Physiological acclimation of *Pinus flexilis* to drought stress on contrasting slope aspects in Waterton Lakes National Park, Alberta, Canada

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Abstract: Photosynthetic gas-exchange characteristics were measured in *Pinus flexilis* James (limber pine) during two drought years in a xeric, subalpine ecosystem of the Rocky Mountains. Limber pine exhibited conservative water-use traits, including low specific leaf area, leaf nitrogen, stomatal conductance, transpiration (E), and light-saturated net photosynthesis (A_{max}), but exhibited high needle longevity, water-use efficiency (A_{max}/E), and stable carbon isotope composition. Net photosynthesis declined strongly with leaf-to-air vapour pressure deficit, resulting in a bimodal seasonal pattern of A_{max}. Although very little gas exchange was observed in late summer, photosynthetic activity extended into October. The avoidance of gas exchange during high atmospheric demand maximized whole-season water-use efficiency. Leaf temperature and leaf-to-air vapour pressure deficit were higher on south-facing slopes during both moderate (2006) and severe (2007) drought. Severe drought caused lower stomatal conductance and E on the southeast-facing slope, but neither A_{max} nor canopy reflectance indices differed among slope aspects. Although A_{max} was lower in 2007 than 2006, branch-length increment did not differ. Foliar stable carbon isotope composition was higher in needles produced in dry years but did not vary among slope aspects. These results indicate that physiological acclimation to water stress prevented among-aspect differences in A_{max} and that shoulder-season photosynthesis may become increasingly important in a warmer climate.

Résumé : Les caractéristiques des échanges gazeux associés à la photosynthèse ont été mesurées chez le pin flexible (*Pinus flexilis* James) pendant deux années de sécheresse dans un écosystème subalpin xérique des montagnes Rocheuses. Le pin flexible avait des traits associés à une utilisation conservatrice de l'eau, incluant une surface foliaire spécifique, un contenu en azote foliaire, une conductance stomatale (g_s), une transpiration (E) et une photosynthèse nette à saturation lumineuse (A_{max}) faibles, mais une longévité des aiguilles et une efficacité d'utilisation de l'eau (WUE); A_{max}/E) élevées ainsi qu'une composition isotopique stable du carbone ($\delta^{13}C$). La photosynthèse nette a fortement diminué avec le déficit de saturation, ce qui a entraîné un patron saisonnier bimodal de A_{max}. Bien que très peu d'échanges gazeux aient été observés à la fin de l'été, l'activité photosynthétique s'est poursuivie jusqu'en octobre. L'arrêt des échanges gazeux lorsque le déficit de saturation était élevée a maximisé WUE sur l'ensemble de la saison. La température des feuilles et le déficit de saturation étaient plus élevés sur les pentes exposées au sud en période de sécheresse tant modérée (2006) que sévère (2007). La sécheresse sévère a réduit g_s et E sur les pentes exposées au sud-est mais ni A_{max}, ni l'indice de réflectance de la canopée n'étaient différents peu importe l'orientation de la pente. Même si A_{max} était plus faible en 2007 qu'en 2006, l'accroissement en longueur des branches était semblable. La composition foliaire en $\delta^{13}C$ était plus élevée lors des années sèches mais ne variait pas selon l'orientation de la pente. Ces résultats indiquent que l'acclimatation physiologique au stress hydrique a éliminé les différences de A_{max} dues à l'orientation et que la photosynthèse qui a lieu durant le printemps et l'automne pourrait devenir de plus en plus importante sous un climat plus chaud.

[Traduit par la Rédaction]

Introduction

Limber pine (*Pinus flexilis* James) is a long-lived, drought-tolerant tree, distributed over a wide altitudinal and latitudinal range in subalpine ecosystems of western North America. In the Rocky Mountains of Waterton Lakes National Park (WLNP), limber pine occurs from 1500 to 2000 m a.s.l., but it is found at elevations ranging from 870 m in North Dakota (Steele 1990) to 3700 m a.s.l. in the Sierra Nevada of southern California (Millar et al. 2007).

Despite its broad distribution, *P. flexilis* is shade intolerant and is primarily restricted to xeric patches of rocky, wind-swept terrain (Rebertus et al. 1991). Its growth and reproduction strategy is conservative, with longevity reported at over 600 years in southern Alberta (Webster and Johnson 2000) and reproductive maturity delayed to 50 years (Schoettle 2004).

The ability to colonize extreme environments and to withstand long-term climate variability make limber pine an ideal species for plant-stress research in the context of con-

temporary global climate change. Optimal elevation ranges of plant species have increased with twentieth century warming (Lenoir et al. 2008), but upslope migration is dependent upon a variety of limitations, including geologically unfavourable terrain (Maher and Germino 2006; Malanson et al. 2007), resistance of existing vegetation to invasion, low growth rates, long generation times, and slow dispersal (Dullinger et al. 2004). Where migration is inhibited, all trees are forced to adapt in situ to warmer conditions and increasing stand density (Klasner and Fagre 2002). Under these conditions, limber pine tends to be competitively excluded by more shade-tolerant spruce and fir, as observed in *Pinus contorta* stands (Veblen et al. 1991), but it achieves high longevity where dry conditions prevent succession (Langor 2007).

