, as in a mature stand the crowns maintain a buffered environment that limits exposure to the identified stress factors (temperature, light, wind).

In recent decades, Douglas-fir has exhibited an earlier budburst of approximately 5 days per decade in France (Bastien 2020). Earlier date of budburst increases the probability of late-freezing events, resulting in higher frost damages due to early cold deacclimation (Chamberlain and Wolkovich 2021; Mura et al. 2022). Among the various provenances growing in France, California has the earliest bud burst, occurring 20 days before the others (Bastien 2020). To prevent spring damages, other provenances with later date of budburst should be planted, such as Washington 2 and Luzette, especially under warm and highly fluctuating conditions (Bastien 2020).

4 Putative physiological background of winter reddening

Based on the above observations, the physiological perturbations involved in the development of winter reddening in trees are most likely related to three potential processes: (i) hydraulic failure due to winter drought stress, (ii) photo-oxidative stress in shade-acclimated trees or (iii) early cold deacclimation during warm periods. The following section describes the sequence of physiological events that can lead to winter reddening by these different processes, bearing in mind that separate processes may also interact in the development of reddening.

4.1 Winter hydraulic failure

Winter reddening occurs under specific conditions such as low nighttime temperatures, mild daytime temperatures, cold or frozen soil, high irradiance and moderate to strong wind speed (Sakai 1970; Herrick and Friedland 1991; Malmqvist et al. 2018). The relatively warm conditions during the daytime maintain transpiration, which may not be adequately compensated by water uptake by the root system because the soil is cold or frozen, a typical pattern of winter drought (Tranquillini 1982).

4.1.1 Low soil temperature reduces root water uptake

A typical pattern of dehydration in autumn and rehydration in spring is observed in plants, under the control of soil temperature (Charrier et al. 2013a; Charrier and Améglio 2024). At temperatures below 8°C, root water uptake is strongly limited (Améglio et al. 2002; Mellander et al. 2006). The entry point of water into plants is through the symplastic pathway as the apoplastic pathway is interrupted by barriers in roots such as the one created by exo- and endodermal Casparian bands in the endoderm (Lee et al. 2005). In symplastic pathway, water molecules pass through the plasma membrane whose permeability is mainly driven by the channel aperture of aquaporins (Lee et al. 2012). Low soil temperature affects aquaporin synthesis, but also their three-dimensional conformation which is constrained by the rigidity of acyl groups at low temperature (Ranganathan et al. 2016). If transpiration is maintained, the subsequent water deficit also increases the resistance to water flow by increasing the viscosity of the cytoplasm due to more concentrated intracellular sap (Améglio et al. 1990).

The sensitivity of root water uptake to low soil temperature may be further limited by the distribution of the root system in superficial soil layers (e.g., young plants or those with an unbalanced root system conformation;

Cox and Zhu 2003; Lopushinsky and Kaufmann 1984). Soil temperature in the superficial layers exhibits overall larger thermal fluctuations and can reach damaging freezing temperature. A shallow root system is therefore more at risk due to an increased exposure to frost damage (Jalkanen 1990). Finally, if part of the root system is damaged, root water uptake is further limited (Malmqvist et al. 2018). Although cold limitation of water uptake is more likely on northern slopes, a deficit in soil water content can have a similar effect on southern slopes, if winter precipitations are limited.

4.1.2 Anticyclonic conditions can exacerbate water losses

Warm daytime temperatures, high light intensity and dry air induce higher needle transpiration rates. Although, under drought stress, water loss by needles is limited by stomatal closure, non-controlled fluxes through leaky stomata, leaf cuticle or bark can further increase plant water deficit (Duursma et al. 2019). During winter, stomatal control may not be as effective as during the growing season (Zweifel et al. 2007). Changes in the lipid composition of the plasma membrane may alter the efficiency of guard cells to regulate stomatal opening (Rueland et al. 2009).

Water losses can be enhanced by short-term thermal fluctuations for the organ above the snow cover or the understorey vegetation. Warm periods may suddenly release the inhibition of stomatal responsiveness to cold temperature, resulting in enhanced water losses and a drop in xylem water potential. When a critical threshold is reached, a bubble is formed in the sap and propagates to adjacent xylem conduits by air-seeding (Cochard et al. 1992; Cochard 2006). Air-filled vessels do no longer contribute to the transfer of water to the needles, increasing further needle dehydration and ultimately leading to drought-induced reddening (Cochard et al. 2009). Finally, the concomitance of cold soil temperature with warm air temperature is likely to induce winter drought by limiting root water uptake whereas water losses are enhanced. Although some conifer species can resist to such seasonal stress, even under harsh conditions (Chang et al. 2021; Charrier et al. 2017), the sensitivity of Douglas-fir to maintain its hydraulic integrity by preventing water losses may explain why this species would be more vulnerable to winter reddening than other species.

