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Seasonal Frost Tolerance of Trees in the New Zealand Treeline Ecotone

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Introduction

New Zealand treelines are lower in altitude than treelines at similar latitudes in the northern hemisphere (Körner 1998), and are anomalously warm compared to their northern counterparts (Körner and Paulsen, 2004). Most species forming northern hemisphere treelines are extremely frost tolerant, whereas their equivalents in the southern hemisphere are not (Bannister and Neuner, 2001). As several northern hemisphere treeline conifers grow above the native New Zealand treeline and are capable of aggressive spread (Wardle 1985a, 2008), there can be no doubt that they can overcome some aspect(s) of the environment that prevent(s) upward expansion of native trees. Frost has been argued to be the most likely candidate (Wardle, 2008) as growing season warmth is sufficient for exotic trees to reach at least 200 m higher in altitude than the natives.

The New Zealand treeline climatic regime is highly oceanic and, compared with northern temperate areas with similar mean annual temperatures at treeline, the growing season tends to be longer but not as warm at its peak, and winters shorter and milder (Mark et al., 2000). Growing seasons (estimated by phenology) at New Zealand treelines may be nearly twice as long as those in Europe (Benecke et al., 1981). Although winters are not severe, spring and early summer temperatures tend to be highly variable. Introductions and experimental plantings at and above the native New Zealand treeline have shown that several exotic treeline species generally considered to tolerate low winter temperatures, including Picea engelmannii and Larix decidua, are highly susceptible to summer frosts (Benecke et al., 1981; Wardle, 1985a). Wardle (2008) argued that, because of the low frost resistance of New Zealand trees, occasional severe frosts in spring and autumn killed unhardened tissue, and frost-related dieback over winter prevented permanent height gains of trees. He argued that this low frost resistance was a consequence of the short time available for its evolution in New Zealand, a view supported by Körner and Paulsen (2004).

The hypothesis that the native trees are limited by frost at their upper altitudinal limit has been supported by transplant experiments in the Craigieburn Range, New Zealand, in which frost-sheltered Nothofagus seedlings and saplings were shown to grow 150 m above the natural treeline (Wardle 1985a). However, old, well-established plants were incapable of extending branches outside of the 30 cm high shelters without suffering dieback.

Despite the clear importance of frost in New Zealand treeline studies, little research has been done on this subject since Peter Wardle’s pioneering studies in the 1970s and 1980s. Frost tolerance has been measured on most of the major treeline species (Wardle, 1991), but often using material not sourced at treeline. Techniques used to assess frost damage have varied from study to study, and it is difficult to compare results. The temporal variation of frost tolerance reduces comparability and is poorly studied (Bannister, 2007), even though it is known that seasonality greatly affects frost tolerance (foliage is most resistant in winter and de-hardens towards summer; e.g., Alberdi et al., 1985; Bannister and Neuner, 2001). Additionally, it may not be solely frost that affects foliage: if combinations of frost and other environmental factors, such as high radiation or strong winds, damage tree foliage. The severity of such combinations increases with altitude, and one would expect an altitudinal trend of decreasing performance, as well as a decrease in performance in the seasons in which the environmental conditions are most severe. This can be quantified by chlorophyll fluorescence measurements that indicate changes in photosystem efficiency (Richardson et al., 2001).

In this paper, we critically examine the suggestion that frost affects tree performance at New Zealand treelines and that this in turn is a significant factor in determining treeline altitude. We use two contrasting treelines. On the eastern side of the Southern Alps,
Nothofagus solandri var. cliffortioides dominates at treeline, with a subdominant coniferous tree, Phyllocladus alpinus. These treelines are typically abrupt and abut alpine tussock grassland. Exotic Pinus contorta is invading above these treelines. In the central western districts of the Southern Alps, a diverse, diffuse tree line forms, in which the conifers Halocarpus biformis and Libocedrus bidwillii are dominant. If frost is a determinant of treeline position, we hypothesize that the treeline species should extend close to the minimum isotherm at which frost damage occurs, and that foliage becomes more tolerant to frost as treeline is approached. If this is not the case, then other factors must be more important in limiting tree performance. If foliage is progressively more stressed by environmental conditions closer to treeline, chlorophyll fluorescence values should decrease with altitude. Periods of low photosystem efficiency, as indicated by fluorescence measurements across seasons and altitudes, will hence provide information regarding the timing and candidate processes involved in foliar damage.