In patches of rocky terrain ranging from the lower to upper treeline limits, physiological acclimation to a broad range of environmental stresses has enabled older limber pine specimens to survive both the Little Ice Age and recent warming. By observing the physiological response of this successful colonizing species to variable environmental controls, we gain insight into the growth strategy that facilitates the long-term survival of a subalpine conifer in a xeric, montane environment. To ascertain how *P. flexilis* acclimates physiologically to drought stress, we measured seasonal patterns of microclimatic conditions, photosynthetic gas exchange, stable carbon isotope (δ^{13}C) composition, chlorophyll fluorescence, and canopy reflectance characteristics on trees of contrasting slope aspects.

Water-use characteristics of conifers

Coniferous tree species tend to exhibit physiological and structural traits that facilitate greater resistance to drought stress compared with broadleaf deciduous trees. Such characteristics include lower osmotic potential, lower soil-to-leaf conductance, and limitation of water loss owing to the sunken position and lignification of guard cells (Gao et al. 2002). Limber pine needles exhibit several related traits, including high needle longevity (4.4–9.6 years; Schoettle and Rochelle 2000), low mass-based leaf nitrogen content (N_{leaf}, 0.9%–1.3%; Barrick and Schoettle 1996), low stomatal density (45–56 mm^{-2}; Schoettle and Rochelle 2000), and low specific leaf area (SLA, 20–37 cm$^2 \cdot$g^{-1}; this study). Plants with low SLA and mass-based N_{leaf} but high leaf longevity tend to display a low light-saturated net photosynthesis rate (A_{max}) and stomatal conductance (g_s; Reich et al. 1999; Wright et al. 2004). Low g_s is also associated with higher photosynthetic water-use efficiency (WUE; A_{max}/E) and stable carbon isotope (δ^{13}C, ‰) ratios because of the impact of lower ratios of internal to atmospheric CO_2 concentration (c_i/c_a; Brugnoli et al. 1988) on carbon isotope discrimination during photosynthesis. Although each of these traits helps to characterize the photosynthetic water-use strategy of a given species, they are each affected by microclimatic conditions, which determine the degree to which stomatal regulation is required to limit water loss.

Slope aspect effects on microclimate and physiological stress

Topographic heterogeneity introduces microclimatic variability at fine scales in montane ecosystems, because of the

impact of slope aspect on solar insolation (Daly et al. 2007). Solar insolation increases soil temperature and near-surface air temperature (T_a), resulting in higher evapotranspiration (Fisher et al. 2005) and lower volumetric soil water content (θ_v; Leij et al. 2004). However, θ_v is also influenced by synergistic interactions among other environmental variables, including hillslope form, hydrogeology, vegetation cover, soil texture, and soil depth (Tromp-van Meerveld and McDonnell 2006). To assess the impact of slope aspect on plant physiological stress, it is therefore necessary to compare hillslopes that are geologically, biologically, and pedologically similar.

The impact of slope aspect on seasonal patterns of plant physiological stress depends on the limiting environmental controls at a given site. Near treeline, suboptimal temperature is often the primary growth-limiting factor (Körner and Paulsen 2004). As a result, Danby and Hik (2007) observed higher branch-length increment and radial growth in south-facing white spruce (*Picea glauca*) at a 1300 m a.s.l. treeline site in southwest Yukon. The lower growth rates of north-facing trees were attributed to low soil temperature. However, in the same study, winter needle mortality was higher on the south-facing slope and the photochemical efficiency of Photosystem II (Φ_{PSII}) was lower. Slot et al. (2005) also observed a lower Φ_{PSII} in south-facing pines (*Pinus sylvestris*) owing to the activation of photoprotective xanthophyll cycle pigments to prevent cold temperature photoinhibition. Also, when water availability is limiting, the higher solar radiation and evaporation rates of southerly aspects can aggravate the physiological impacts of soil dryness. Consequently, Oberhuber and Kofler (2000) found that the impact of dry years on radial growth increment was most severe in *P. sylvestris* on south-facing slopes.

The goals of this study are (*i*) to quantify among-aspect differences in T_a and vapour pressure deficit (VPD) during the growth season at Lakeview Ridge, WLNP; (*ii*) to characterize the photosynthetic water-use strategy of limber pine, based on field measurements of leaf gas exchange, reflectance, and chlorophyll fluorescence; and (*iii*) to assess slope-aspect-related differences in seasonal acclimation to drought stress. Three hypotheses were tested. Firstly, on the basis of the leaf traits and xeric habitat of *P. flexilis*, we expected that tight stomatal regulation would be observed, enabling the trees to maintain a positive A_{max} throughout the dry periods of the growth season. Secondly, we hypothesized that soil moisture stress would be more severe on south-facing slope aspects, resulting in lower g_s, A_{max}, c_i/c_a, maximum quantum efficiency of Photosystem II (F_v/F_m), and photochemical reflectance index (PRI), but higher WUE (A_{max}/E) and δ^{13}C composition. Thirdly, we predicted that the shoulder seasons (spring and fall) would be more important on south-facing slopes because of the heating associated with higher insolation during relatively moist periods with suboptimal temperature.