4.1.3 Interaction between nighttime freezing events and daytime passive water losses.

Night temperatures below -2°C can induce freezing of sap around a nucleus in xylem elements, resulting in the formation of air bubbles in the frozen sap (Zimmermann 1964; Špulák and Balcar 2013; Charra-Vaskou et al. 2023).

As night freezing events appears to be a key parameter in triggering winter reddening, a significant decrease in xylem water potential at the solid-liquid interface is expected, although it may not be low enough to induce bubble expansion and embolism formation (Charrier et al. 2015b). However, in dehydrated samples, ice nucleation occurs in the xylem at colder temperatures than in saturated branches (Lintunen et al. 2013). The water potential generated at the solid-liquid interface is temperature dependent ($-1.18\text{MPa}\cdot\text{K}^{-1}$; Rajashekar et al. 1983) and induces greater water fluxes towards ice nucleation sites under pre-existing water stress (Ball et al. 2006; Charra-Vaskou et al. 2016). Indeed, a dehydrated xylem would be more vulnerable to the formation and propagation of gas bubbles in xylem conduits (Sperry and Sullivan 1992; Mayr and Charra-Vaskou. 2007; Charrier et al. 2014, 2015b). Successive freeze-thaw cycles promote air seeding and bubble expansion from conduit to conduit in dehydrated trees (Charrier et al. 2015b; Charra-Vaskou et al. 2023).

In natura, ice nucleation usually occurs at the basal part of the branch, which can draw liquid sap from the distal part towards the basal part, creating a water potential gradient (Charrier et al. 2017). At tree level, the temperature at the base is more constant so the base remains frozen for a longer time than the upper crown which is exposed to more freeze-thaw cycles (Mayr et al. 2003). Needle tissue experience severe water stress after several days of freezing, even under negligible transpiration (Kozłowski 2012). Furthermore, younger and distal twigs tend to dehydrate more rapidly than older parts (Lemoine et al. 1999). This may be due to their lower relative water content and cuticular resistance to water loss (Herrick and Friedland 1991). Under severe water deficit, chlorophyll a may be degraded, resulting in a colour change of the needle from green to red-brown. (Guadagno et al. 2017). Water stress is more pronounced in younger shoots, causing Douglas-fir needles to gradually dehydrate from the top to the base of the tree, resulting in the coloration pattern from the crown to the base.

4.1.4 How to induce experimental winter hydraulic failure in young Douglas-fir?

To test the effects of cold soil on plant water relations, a differential conditioning with cold soil and warm air temperature would induce winter drought by limiting water uptake while transpiration is maintained (Lopushinsky and Kaufmann 1984; Wang and Hoch 2022). New insights into the physiological behaviour of Douglas-fir under such conditions would be provided by measuring stomatal conductance, water status and hydraulic conductivity along the soil-plant-atmosphere continuum as

well as growth resumption. A complementary experiment could test the effects of freeze-thaw cycles to amplify winter drought and increase the rate of hydraulic failure in the xylem by exposing single branches or part of the crown to successive freeze–thaw cycles.

4.2 Photo-oxidative stress

Winter reddening may occur when low temperature and high irradiance coincide, disturbing the photostasis i.e., the energy balance between the photo-physical (from the electron chain transport) and photochemical processes that transform light and the metabolic sinks that consume the energy (Calvin-Benson cycle and related primary metabolism; Öquist and Hüner 2003; Ensminger et al. 2006). In response to changing irradiance, plants can rapidly engage or disengage dissipatory pathways, which facilitates sustained thermal energy dissipation.

4.2.1 Low temperatures and high irradiance imbalance photostasis

Low temperature reduces the rate of electron transport, by decreasing membrane fluidity and the rates of enzymatic reactions involved in the Calvin-Benson cycle, whereas high irradiance enhances photochemistry (Hüner et al. 1998; Yamazaki et al. 2007). If there is an imbalance in the energy flow between the rapid, temperature-insensitive reactions of photochemistry and the slow, temperature-dependent reactions of CO₂ reduction, the capacity to modulate this balance may be overwhelmed (Ensminger et al. 2006). Thus, a potential energy imbalance is induced by the down-regulation of the biochemical reactions of the Calvin-Benson cycle by low temperature that cannot be compensated by heat dissipation.