Methods

SITES AND PLANT MATERIAL

Altitudinal gradients through the treeline ecotone were selected at two sites in New Zealand, one on the east and one on the western slopes of the Southern Alps (Fig. 1). Treelines at both sites have not been depressed by human activities or avalanches, and they represent the local natural climatic tree limit (Wardle, 2008). The sites experience similar thermal conditions during the growing season, but the eastern site has markedly lower winter temperatures and longer lasting snow cover than the more oceanic western site (Cieraad, 2011). Five species were sampled at different altitudes depending on their altitudinal distribution and the local treeline altitude (Table 1). Foliage of the evergreen broadleaf species, Nothofagus solandri var. cliffortioides (Hook.f.) Poole (Nothofagaceae), and of two evergreen conifers, Phyllocladus alpinus Hook.f. (Podocarpaceae) and the exotic Pinus contorta Loudon subsp. contorta (Pinaceae), was collected from Craigieburn on the east side of the Southern Alps. N. solandri dominates treeline forests on the eastern sides of the North and South Islands (Wardle, 2008). This species forms a clear abrupt treeline, where the closed canopy forest (ca. 5 m tall) is abruptly replaced by alpine tussock grassland (Wardle, 2008). Although N. solandri is the main tree species at the site, some shrubs are also present (including P. alpinus), and exotic species are spreading from nearby plantations. Most notably, Pinus contorta subsp. contorta has successfully established as tree-sized individuals (Ledgard, 2001) some 150 altitudinal meters above the...
highest *N. solandri* trees, and *P. contorta* seedlings can be found for an additional 200 m (Wardle, 2008).

Foliage of *Halocarpus biformis* (Hook.) Quinn (Podocarpaceae) and *Libocedrus bidwillii* Hook.f. (Cupressaceae) was collected at Camp Creek on the west side of the Southern Alps. These co-existing conifer species are evergreen and long-lived (Wardle, 1991) and often form the tallest trees in the mixed conifer-broadleaved hardwood tree line ecotone communities that dominate in western areas where *Nothofagus* species are absent (Reif and Allen, 1988). The species will hereafter be referred to by their generic name only, and the sites as West Alps and East Alps, respectively. Trees were sampled at 100 m vertical intervals over the whole tree line ecotone, i.e., the gradient from tall closed canopy forest stands to the tree line (uppermost limit of >3-m-tall trees) (Table 1). Where the species were present above the treeline (<3 m tall), samples were also taken. In the case of *Phyllocladus*, no trees >3 m were present, so shrubs were sampled along the whole gradient.

Material was collected from the sites three times during the year: at the end of the growing season when hardening had started (autumn: 4 and 11 April 2010 for West Alps and East Alps, respectively); in spring, before leaves had flushed (30 and 31 October 2010); and in early summer (9 and 15 January 2011). A mid-winter collection at the sites was not feasible because of difficulties with access. On a northwest-facing slope of ca. 30° inclination, at each altitude, five mature individuals were selected (different trees each season). From each individual, a sample was taken consisting of eight short shoots with the most recent, fully expanded foliage. Material was consistently collected from fully sunlit branches in the top half of the crown (shotgun sampling where necessary), as frost tolerance and stress levels may differ between sun and shade leaves (e.g., Stecher et al., 1999). Samples were kept in polythene bags in an insulated container during transport to the laboratory where they were held in a refrigerator at ca. 4 °C overnight. Shoots were then allocated randomly to eight frost treatments (see below). **FREEZING TREATMENTS**

Freezing treatments followed Bannister et al. (2005). Small shoots (ca. 5 cm) with foliage were placed on damp paper towel inside polythene bags and cooled to seven treatment temperatures (0, −3, −6, −9, −12, −15, and −18 °C) at a rate of 5 °C h⁻¹. Control samples were held in a refrigerator at +4 °C. The range of target temperatures was selected so that the least severe treatment was likely to inflict no damage, whereas the most severe treatment would be at a temperature lower than those experienced in the field. The target temperature was held for 4 h (Taschler et al., 2004); the combination of this length of time and a damp towel with the sample prevented supercooling (Bannister and Lord, 2006). Diurnal temperature records at New Zealand treeline show daily extreme minimum temperatures are generally attained in the early morning and remain at similar values for 2–4 h before dawn (Cieraad, 2011). The material was then thawed to +4 °C at a rate of ca. 5 °C h⁻¹ before being placed at room temperature (ca. 20 °C) in the dark. Freezer air temperatures were measured using a calibrated thermocouple (type T) and logged at one-second intervals using a data logger (CR10X, Campbell Scientific Inc., Logan, Utah, U.S.A.). An on/off control relay, activated by the data logger, was used to keep two freezers within 0.3 °C of their respective target temperatures. Cold air stratification inside the freezers was eliminated by the use of a small fan. The seven frost treatments were allocated randomly to the two freezers, and were conducted over 2 d.