Materials and methods

Site description

The study was carried out on southeast, southwest, northeast, and northwest slopes (35°–50°) of a subalpine krummholz peak on Lakeview Ridge, WLNP (49°09′22″N,

113°55′01″W; 1890–1937 m a.s.l.; Fig. 1). Using the Waterton Park Gate and Waterton River Cabin climate normals (1950–2005; Environment Canada 2008), mean annual T_a and precipitation are approximately 1.2 °C and 816 mm, respectively, at the Lakeview Ridge peak, based on T_a and precipitation lapse rates of 6.3 °C·km^{-1} (Pigeon and Jiskoot 2008) and +84 mm·year^{-1}·km^{-1}, respectively (Shea et al. 2004). Lakeview Ridge extends from southwest to northeast from the eastern front of the Lewis and Clark ranges of the Rocky Mountains of WLNP. Located 18.5 km to the east of the continental divide between the Pacific Ocean and Hudson Bay drainage basins, the study site is characterized by persistent, strong, and downsloping west-southwesterly winds, especially during chinook events (Nkemdirim 1996), which often clear exposed surfaces of snow cover in mid-winter.

At the Lakeview Ridge peak (Fig. 1), the southeast and southwest slopes consisted of ∼30%–50% bare rock and ∼50%–70% vegetation cover. The proportion of vegetation cover was lowest on the northwest-facing slope (∼30%) and highest (∼90%) in the small patch of northeast-facing terrain, which was sheltered from the prevailing wind and received heavy snow accumulation in winter (>2 m). The regosol soil depth beneath the vegetation cover was 0–4 cm, except beneath trees, where depths ranged from 0 to 12 cm, but roots extended downward into rock fissures. Surface vegetation cover is dominated by *Arctostaphylos uva-ursi* and *Juniperus horizontalis*, but *Dryas octopetala* is also prevalent on the more sparsely vegetated northwest slopes. Diverse grasses, sedges (*Carex* spp.), severely stunted deciduous shrubs (e.g., *Shepherdia canadensis*, *Rosa woodsii*, and *Dasiphora fruticosa*), and forbs (e.g., *Achillea millefolium* and *Astragalus* spp.) were also common. *Pinus flexilis* was the dominant tree on the southeast, southwest, and northwest slopes, and was found with consistent stand density. It was also found in canopy gaps of the northeast slope. All specimens were less than 3 m in height, with shoots present to ground level. A few stunted specimens of *P. contorta* (lodgepole pine) were found on each slope aspect, but were sparsely distributed compared with *P. flexilis*. Patchy stands of stunted Douglas fir (*Pseudotsuga menziesii*) were found behind ridgelines that accumulated snow in winter on northeast and northwest slopes. Isolated, stunted, and wind-abraded fir specimens were also found on southeast and southwest slopes, but fir trees were dominant only on the northeast slope, reaching heights of up to 3 m.

Microclimatological monitoring

Meteorological measurements were taken at 1902 m a.s.l., on southeast, southwest, northeast, and northwest faces of the Lakeview Ridge peak, from day of year 200 to 253 (2006) and day of year 172 to 253 (2007). Data were recorded with HOBO Micro Station Data Loggers (Onset Computer Corporation, Bourne, Massachusetts, USA). T_a and relative humidity (RH) were measured at heights of 7.5 and 100 cm, using HOBO Temperature/RH Smart Sensors housed within Gill radiation shields. The T_a sensor is accurate to ±0.7 °C from 0 to 50 °C. The RH sensor is accurate to ±3%, except to ±4% near 100% RH. ECHO Dielectric Aquameter soil moisture probes (Decagon Devices, Inc.,

Fig. 1. Map of Lakeview Ridge study site, Waterton Lakes National Park, Alberta, Canada.
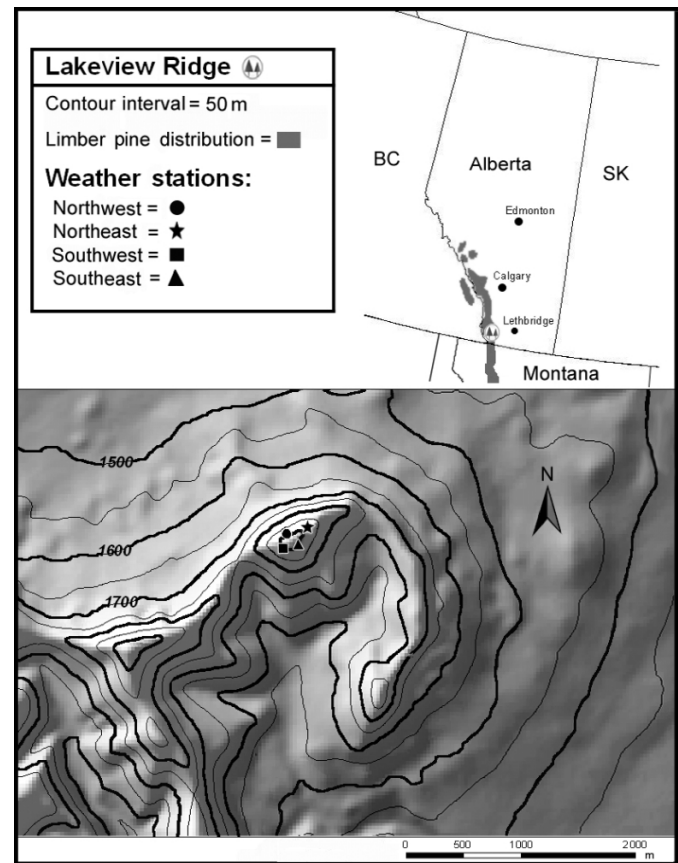
Pullman, Washington, USA) were used to measure θ_v beneath *P. flexilis* trees, at –10 cm.