4.2.2 Excess light energy and induction of a photo-oxidative stress

Excess light is transferred to the ever-present oxygen (Ledford and Niyogi 2005), resulting in the formation of reactive oxygen species (ROS). More specifically, singlet electrons can generate ROS, resulting in photo-oxidative damage to the photosynthetic apparatus (Soitamo et al. 2008; Ruelland et al. 2009; Yun et al. 2010). ROS are known to induce damages to thylakoid membranes (Levitt 1980), to alter the conformation of proteins associated with photosystems (Ogren and Öquist 1985; Gamon and Pearcy 1990; Zwiazek et al. 2001; Dellerio et al. 2016), and to degrade PS2 reaction centres via lipid peroxidation and chlorophyll bleaching (Yamazaki et al. 2007). In some cases, ROS may lead to cell and leaf death (Suzuki and Mittler 2006; Petrov et al. 2015). To prevent the accumulation of ROS, plants have developed photo-protective

processes by enhancing alternative energy dissipation pathways (Demmig-Adams et al. 1996, Adams et al. 2006).

4.2.3 Winter protective processes to limit photo-oxidative stress

During the dormant period, the photosynthetic capacity of the Douglas-fir is significantly reduced due to a decrease in the sink demand for photo-assimilates (Adams et al. 2006; Öquist and Hüner 2003). Faced with a down-regulation of the photosynthetic capacity and the saturation of photochemistry, evergreen conifers have evolved protective processes to dissipate the excess energy (Chang et al. 2021; Gillies and Vidaver 1990; Yamazaki 2003).

Photoprotection is mainly provided by the thermal energy dissipation, whereby excess energy absorbed by chlorophyll is safely dissipated as heat rather than being transferred to oxygen, resulting in ROS production. Non-photochemical quenching (NPQ) dissipate excess energy not used in photosynthesis as heat either through antenna quenching via the xanthophyll cycle (Demmig-Adams et al. 2014) or reaction center quenching through PSII charge recombination (Hüner et al. 2012). Under the conditions that trigger reddening, this ability may be overwhelmed by a combination of cold temperatures and relatively high irradiance. This protective mechanism is key in winter, as the pool of xanthophyll cycle carotenoids is higher than in summer in the needles of the Douglas-fir (Adams et al. 2004). Other secondary protective processes include structural adaptations through the rearrangement of the light harvesting complexes, the degradation of PSII reaction centres (Ottander et al. 1995) or cyclic electron transport around PSI, which acts as an alternative electron sink (Ivanov et al. 2001).

4.2.4 Overwhelming of the photoprotection mechanism may result in photodamage and subsequent reddening

When environmental stress increases (frozen/cold soil, freezing day/night temperatures and high irradiance), the efficiency of the photoprotective mechanism decreases (Gillies and Vidaver 1990) by altering the biophysical properties of the thylakoid membrane and limiting the function of the xanthophyll cycle (Zhang et al. 2016). Long-lasting photoinhibition due to the combination of high irradiance and low temperature may induce permanent damages to PSII and needle discoloration (Robakowski 2011), with the uncontrolled production and accumulation of ROS (Suzuki and Mittler 2006; Petrov et al. 2015). When photo-damages occurs, repairing processes involve photoprotective proteins and enzymes, such as the D1 protein to maintain photochemistry (Aro et al. 1993; Ottander et al. 1995).

Winter reddening usually initiates on the youngest needles, from the top to the base of the branch. In the case of Norway spruce, the current year's needles has a lower ratio of xanthophyll and alpha-tocopherol to chlorophyll (Tegischer et al. 2002). Alpha-tocopherol being the main lipophilic antioxidant (Fryer 1992), the potential photoprotection of the current year's needles is reduced compared to the previous year's needles (Tegischer et al. 2002). The upper crown of a Douglas-fir, which bears younger needles and is more exposed to high irradiance and low temperature, is thus more susceptible to photo-oxidative stress. Mature trees have a higher proportion of older needles, which are generally better protected than the younger ones, by a higher concentration of the xanthophyll pool and antioxidants (Tegischer et al. 2002). Older trees are therefore less vulnerable to photooxidative stress than seedlings and young trees.