### DAMAGE ASSESSMENT

Foliage damage was assessed by chlorophyll-α fluorescence measurements 3 d after frost treatments. The ratio of variable to maximum fluorescence of the sample (*Fv/Fm*) of dark-adapted photosynthetic systems (>30 min) (Maxwell and Johnson, 2000) was determined using a portable infrared gas analyzer with chlorophyll fluorescence attachment (Li6400 and Li6400-40 LCF, Lincoln, Nebraska, U.S.A.). As dead material effectively has an *Fv/Fm* of zero, the degree of damage was calculated as the photoinactivation ratio (*PhI*) as described by Larcher (2003):

\[
PhI = 1 - \frac{F_{s}}{F_{m}}
\]

where *F_{s}*/ *F_{m}* is the *F_{s}/F_{m}* of the sample exposed to a freezing temperature *T*, and *F_{m}* is the maximum value of *F_{s}/F_{m}* for all samples of each tested species.

The temperature producing 50% damage (*LT₅₀*) was determined by linear interpolation using the temperature causing the highest *PhI* of <50% and the temperature causing the lowest *PhI* of >50% (Bannister et al., 1995, 2005; Sierra-Almeida et al., 2009). Extrapolation was used only if the *F_{s}/F_{m}* from the coldest treatment approached 50% of *F_{m}*. In cases where the lowest temperature

<table>
<thead>
<tr>
<th>Site</th>
<th>Species</th>
<th>Altitudinal range sampled (m a.s.l.)</th>
<th>Highest altitude attained by trees (&gt;3 m) (m a.s.l.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>East Alps: Craigieburn</td>
<td><em>Nothofagus solandri</em> var. elffortoides</td>
<td>1250–1450</td>
<td>1350</td>
</tr>
<tr>
<td>(43.12°S, 171.70°E)</td>
<td><em>Phyllocladus alpinus</em></td>
<td>1250–1480</td>
<td>NA (only shrubs; no trees &gt;3 m present)</td>
</tr>
<tr>
<td></td>
<td><em>Pinus contorta</em></td>
<td>1250–1550</td>
<td>1450</td>
</tr>
<tr>
<td>West Alps: Camp Creek</td>
<td><em>Halocarpus biformis</em></td>
<td>850–1250</td>
<td>1150</td>
</tr>
<tr>
<td>(42.72°S, 171.57°E)</td>
<td><em>Libocedrus bidwillii</em></td>
<td>850–1120</td>
<td>1120</td>
</tr>
</tbody>
</table>
treatment (−18 °C) caused less than 50% damage to a sample, that temperature was taken as the best estimate of freezing tolerance.

Alternative methods of frost damage assessments include electrolyte leakage and visual estimates. Electrolyte methods have been tried on New Zealand subalpine plant species, but with limited success, as a number of the species tested (including Phyllocladus alpinus, also in this study) showed no perceptible electrolyte release, probably due to their thick cuticle (Reitsma, 1994). Although chlorophyll fluorescence measurements may overestimate the degree of frost resistance, particularly if readings of Fv/Fm are taken soon after thawing, they are similar to visual estimates when leaves are allowed to develop damage over several days after thawing (see review in Bannister, 2007).

PHOTOSYSTEM EFFICIENCY

The Fv/Fm of control (unfrozen) dark-adapted samples measured the day after collection provided a measure of the efficiency of photosystem II. Fv/Fm values for healthy plants are typically around 0.83 (Bjorkman and Demmig, 1987), and lower values indicate damaged photosystems, for example through photoinhibition (Maxwell and Johnson, 2000).