Assessment of stand, tree, and needle characteristics

Stand density was determined in three 30 m × 30 m plots on each slope aspect as the mean number of specimens that was taller than 0.5 m per unit area. Tree height and canopy width were measured with a tape measure. Five trees were measured on each aspect. For trees growing within clusters, canopy width was determined separately for each individual along its axis of maximum diameter. Basal diameter and annual branch increment were determined on five individuals of each slope aspect, using digital calipers. Some trees possessed large woody knobs at their base. In such cases, basal diameter was determined just above these features. In the spring of 2008, branch-length increment was determined for each growth year from the means of three year 1 (y1; produced in 2007), three year 2 (y2; 2006), and three year 3 (y3; 2005) internode lengths of five trees of each slope aspect. All branch increment measurements were taken at the apical position of the branches at 0.5–1.5 m above ground. Needle life-span was determined on the same branches as the branch increment measurements, as the number of internodes with at least two green needle pairs

Photosynthetic gas-exchange measurements

Photosynthetic gas exchange was measured using a TPS-1 Photosynthesis System, with a 25 mm × 18 mm leaf cuvette

(PLC6, PP Systems, Hitchin, UK). System calibration was performed by the manufacturer in April 2006 and 2007 (PP Systems, Amesbury, Massachusetts, USA). In 2006, measurements were taken on y2 needles (produced in 2005) of all four aspects, on nine dates from day of year 144 to 274. In 2007, measurements were taken on y2 (2006) and y3 (2005) needles of southeast and northwest slopes on five dates from day of year 138 to 275, and on y1 needles (2007) after full emergence and expansion (day of year 204, 243, and 275). All measurements were taken on needles of healthy shoots of branches found 0.5–1.5 m above ground.

Five gas-exchange measurements were taken on each slope aspect, and each measurement was from a different individual limber pine tree growing in a separate vegetation cluster. Within both years, the same five trees were examined on each measurement date. Each measured value was determined from the mean of 10 readings obtained on a single group of needles densely packed into the leaf cuvette without any overlapping. On each measurement date, mean gas-exchange values were obtained for each slope aspect from its five respective measurements. All measurements were taken with natural light and in full sun, from 1100 to 1600. Readings and measurements were discarded from the dataset if photochemical photon flux was less than 800 $\mu mol \cdot m^{-2} \cdot s^{-1}$. In 2006, the total number of measurements used from the nine measurement dates was as follows: southwest (41), southeast (44), northwest (40), and northeast (38). In 2007, the total number of measurements used from five measurement dates (except three measurement dates for y1 needles) was southeast (57) and northwest (53). The mean CO_2 concentration in the leaf cuvette was lower on 2006 measurement dates (394 ± 6 ppm) than on those of 2007 (404 ± 3 ppm; $P < 0.01$), with a July minimum in 2006, but no seasonal pattern in 2007.

Leaf reflectance and chlorophyll fluorescence measurements

Canopy reflectance measurements were taken from a height of 1 m directly above each tree in 2007. Measurements were taken near solar noon with the Unispec-SC Spectral Analysis System (PP Systems, Amesbury, Massachusetts, USA), equipped with a straight fibreoptic cable (UNI684, PP Systems) with a 28° angular field of view or a 49.9 cm linear field of view. The spectral range of the Unispec-SC is 400–1000 nm. Spectral resolution is 10 nm full width at half maximum, with 16-bit radiometric precision. To minimize instrument noise effects, a dark current scan was performed before each measurement. A polytetrafluoroethylene calibration disk (UNI420) was also scanned before each measurement to provide a white reference. Leaf reflectance values were calculated as the leaf radiance divided by the irradiance of the polytetrafluoroethylene disk. Vegetation indices were calculated from 1 nm bandwidths, which were linearly interpolated from the reflectance data.