Needles that have been acclimated to full light conditions have a four to five times larger xanthophyll pool than shade-developed needles (Adams and Demmig-Adams 1994). After clearcutting, young trees that were acclimated to shade conditions are suddenly exposed to high irradiance and may be exposed to photo-oxidative stress and reddening (Adams and Demmig-Adams 1994).

Although many evergreen species are able to down-regulate photosynthesis during winter, Douglas-fir maintains photosynthesis under favourable conditions. From October to March, photosynthesis can account for up to 30% of annual net assimilation at the branch level (Guehl et al. 1985; Choisnel et al. 1990). Douglas-fir is probably more vulnerable to winter reddening than other conifer species because of its ability to maintain the integrity of the photosynthetic apparatus during winter. Although this ability enables a rapid resuming of photosynthesis as soon as temperature and irradiance become favorable, when the photosystem is saturated but temperature low, photo-oxidative stress is to be expected.

4.2.5 How to induce experimental photo-oxidative stress in young Douglas-fir?

To characterize the risk of photo-oxidative stress in young trees through their contents in xanthophyll, carotenoid, lutein and antioxidants, seedlings may be exposed to different treatments that combine high irradiance and chilling temperature.

4.3 Early cold deacclimation

During the winter season, trees transiently increase their tolerance to freezing temperatures through the process of cold acclimation / deacclimation. Cold acclimation is induced by cold temperatures in autumn, while cold deacclimation is induced by warm

temperatures in late winter to early spring (Charrier et al. 2011; 2015a). Anticyclonic conditions or a period of mild temperature in late winter may increase the vulnerability to the following cold spell, by inducing cold deacclimation before budbreak (Man et al. 2009).

4.3.1 Warm spell induces Douglas-fir cold deacclimation

In Douglas-fir, the period of maximal cold hardiness usually occurs in December before trees deacclimate to cold under the control of environmental factors (Stevenson et al. 1999). Cold deacclimation is mainly driven by mild/warm air temperature rather than water availability, day length or light intensity (Driessche 1969; Kalberer et al. 2006; Charrier et al. 2018a). In Douglas-fir, air temperature above +5°C induces cold deacclimation, after endodormancy is released (Bailey and Harrington 2006). Short periods of warm temperature can induce cold deacclimation in *Picea rubens* (i.e. 45 h at 15°C or 18 h at 24°C; DeHayes et al. 1990). Over a longer period, warm temperature promotes the resumption of growth. A significant increase in water content is observed in buds and in other living tissues, before bud break, as growing tissues require higher water content to reach the cell turgor necessary for cell enlargement (Pagter et al. 2011; Charrier et al. 2013a).

Cold acclimation is induced, at the cellular level, by a decrease in water content and an increase in various compounds such as soluble carbohydrates, raffinose family oligosaccharides (Charrier et al. 2013b; Baffoin et al. 2021; Deslauriers et al. 2021). Cold deacclimation is associated with significant changes in cell and tissue water relations, as well as carbohydrate metabolism, which can be affected by temperature-induced changes in respiratory metabolism (Pagter and Arora 2012; Charrier et al. 2018b). The depletion of cryoprotectants induced by warm temperatures, using rapid metabolic pathways, cannot be reversed quickly and at low energy cost (Kalberer et al. 2006). Deacclimation is therefore much faster than reacclimation, taking several days or weeks (e.g. 10 to 20 days in *Picea rubens*; Strimbeck et al. 1995) to return to previous levels of frost hardiness. Following a warm period, trees are therefore vulnerable to a sudden drop in temperature.

4.3.2 Frost damages can lead to winter reddening

The use of cumulative growing degree days (GDD) since 1 January is a relevant proxy of ontogenic development during ecodormancy and concomitant cold deacclimation, regardless of the date of endodormancy release (Man et al. 2009). In 2007 in Northeastern Ontario, trees reached critical values of 100 GDD a

week earlier than during the 1981–2007 period. The occurrence of extremely cold nights at this period induced severe winter freezing injuries on conifer trees (Man et al. 2009). Freezing events occurring before budbreak affect more severely young trees of different sizes and species over large areas than after budbreak (Man et al. 2009). Colder conditions (eg. on norther slopes) would decrease the vulnerability of living cells but increase the exposure to frost hazard, compared to southern slopes.