CLIMATIC DATA

Frost tolerance (LT10 and LT50) data were compared with temperatures experienced at treeline to assess if (and if so, how frequently) these temperatures exceed the frost tolerance and can cause damaged photosystems, for example through photoinhibition. Average monthly lapse rates of Tmin varied from 1.2 to 5.6 °C km−1. A model only containing Hokitika Tmin explained only 54% of the variation in treeline Tmin, whereas a more comprehensive model (selected using AIC) explained 71% of the variation. The latter model included the following significant parameters: Hokitika Tmin, wind direction (included as a combination of cosine and sine to account for the circularity of this variable), wind speed, atmospheric pressure, and month (as a factor). This model was used to predict Tmin at the Camp Creek treeline from the Hokitika data for the period 1971–2010, and the results again summarized by month.

For the West Alps site, a similar approach was adopted: temperature data were collected at a weather station at the Camp Creek treeline (ca. 100 m from the treeline collection site in this study) between March 1978 and April 1984 (Ian Payton, unpublished data; Payton, 1989). These data were modeled using data recorded by the Hokitika weather station (ca. 40 km to the west, 39 m a.s.l.). Average monthly lapse rates of Tmin varied from 1.2 to 5.6 °C km−1. A model only containing Hokitika Tmin explained only 54% of the variation in treeline Tmin, whereas a more comprehensive model (selected using AIC) explained 71% of the variation. The latter model included the following significant parameters: Hokitika Tmin, wind direction, and month (as a factor). This model was used to predict Tmin at the Camp Creek treeline from the Hokitika data for the period 1971–2010, and the results again summarized by month.

STATISTICAL ANALYSES

The effects of altitude and time of sampling (season) on frost tolerance and photosystem efficiency were assessed in a linear model in R v. 2.12.2 (R Core Development Team, 2011). Following Crawley (2002), models were simplified by progressively removing non-significant terms and selecting the minimum adequate model. At each step of this backward selection procedure, the non-significant term with the smallest associated effect size was eliminated. Main effects were removed only after relevant interactions had been eliminated. Differences in photosystem efficiency and frost tolerance between treeline individuals and short-statured individuals occurring above the treeline were assessed using t-tests.

Results

FROST TOLERANCE

At the East Alps site, all species were more tolerant of frosts in autumn than in spring or summer (Fig. 2). Altitude did not affect the frost tolerance of Nothofagus or Phyllocladus, although both species showed significant seasonal variation in frost tolerance (autumn LT50 was −11.2 °C and −15.6 °C for Nothofagus and Phyllocladus, respectively, compared to −10.8 °C and −12.9 °C in spring, and −5.5 °C and −5.7 °C in summer) (Fig. 2). Pinus had a frost tolerance of −18 °C at most altitudes in autumn (i.e. no sign of damage at the lowest temperature tested), although decreased tolerance at the highest altitude site resulted in a slight but significant positive altitudinal trend. In summer, altitude did not affect frost tolerance of Pinus (−6.8 °C), whereas in spring tolerance increased significantly with altitude, from −10.5 °C to −16.5 °C.
East Alps), there was a significant negative trend of photosystem efficiency ($F_{\text{m}}/F_{\infty}$) with altitude. For Halocarpus, the time of sampling affected this trend, with no significant altitudinal trend in autumn (Fig. 4, Table 3). All species showed decreasing $F_{\text{m}}/F_{\infty}$ in spring compared to autumn, and an intermediate level in summer (Fig. 4, Table 3). For Pinus, $F_{\text{m}}/F_{\infty}$ did not differ significantly over the entire altitudinal range. However, if the highest site (where individuals were <3 m tall) was excluded, $F_{\text{m}}/F_{\infty}$ decreased significantly with altitude in all seasons (data not shown). Only in spring was $F_{\text{m}}/F_{\infty}$ of low-statured Pinus at the highest altitude site significantly different from $F_{\text{m}}/F_{\infty}$ at the treeline site ($t$-test: mean $F_{\text{m}}/F_{\infty}$ at treeline = 0.586, mean 100 m above treeline 0.670; $t = -3.70$, df = 5.62, $p = 0.01$). Photosystem efficiency did not differ between treeline individuals and low-statured Halocarpus 100 m above treeline in any season.