The chlorophyll index (CI; Gitelson and Merzlyak 1994) and the normalized difference vegetation index (NDVI; Tucker 1979) are "greenness" indices that vary with foliar chlorophyll content and the amount of light absorbed by live vegetation (Sims et al. 2006). The PRI is sensitive to xanthophyll cycle pigment activity and tends to decline when the ratio of carotenoid to chlorophyll pigments in-

creases, to protect the leaves from photoinhibition (Gamon et al. 1997). The water band index (WBI; Peñuelas et al. 1997) is positively correlated with the water content of fine tissues in plant canopies (Sims and Gamon 2003). The reflectance indices were calculated as follows:

$$[1] \quad CI = \frac{r_{750} - r_{705}}{r_{750} + r_{705}}$$

$$[2] \quad NDVI = \frac{r_{800} - r_{670}}{r_{800} + r_{670}}$$

$$[3] \quad PRI = \frac{r_{531} - r_{570}}{r_{531} + r_{570}}$$

and

$$[4] \quad WBI = \frac{r_{900}}{r_{970}}$$

where r is reflectance, and the subscripts refer to wavelengths in nanometres.

The maximum quantum efficiency of Photosystem II (F_v/F_m) was measured on tightly clustered y1, y2, and y3 needles of northwest and southeast slopes on day of year 172, 2007. The readings were obtained with a pulse-modulated chlorophyll fluorimeter (FMS-2, Hansatech Instruments, King's Lynn, UK). All needle clusters were dark-adapted for a minimum of 30 min, using the FMS-2 dark-adaptation clip system, before the measurements were taken.

Stable carbon isotope ($^{13}C/^{12}C$) composition

The projected needle area was determined with an LI-3100C Area Meter (Li-cor Biosciences, Lincoln, Nebraska, USA). Needle samples were then dried for 48 h at 70 °C and weighed to determine SLA. A selection of needles from both 2006 (day of year 144, 199, and 254) and 2007 (day of year 138, 172, 204, 243, and 275) were crushed into a powder using a mortar and pestle, for $\delta^{13}C$ analysis. Liquid nitrogen was applied to expedite this process. The $\delta^{13}C$ composition of needle tissue ($\delta^{13}C_p$) and total N content (%) were determined for 2006 needles by combustion of 1–3 mg samples in an elemental analyzer (NC2500, CE Instruments, ThermoQuest Italia, Milan, Italy) coupled to a gas isotope ratio mass spectrometer (Delta^{plus}, Finnigan Mat, Bremen, Germany), running in continuous flow mode. The same process was carried out in 2007, but 6 mg samples were combusted into the elemental analyzer (NC2100, CE Instruments), which was coupled to an Optima mass spectrometer (VG Isotech, Cheshire, UK). $\delta^{13}C_p$ was calculated from the ratio, R ($^{13}CO_2/^{12}CO_2$):

$$[5] \quad \delta^{13}C_p = \left[\frac{R_{sample}}{R_{standard}} - 1 \right] \times 1000$$

where $R_{standard}$ is the molar ratio of the international standard Pee Dee Belemnite (Craig 1957).

Statistical analysis of physiological data

Among-aspect differences in T_a and the leaf-to-air vapour pressure difference (LAVD) were assessed by unpaired t tests. Among-aspect differences in limber pine stand, individual and needle traits were assessed using one-way analy-