In Douglas-fir, the provenances that exhibit earlier dormancy release and growth initiation are more vulnerable to frost damages (Stevenson et al. 1999). Usually, late freezing events mainly damage buds and needles (Tinus et al. 2000), which are more sensitive than stems (Burr et al. 1986). Late freezing events, commonly referred to as false springs, occur close to the time of bud break and cause frost injury when exposure to cold temperatures exceeds the frost hardiness of the plant (Mura et al. 2022). However, frost damage can also be observed on the cambium, affecting the timing and the pattern of secondary growth resumption, noticeable by a frost ring along the growth ring chronology (Glerum and Farrar 1966). In this case, the new ring formation and consequently the restoration of hydraulic conductivity may be limited by the cambium damage, increasing the dehydration of the needles, which may turn red.

Frost injury depends not only on frost hardiness but also on the duration and intensity of the frost event, the number of freeze-thaw cycles and the rate of cooling and thawing (Charrier et al. 2015a). The upper part of the crown is usually exposed to lower temperatures and tends to be more severely affected with killed buds (Redfern et al. 1987; Kamp and Worrall 1990). Young Douglas-fir saplings, less than 2 m tall, are at high risk as their upper part is exposed to cold air (Morén and Perttu 1994). Perennial understorey plants can act as a shelter and protect the seedling from very low temperatures. Thus, frost causes cellular damage to the upper parts of the trees, which can impact the photosynthetic apparatus and degrade chlorophyll a. As a result, needles, which are particularly vulnerable to cold, may change colour from green to red.

4.3.3 How to experimentally induce frost damages in young Douglas-fir?

To test this hypothesis, young saplings or isolated branches of Douglas-fir could be stored at different temperature after endodormancy release in late winter to build deacclimation curves (Man et al. 2017). The whole plant or individual branches can also be exposed to different types of freezing events (intensity,

duration) in a controlled environment at varying levels of cold hardiness to clarify the damaging process (Man et al. 2021). The objective would be to induce localized damage to needles and/or cambium. Needle colour and the resumption of primary and secondary growth after exposure to a stress condition could shed light on the relevance of this mechanism.

4.4 Potential interaction between physiological stresses

The three processes described above, namely winter hydraulic failure, photo-oxidative stress, and early cold deacclimation, are not necessarily mutually exclusive. For instance, freezing nighttime temperature combined with high irradiance and warm daytime temperature can trigger the formation of freeze-thaw induced embolism in pre-stressed trees. Additionally, frost damage to the cambium can prevent the restoration of hydraulic function. Another interaction between stomatal closure, induced by either water stress or low temperature, and photosynthetic carbon assimilation would primarily maintain the hydration status of the tree. This would occur at the cost of photo-oxidative stress in the needles under high light. The variation in vulnerability between young and mature trees indicates that either ontogenic changes are taking place in the upper crown, where more stressful conditions are present, such as lower temperatures, higher wind speeds, and greater irradiance, or the vertical growth of the root system enables mature trees to escape drought stress during the winter season. Although winter drought due to low soil temperature is observed in *P. abies* with only minor consequences at the photo-oxidative and hydraulic levels (Day et al. 1991; Delucia 1986; Charrier et al. 2017), Douglas-fir could be highly sensitive to this stress. The differences could lie in the maintenance of photosynthesis and stomatal regulation, or the ability to limit air seeding during freeze-thaw cycles.

5 Conclusion

Under atypical anticyclonic conditions in late winter, Douglas-fir trees may experience winter reddening due to various abiotic factors. The interaction between warm/dry and cold temperatures can make trees vulnerable to stress at the cellular and hydraulic level.

Cold soil or soil water deficit can reduce root uptake, leading to a drop in water potential and subsequent stomatal closure. Clear nights and freezing events, combined with warm, sunny days, can affect stomatal regulation, damage living cells, promote the biosynthesis of photoprotective pigments and alter water flow within the crown of the tree. A hydraulic gradient can cause dehydration of the apexes, diameter contraction,

and a drop in water potential. This can result in massive embolism in the most exposed parts of the tree, such as the top of the tree, while the base remains frozen for longer. The reduced water content of the needles may result in chlorophyll degradation and the emergence of secondary pigments. Although whole crown reddening is usually irreversible, moderate colouration may disappear with increasing air temperature and resumption of growth in spring, suggesting that this symptom is related to the photoprotective response. To confirm the mechanism of reddening, measurements of the changes in diameter, water potential, hydraulic conductivity should be taken at various heights in the crown to demonstrate the presence of a hydraulic gradient. Finally, to demonstrate the loss of stomatal regulation, gas exchange measurements and residual transpiration should be measured before and after one or more freeze-thaw cycles, in combination with pigment extraction.