**DISCUSSION**

All five New Zealand treeline species in this study were least frost tolerant in early summer, which is the most intensive growth period (Benecke and Havranek, 1980), as has been described by others (Tranquillini, 1979; Bannister and Neuner, 2001; Larcher, 2005). Although exact comparisons with previous frost tolerance studies are difficult because of different damage assessment techniques, collection location, and timing, $LT_{50}$ values generally concurred with those from previous studies. The maximum frost toler-

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For two species (Halocarpus at West Alps and Nothofagus at East Alps), there was a significant negative trend of photosystem efficiency ($F_{\text{m}}/F_{\infty}$) with altitude. For Halocarpus, the time of sampling affected this trend, with no significant altitudinal trend in autumn (Fig. 4, Table 3). All species showed decreasing $F_{\text{m}}/F_{\infty}$ in spring compared to autumn, and an intermediate level in summer (Fig. 4, Table 3). For Pinus, $F_{\text{m}}/F_{\infty}$ did not differ significantly over the entire altitudinal range. However, if the highest site (where individuals were <3 m tall) was excluded, $F_{\text{m}}/F_{\infty}$ decreased significantly with altitude in all seasons (data not shown). Only in spring was $F_{\text{m}}/F_{\infty}$ of low-statured Pinus at the highest altitude site significantly different from $F_{\text{m}}/F_{\infty}$ at the treeline site ($t$-test: mean $F_{\text{m}}/F_{\infty}$ at treeline = 0.586, mean 100 m above treeline 0.670; $t = -3.70$, df = 5.62, $p = 0.01$). Photosystem efficiency did not differ between treeline individuals and low-statured Halocarpus 100 m above treeline in any season.

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### TABLE 2
Linear model results for frost tolerance (LT<sub>50</sub>) for each species.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Sum of Squares</th>
<th>Mean Squares</th>
<th>F</th>
<th>p</th>
</tr>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>Halocarpus</td>
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<td>93.7</td>
<td>46.8</td>
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<td>45.2</td>
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<td>248.1</td>
<td>4.5</td>
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<td><strong>East Alps</strong></td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Nothofagus</td>
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<td>310.3</td>
<td>155.1</td>
<td>96.2</td>
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<td>42</td>
<td>67.7</td>
<td>1.6</td>
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<td>Phyllocladus</td>
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<td>392.7</td>
<td>251.2</td>
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<tr>
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<td>43</td>
<td>67.2</td>
<td>1.6</td>
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<td>43.8</td>
<td>31.1</td>
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<td>614.8</td>
<td>436.8</td>
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<td>Altitude:Season</td>
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<td>80.1</td>
<td>40.1</td>
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<td>55</td>
<td>77.4</td>
<td>1.4</td>
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</table>

**FIGURE 3.** Frost tolerance temperature (mean temperature at which 10% and 50% of the foliage is damaged, LT<sub>10</sub> and LT<sub>50</sub>, respectively, ±1 standard error) of trees of five species at treeline in relation to estimated monthly extreme minimum air temperatures at the West Alps (left) and East Alps (right) sites for the last 10, 20, and 40 years.
FIGURE 4. Effect of season and altitude on photosystem efficiency ($F_v/F_m$, mean ± 1 standard error) of treeline species at the West Alps (top) and East Alps (bottom) sites. Efficiency is highest for all species in autumn and lowest in spring. *Nothofagus* and *Halocarpus* show a significant decline in efficiency with altitude.

TABLE 3
Linear model results for photosynthetic efficiency ($F_v/F_m$) for each species.

<table>
<thead>
<tr>
<th></th>
<th>df</th>
<th>Sum of Squares</th>
<th>Mean Squares</th>
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<tr>
<td><em>Halocarpus</em></td>
<td></td>
<td></td>
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<tr>
<td>Altitude</td>
<td>1</td>
<td>0.0290</td>
<td>0.0290</td>
<td>47.58</td>
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<tr>
<td>Season:Altitude</td>
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<td>Residuals</td>
<td>66</td>
<td>0.0403</td>
<td>0.0006</td>
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<td><em>Libocedrus</em></td>
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<td>Season</td>
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<td><strong>East Alps</strong></td>
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<td><em>Nothofagus</em></td>
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<td><em>Pinus</em></td>
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ance for _Nothofagus solandri_ var. _cliffortioides_ in this study (−11.2 °C) fell within the range of −10 to −13 °C reported by previous studies (Wardle and Campbell, 1976; Sakai and Wardle, 1978; Sakai et al., 1981; Greer et al., 1989). The summer tolerance of ca. −5.5 °C fell between −3.5 and −7 °C, previously reported for seedlings (Greer et al., 1989) and mature trees (Wardle and Campbell, 1976), respectively. _Phyllocladus alpinus_ tolerated temperatures down to −15.6 °C in autumn, close to −16.3 °C reported by Reitsma (1994), but not as extreme as the −18 to −20 °C recorded by Sakai and Wardle (1978). Foliage of closely related _Phyllocladus aspleniifolius_ near the treeline in Tasmania tolerated frosts down to −14 °C (Read and Hill, 1988). In its native range, _Pinus contorta_ can withstand temperatures down to −35 °C in winter and −9 °C in summer (Bigras et al., 2001); in this study, the species showed no damage in autumn at the lowest temperature measured (−18 °C), but LT$_{50}$ increased up to ca. −7 °C in summer.