Fig. 2. Environmental conditions on northeast and southwest slopes of the Lakeview Ridge study site during the 2006 and 2007 growth seasons.
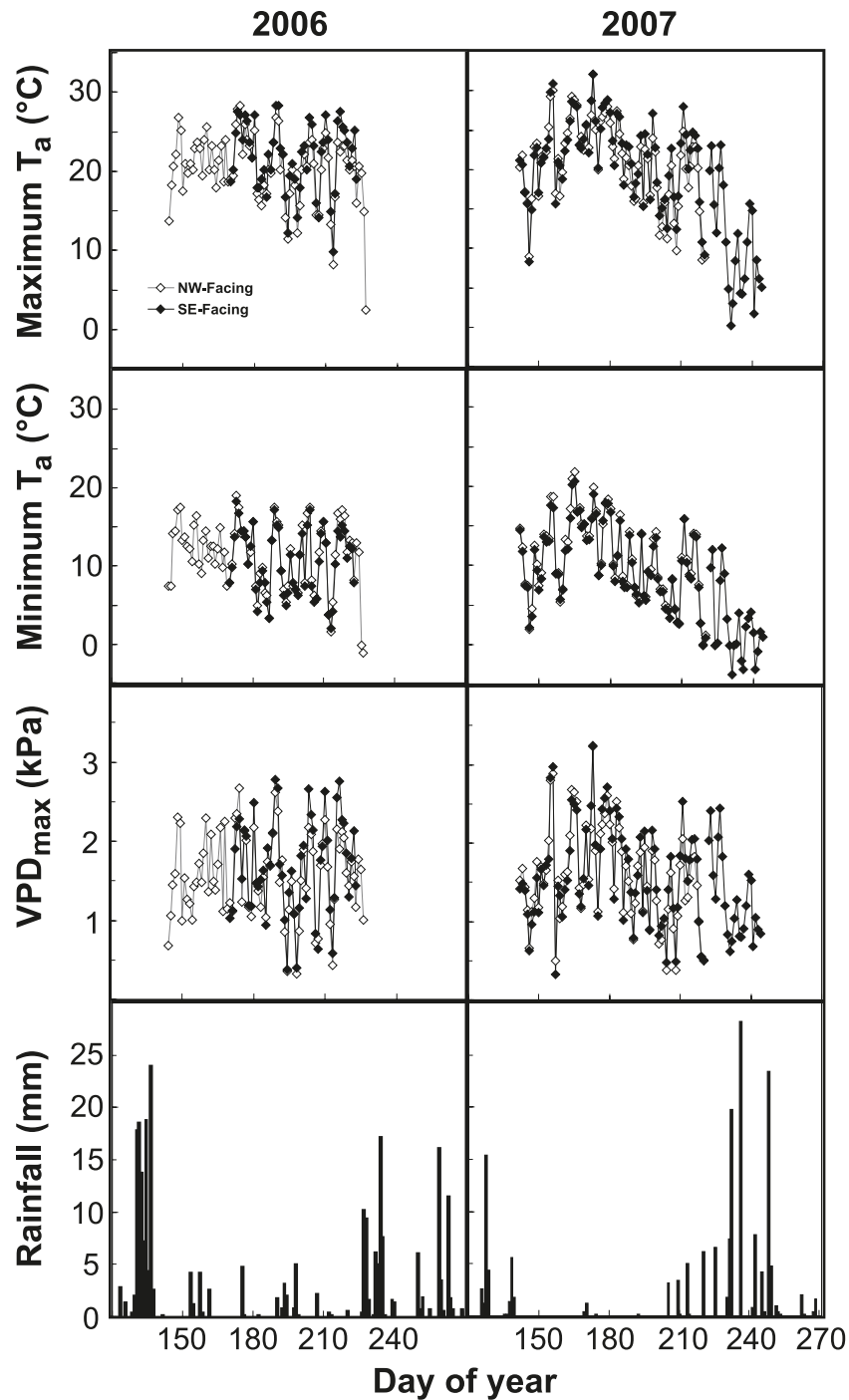
sis of variance (ANOVA), with post hoc evaluation of significant differences carried out with Tukey's HSD test. Mixed-model repeated measures ANOVA was used to assess among-aspect and among-needle year differences in seasonal patterns of photosynthesis and reflectance variables, using JMP (SAS, Cary, North Carolina, USA). Day of year was input as the repeated measure, with aspect specified as a fixed effect, aspect × day of year interaction in-

cluded, and post hoc analysis of differences assessed using the Tukey's HSD test.

Results

Environmental conditions

Similar seasonal patterns of T_a and VPD were observed in 2006 and 2007 at the Lakeview Ridge study site (Fig. 2).

Table 1. Between-aspect comparison of mean daily maximum T_a, minimum T_a, and maximum vapour pressure deficit (VPD) at the Lakeview Ridge study site (1902 m a.s.l.), with data from the 2006 (day of year 200–253) and 2007 (day of year 172–253) growth seasons pooled.

Variable	Height (cm)	Aspect	June	July	August	September	Overall	P value*
Maximum T_a (°C)	7.5	SE	20.8±4.3	29.0±4.3	**25.7±5.0**	25.6±5.6	**26.4±5.2**	
		NW	22.2±5.5	28.3±4.1	**22.6±4.9**	21.9±5.1	**24.4±5.5**	0.01
	100	SE	18.0±4.4	24.5±3.8	20.4±4.5	*21.3±5.2*	21.7±4.8	
		NW	18.4±4.4	24.2±3.9	18.9±4.4	*19.2±4.6*	20.6±4.9	0.04
Minimum T_a (°C)	7.5	SE	6.9±3.8	*11.6±3.8*	7.0±3.3	7.8±4.0	8.6±4.1	
		NW	7.8±3.7	*12.9±3.6*	8.5±3.6	8.7±4.5	9.9±4.3	0.01
	100	SE	8.3±3.8	13.5±4.2	8.9±4.1	9.5±4.9	10.5±4.6	
		NW	8.8±3.9	13.9±4.0	9.3±4.2	10.3±5.4	10.9±4.8	0.22
Maximum VPD (kPa)	7.5	SE	**1.39±0.30**	2.38±0.80	**2.10±0.83**	**2.22±0.84**	**2.15±0.82**	
		NW	**1.72±0.48**	2.38±0.91	**1.74±0.75**	**1.71±0.60**	**1.94±0.83**	0.02
	100	SE	1.24±0.32	1.86±0.63	*1.55±0.62*	1.71±0.62	1.65±0.62	
		NW	1.35±0.34	1.87±0.62	*1.41±0.57*	1.50±0.48	1.57±0.59	0.15

Note: All values are the means ± standard deviations. Values in bold are significantly different between slopes at $P < 0.05$. Values in bold italic are significantly different between slopes at $P < 0.01$.

*The overall slope aspect differences were considered significant at $P \leq 0.05$.

Table 2. Characteristics of the *Pinus flexilis* trees and needles examined on four distinct slope aspects, including stand density, tree height, maximum canopy width, basal trunk diameter, branch-length increment, needle life-span, and specific leaf area.