All provenances are potentially exposed to reddening, but phenological traits exhibiting intraspecific variability such as the dates of bud burst in relation to cold deacclimation, may amplify winter reddening. To minimize the risk, forest managers must focus on three key points:

selecting local factors at the massif scale, maintaining a forest atmosphere in the stand, and promoting a regular root system at the tree scale.

Douglas-fir is at a higher risk of reddening if it is located between 400 and 800 m asl, on the top of north-facing slopes, ridges, or flatlands. In high-risk locations, saplings can be protected by placing them under shelter. Additionally, selecting perennial understorey vegetation, such as broom, or keeping small shrubs can protect young trees from large fluctuations in environmental factors such as light, temperature, and wind. Finally, when planting a new stand, it is important to take care to allow the tree to develop its root system evenly and to avoid rough planting. Finally, this raises questions about the sustainability of the silvo-industrial system. The system promotes clear-cutting and relatively invasive replanting, which may have negative impacts on the environment. To minimize the risk of reddening, it is recommended to adopt an approach that is more respectful of natural balances. This can be achieved by allowing natural regeneration without undue stress on the root system and by maintaining a forest atmosphere through accompanying vegetation that buffers the abrupt shocks of the weather.

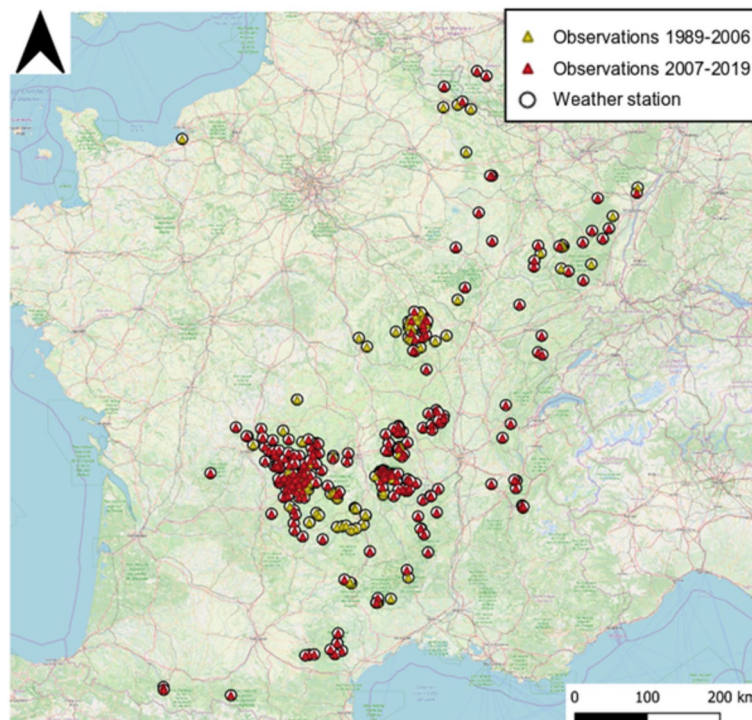


Fig. 6 Locations of 426 different Douglas-fir stands in France where reddening was observed between 1989 and 2019. The yellow triangles represent plots with reddening from 1989 to 2006. The red triangles represent reddening observations from 2006 to 2019. Triangles surrounded by a circle are Douglas-fir stands with meteorological data – DSF data

5.1 Frame 1. Observation database of the French Forest Health Department

The data collected by the Forest Health Department's observer correspondents using standard forms in mainland France, in regions where Douglas-fir is present and affected by the physiological reddening phenomenon between 1989 and 2019 (Fig. 6). Although the provenances are not always known, the Luzette provenance accounts for around 80% of the resource. The various information reported concerns the date of observation, the location of the plot, its altitude, surface area, age, type of forest (public/private), stand type, dominant species, species affected by the health problem, percentage of stems affected and degree of severity (scale from 0 to 4). The presentation of site factors linked to reddening (Figs. 4 and 5) focuses on observations between January and May, to ensure that summer reddening data are not included in the analysis. Geographical and topological information, such as altitude, exposure and topology, was added using the Geoportail (<https://www.geoportail.gouv.fr>) and Google Earth (<https://earth.google.com>) portals.

Code availability

Not applicable.

Authors' contributions

MVR wrote the paper under the supervision of GC, with inputs from NB, OB and TA. All authors read and approved the final manuscript.

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Availability of data and materials

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Declarations

Ethics approval and consent to participate

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