The maximum frost tolerance values in this study for the West Alps conifers, _Halocarpus biformis_ and _Libocedrus bidwillii_, were ca. 4 °C warmer (−9.0 °C and −8.8 °C, respectively) than the −13 °C recorded for both species by Sakai and Wardle (1978). Collection locations are likely to explain this difference, as their material came from east of the Main Divide, and thus was exposed to more extreme winter conditions, likely resulting in increased hardening. Additionally, the maximum frost tolerance in this current study was determined in autumn, whereas the species may continue to harden further into the winter (Sakai and Wardle’s samples were collected mid-winter).

The frost tolerance of native species in this current study did not vary with altitude. Alberdi et al. (1985) found _Nothofagus_ spp. from lower altitudes in southern Chile had only a slightly lower tolerance than those near treeline. In contrast, exotic _Pinus contorta_ showed a significant altitudinal trend in frost tolerance, but only in spring. At this time of sampling, the de-hardening process had begun at the lower altitudes, with frost tolerance values approaching summer values. In contrast, at higher altitudes, lower temperature postponed de-hardening, resulting in similar spring and autumn values of ca. −16 °C for the shrub-statured individuals 200 m above the native treeline.

Frost tolerance of _N. solandri_ var. _cliffortioides_ in this study is similar to that of related species at treeline in other southern hemisphere regions. Its foliage can withstand frosts of similar severity as foliage of _N. dombeyi_ (−8.5 to −12.8 °C), and is much more tolerant than _N. nitida_ (−6.2 to −8.5 °C) (Alberdi et al., 1989; Reyes-Díaz et al., 2005; Bannister, 2007). Both these evergreen species are common in upper montane forests in Chile, but do not reach the local treeline. In Tasmania, evergreen _N. cunninghamii_ at treeline is frost tolerant to similar levels (−11 °C) (Feild and Brodribb, 2001). In contrast, in South America, buds of deciduous treeline-forming _N. antarctica_ and _N. pumilio_ can withstand much colder temperatures (−20 to −22 °C) (Sakai et al., 1981; Alberdi et al., 1985). The reduced frost tolerance of New Zealand compared to South American treeline _Nothofagus_ had led Wardle (1998, 2008) to suggest that frost is an important factor in the formation of the lower local treelines in New Zealand (Wardle, 1998, 2008).

However, this study shows that, at all times, the average LT$_{50}$ of all species far exceeded (by at least 4 °C) minimum air temperatures that trees would have experienced at the respective treeline locations over the past 20 years (Fig. 3). Of course, onset of damage (indicated by LT$_{10}$) occurs at higher temperatures, closer to the air temperatures experienced at treeline. Such minor frost damage may be expected regularly for all species at the East Alps site in spring (when LT$_{10}$ are within 0.5 °C of $T_{min}$) and less regularly in summer (when LT$_{10}$ is between 0.6 °C and 1.8 °C lower than $T_{min}$ for these species). This concurs with our field observations and damage descriptions (e.g., Wardle and Campbell, 1976; Wardle, 1985a). Additionally, a minimum of −5 °C occurred once in the summer months in a longer time series of 40 years at East Alps; such a frost would result in foliar damage approaching 50% for all species at this site (Fig. 3). The canopy, however, has a buffering effect and only the small proportion of foliage in the outer canopy will be exposed to these temperatures (McGlone et al., 2004). Hence, the risk of a severely damaging frost during summer is low, and even extreme episodic frosts are unlikely to kill adult trees of the five species at either location.

Lack of frost tolerance has been implicated as a driver of treeline altitudes in tropical regions, where trees cannot avoid the harsh environment of high elevation tropical nights by becoming dormant (Cordell et al., 2000; Rada et al., 2001). Outside the tropics, it remains unclear whether frost is a mechanism that can actually determine temperate treelines. It has been suggested that the limitation of tree growth with increasing altitude may be due primarily to the inability of trees to complete summer growth, with subsequent death because of winter desiccation and frost damage of immature growth (Tranquillini, 1979). Others have suggested that trees at temperate treeline are dormant during seasonally harsh episodes, and that temperate treeline position is rarely determined by freezing temperatures causing injury (Jobbägy and Jackson, 2000; Körner, 2003). This study concurs with the latter proposition, and suggests that, although some damage may occur, it is unlikely that such occasional frosts control the temperate New Zealand treeline position through dieback of adult trees.