Characteristic	Northwest	Southeast	Northeast	Southwest	P value*
Stand density (trees·ha^{-1})	263±28	237±28	256±62	181±57	0.21
Tree height (m)	1.15±0.56	1.38±0.50	1.21±0.76	1.33±0.59	0.85
Maximum canopy width (m)	1.23±0.75	2.26±0.75	1.94±0.59	2.90±0.80	<0.01
Basal diameter (cm)	14.5±3.1	13.5±4.2	14.3±1.7	12.7±2.8	0.40
Branch increment (mm)	27±5	35±6	32±5	38±7	0.03
Specific leaf area (cm^2·(g·dry mass)$^{-1}$)	29.1±4.5	30.3±6.2	30.4±5.6	28.4±4.3	0.81
Needle life-span (years)	5.4±0.8	5.3±0.6	5.5±0.2	5.5±1.0	0.85

Note: Sample size (N) = 5 for each slope aspect, except for stand density (N = 3) and branch increment (N = 15; 5 for each year (2005, 2006, and 2007)). Values are the means ± standard deviation.

*P values indicate the results of a one-way ANOVA to assess among-aspect differences. Differences were considered significant at $P \leq 0.05$.

During the day of year 200–253 period for which data were available for both years on southeast and northwest slope aspects, no significant between-year differences were observed in mean, maximum or minimum T_a, or VPD at either 7.5 or 100 cm (*t* tests, $P > 0.05$). Higher extreme T_a and VPD maxima were observed in 2007 (day of year 203; 32.1 °C and 3.23 kPa at 100 cm) than in 2006. Daily maximum T_a was higher overall on southeast than northwest slopes because of large between-aspect differences in August and September (Table 1). Conversely, daily minimum T_a did not differ between slope aspects at 100 cm and was lower on southeast slopes near the surface. Maximum daily VPD did not differ between slope aspects at 100 cm, except in August, when VPD tended to be higher on southeast slopes. Near-surface VPD maxima were higher on southeast slopes overall, despite lower VPD in June (Table 1).

The May to September period was warmer and drier than normal in both 2006 and 2007. A 12 year climate record was available for Park Gate (WLNP; 49°07′12″N, 113°49′48″W, 1296 m a.s.l.), located 7 km to the southeast of the study site. The mean daily maximum T_a was 21.8 °C in both 2006 and 2007 at Park Gate, which is 2.0 °C above the 1996–2005 mean and represents the highest growing season mean observed to date (Environment Canada 2008).

At 29.5 °C, mean maximum T_a was higher in July 2007 than any other month in the climate record history and was 5.2 °C above the monthly normal. Total precipitation during May to September was 258 mm in 2006 and 207 mm in 2007, or 81% and 65% of the 1996–2005 mean, respectively. July and August were very dry, with 62 day totals of only 34 and 14 mm of rain, representing 51% and 20% of the 1996–2005 mean. In response to drier than normal conditions in July and August 2006, 10 cm θ_v decreased from 0.39 to 0.18 m^3·m^{-3} from mid-June to August on the northwest slopes, but rebounded to 0.24 m^3·m^{-3} in response to light rains in August. On the southeast slope, θ_v decreased from 0.28 to an asymptotic low of 0.12 m^3·m^{-3} between mid-June and early July, but twice briefly increased to 0.18–0.22 m^3·m^{-3} in response to light rains during the dry period.

Stand, structural, and foliar characteristics of *P. flexilis*

The four aspects were similar in terms of limber pine stand density, basal diameter, and tree height, but maximum canopy width was higher on the slopes with higher irradiance (SW > NE, SW > NW, SE > NW, at $P < 0.05$; Table 2). Branch-length increment was lower on the northwest slope than on the southwest slope, but no significant differ-

Fig. 3. Mean daily specific leaf area (SLA) of *Pinus flexilis* needles vs. day of year at Lakeview Ridge, Waterton Lakes National Park. Error bars indicate the standard deviation.
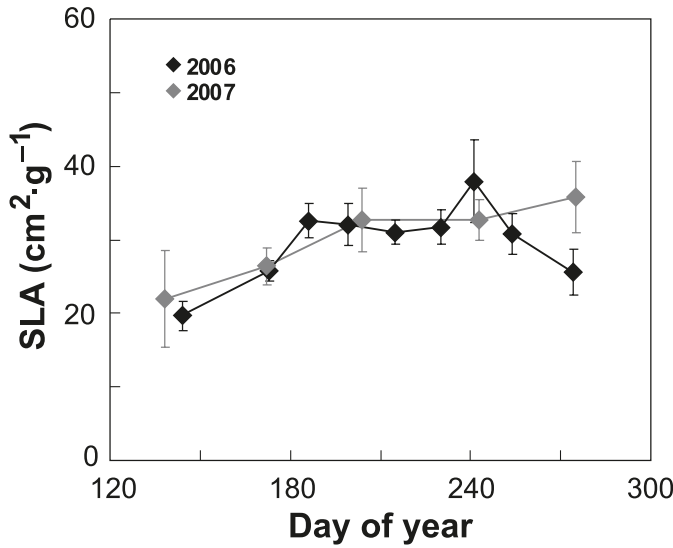
ences were observed among years, with mean values of 29 ± 2, 34 ± 7, and 33 ± 7 mm in 2005, 2006, and 2007, respectively (P = 0.10). No among-aspect differences were observed in either needle longevity or SLA. The mean SLA was 29.7 ± 5.3 cm²·(g dry mass)⁻¹ in 2006 and 29.9 ± 5.3 cm²·(g dry mass)⁻¹ in 2007, increasing with day of year in both years (Fig. 3). The decrease in SLA observed through September 2006 was short-lived, as SLA increased to 28.9 ± 3.6 cm²·(g dry mass)⁻¹ by day of year 325 (not shown). SLA was unrelated to needle year in 2007 but was 1.3 ± 0.4 cm²·(g dry mass)⁻¹ higher in north-facing than south-facing trees, with mean daily SLA data pooled for both years.