Wardle (1973, 1985b) suggested that limiting factors for treeline may be met in the seedling stage. Indeed, in a recent study, Piper et al. (2006) found that _Kageneckia angustifolia_ seedlings at treeline in temperate Chile are less tolerant than the temperatures often encountered at this site, suggesting that the lack of frost tolerance will affect treeline formation in this area (Piper et al., 2006). In New Zealand, no direct comparisons of frost tolerance of seedling and mature foliage exist for any treeline species. However, one study of seedlings (Greer et al., 1989) found LT$_{50}$ comparable to tolerance of mature tree foliage reported in Wardle and Campbell (1976), and suggested that, at least for _Nothofagus solandri_, age does not affect frost tolerance. In Chile, similar adult and seedling of two _Nothofagus_ species also had similar levels of frost tolerance (Reyes-Díaz et al., 2005). Even so, seedlings occupy space closer to the ground surface, and may be exposed to colder (potentially 3 °C lower) temperatures compared to those in the canopy, especially on calm clear nights (Wilson et al., 1987, Wardle, 1985b). Given the 4 °C window between absolute minimum temperatures experienced and frost tolerance values, and the short-lived nature of the seedling stage, we suggest that frost sensitivity during the seedling stage cannot alone be the determining factor of treeline position.
Overall, it appears that the New Zealand treeline species, at least in winter, have excess frost tolerance relative to the risk of damaging frosts at treeline. In winter, the differences in LT50 between the five species are largest (−18 to −8.6 °C). In summer, despite the very different leaf and tree structures, biogeographic and ecological distributions of the species, they show a much narrower range in LT50 (−7.1 to −5.2 °C). This suggests that a minimal level of tissue adaptation secures protection down to −7 to −5 °C, which apparently is relatively easily achieved by most trees capable of growing in the alpine zone. Almost 80% of the 58 New Zealand trees assessed for winter freezing resistance of their leaves can tolerate −5 °C or colder (Wardle, 1991). *Phyllocladus alpinus* is the only native species in this study whose range extends out to the drier, eastern basins where, in winter, daily minimum temperatures colder than −10 °C are commonly experienced. The ability to achieve such levels of winter tolerance, as in *Phyllocladus alpinus* and *Pinus contorta*, may be linked to the permanent structural protection provided by the robust anatomical features of these conifers, as has been suggested for other conifers and winter cereals (Savitch et al., 2002; Oquist and Huner, 2003).

This study does not take into account accounting factors, for example frost desiccation or cold-induced photo-inhibition. In other locations, these processes affect trees near treeline (e.g., Hadley and Smith, 1986; Bader et al., 2007). If combinations of frost and other environmental factors, such as high radiation or strong winds, are damaging to tree foliage, and the severity of such combinations increases with altitude, one would expect an altitudinal trend of decreasing performance. This can be measured by fluorescence measurements that indicate changes in photosynthetic efficiency, $F_v/F_m$ (Richardson et al., 2001). In this study, photosystem efficiency decreased towards treeline for some species: *Nothofagus* in all seasons and *Halocarpus* in autumn and spring (Fig. 4). In contrast, *Phyllocladus* and *Libocedrus* showed no change in $F_v/F_m$ with altitude. In the Himalayas, De Lillis et al. (2004) also found that fluorescence measurements of trees in the treeline ecotone were more strongly related to species than to altitude. In the current study, exotic *Pinus contorta* showed a pattern of decreasing photosystem efficiency towards treeline in spring (or slower recovery after winter), but individuals at the highest site had higher photosystem efficiency than at the treeline site (overall resulting in a non-significant linear altitude effect). A similar pattern has been reported in the U.S.A., where *Picea* and *Abies* showed decreasing photosystem efficiency with altitude towards treeline, although at the highest altitude site efficiency was increased, probably related to the prostrate plant architecture and/or stress-tolerant physiology at this site (Richardson et al., 2001). As the trees at the highest site in this study were not more sheltered, nor prostrate, it seems likely that a more stress-tolerant physiology of *Pinus contorta* may explain this increase in $F_v/F_m$ measurements at the highest site.

$F_v/F_m$ measurements were low (0.65–0.8) in spring and highest (0.8–0.85) in autumn for all species. Although frost alone is not sufficient to kill foliage, these results suggest that adverse environmental conditions in winter and/or spring do cause some foliar damage. The seasonal course of $F_v/F_m$ suggests that over-wintering leaves have accumulated damage through adverse conditions (e.g., a combination of cold temperatures, and high light and wind conditions), whereas the photosynthetic efficiency of newly flushed foliage (first measured in early summer) continued to improve towards autumn. These seasonal differences are particularly visible in the East Alps species (Fig. 4), which occupy an environment with a more seasonal climate than the West Alps species. Similarly, in a study of photosynthetic capacity of five conifers along near treeline in the U.S.A., Koh et al. (2009) found that lower temperatures in autumn were initially correlated with increases in maximal photosynthetic capacity, “as long as the declining temperatures remained in a range that should likely permit further net carbon gain” (p. 320). However, when winter encroached and freezing events became common, strong photosynthetic down-regulation followed (Koh et al., 2009). Stecher et al. (1999) also found an extreme reduction in fluorescence in *Picea abies* and *Pinus cembra* near treeline in winter (down to 10%), and a slow recovery towards summer.

The question of the ability of *Pinus* to grow at higher altitudes than the native treeline species remains to be answered. The results of this study suggest that frost tolerance cannot explain the difference in performance between it and native trees. *Phyllocladus* can endure −16 °C frosts, almost 5 °C colder than *Nothofagus*, yet it barely reaches tree height in the alpine ecotone and grows as a low (<2.0 m in height), layering shrub (Wardle 1969). It is a very slow growing tree, in contrast to *Nothofagus*, and it is possible its high level of frost resistance comes at the price of less rapid growth (e.g., Koehler et al., 2012; Molina-Montenegro et al., 2011). Additionally, the collapse of tissue under moderate water stress, common to Podocarpaceae, may limit the success of *Phyllocladus* in this summer-dry alpine environment (Brodribb, 2011).

As *Nothofagus solandri* seedlings transplanted above the species’ natural treeline (Wardle 1985a, 2008) survived as long as they were kept under shelter, insufficient warmth during the growing season is not the reason for the species’ failure to match the performance of *Pinus contorta*. Instead, we suggest the main difference between these species is the superior ability of *Pinus* to counter a range of exposure-related factors, such as high light, wind, and dryness, whilst continuing growth. At the seedling stage, *Pinus contorta* performs best in full sunlight (Despain, 2001), while *Nothofagus* seedlings are intolerant of high light, and occur only a few meters upslope from the abrupt treeline, where the parent canopy provides shade or shelter (Wardle, 2008). Cold-induced photo-inhibition has been suggested to affect *Nothofagus* at treeline (Ball, 1994), but this has not yet been quantified. In addition, dry conditions (high vapor pressure deficits) strongly reduce stomatal conductance and carbon accumulation of *Nothofagus* (Benecke and Havranek, 1980). In contrast, *P. contorta* subsp. *contorta* favors harsh, dry, high radiation sites in its native habitat (Despain, 2001) and the relative lack of such habitats in New Zealand may have restricted the opportunities for such a specialist to evolve here. On the other hand, it is highly unlikely that *P. contorta* could invade under the intensely oceanic conditions (milder, wetter, cloudier) above the gradual west coast or sub-Antarctic treelines. Here, the New Zealand treeline may not be anomalously low, but reflect a universal limit to tree growth governed by the warmth of the growing season (Körner and Paulsen, 2004).

**CONCLUSION**

Frost tolerance did not show a trend with altitude along the gradient studied here for four New Zealand native trees, and an
alitudinal decrease of photosystem efficiency was found only for some species. There was a significant trend in frost tolerance of the exotic Pinus with altitude, but only in spring. The results of this study suggest that frost is not a major factor limiting the performance of adult trees near treeline in New Zealand. Although occasional frosts, especially in spring, may damage some of the adult foliage, we suggest only a small portion of the tree’s foliage is affected, and that such frosts are unlikely to control treeline position through dieback of adult trees. In the last 20 years, the window of at least 4°C between extreme minimum temperatures and the LT50 of mature foliage suggests that, even if seedlings were significantly more frost sensitive, they would not suffer seriously damaging effects.

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References Cited


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