## Slope aspect effects on photosynthetic gas exchange

During the growth season of 2006, among-aspect differences in both T_{leaf} and LAVD were observed, with higher values on the southeast and southwest slopes than on the northwest slope during May, August, and September (P < 0.02; Table 3). Despite the slightly higher atmospheric demand on southeast and southwest slopes, no among-aspect differences were observed in either photosynthetic gas-exchange characteristics or $\delta^{13}C_p$ (P > 0.05). On all four slope aspects, A_{max} exhibited a bimodal seasonal pattern, with the highest rates observed in late May and early October and the lowest rates observed during the dry period in August (Fig. 4). However, aspect × day of year interaction was evident in both A_{max} and E (P < 0.02). In response to the dry conditions of August and early September, A_{max} was near zero on southeast and southwest slopes (−0.6 to +0.1 µmol·m⁻²·s⁻¹) but remained slightly positive (+1.1 to +2.7 µmol·m⁻²·s⁻¹) on northeast and northwest slopes before rebounding sharply in early October along with a very high WUE (A_{max}/E) in the 5.5–8.9 mmol·mol⁻¹ range. Similarly, E reached lower minima on southeast and southwest slopes (0.2 mmol·m⁻²·s⁻¹) than on opposing slope aspects (0.6–0.8 mmol·m⁻²·s⁻¹) during the dry period. In 2006, photosynthetic gas-exchange characteristics were also measured dur-

Table 3. Summary of repeated measures analysis of variance (ANOVA) on light-saturated net photosynthesis (A_{max}), stomatal conductance (g_s), transpiration (E), water-use efficiency (WUE: A_{max}/g_s and A_{max}/E), leaf nitrogen content (N_{leaf}), stable carbon isotope composition ($\delta^{13}C_p$), leaf temperature (T_{leaf}), and leaf-to-air vapour pressure deficit (LAVD) from days of year 144–274, 2006, including (i) among-aspect differences and (ii) aspect × day of year interaction.

Variable	Among-aspect differences (least-square mean±standard error)						Aspect × day of year		
	Northwest	Southeast	Southwest	Northeast	F ratio	P value	df	F ratio	P value*
A_{max} (µmol·m⁻²·s⁻¹)	6.2±0.4	5.2±0.4	5.5±0.4	6.1±0.4	1.48	0.22	24	1.81	0.02
g_s (mmol·m⁻²·s⁻¹)	92±7	88±6	88±7	94±7	0.21	0.89	24	1.47	0.09
E (mmol·m⁻²·s⁻¹)	1.81±0.12	1.59±0.11	1.75±0.12	1.88±0.12	1.17	0.32	24	1.93	0.01
WUE (mmol·mol⁻¹)									
A_{max}/g_s	0.072±0.004	0.068±0.004	0.069±0.004	0.068±0.006	0.36	0.78	18	0.74	0.76
A_{max}/E	4.4±0.3	3.9±0.2	3.6±0.2	3.6±0.3	2.38	0.07	18	1.14	0.33
N_{leaf} (%)	1.14±0.04	1.08±0.04	0.98±0.04	1.05±0.04	2.67	0.06	6	0.94	0.48
$\delta^{13}C_p$ (‰)	−24.94±0.22	−25.26±0.20	−25.54±0.20	−25.53±0.22	1.67	0.19	6	1.03	0.42
T_{leaf} (°C)	23.3±0.3	25.5±0.3	25.4±0.3	24.9±0.3	12.80	<0.01	24	3.01	<0.01
LAVD (kPa)	1.93±0.05	2.13±0.05	2.14±0.05	2.10±0.06	3.41	0.02	24	3.74	<0.01

*Differences were considered significant at $P \leq 0.05$.

Fig. 4. Chamber conditions (leaf temperature (T_{leaf}) and leaf-to-air vapour pressure deficit (LAVD)), photosynthetic gas-exchange characteristics (stomatal conductance (g_s), transpiration (E), and light-saturated net photosynthesis (A_{max})), and leaf nitrogen (N_{leaf}) concentrations of *Pinus flexilis* trees on four slope aspects in 2006 and two slope aspects in 2007. Error bars indicate the standard error.
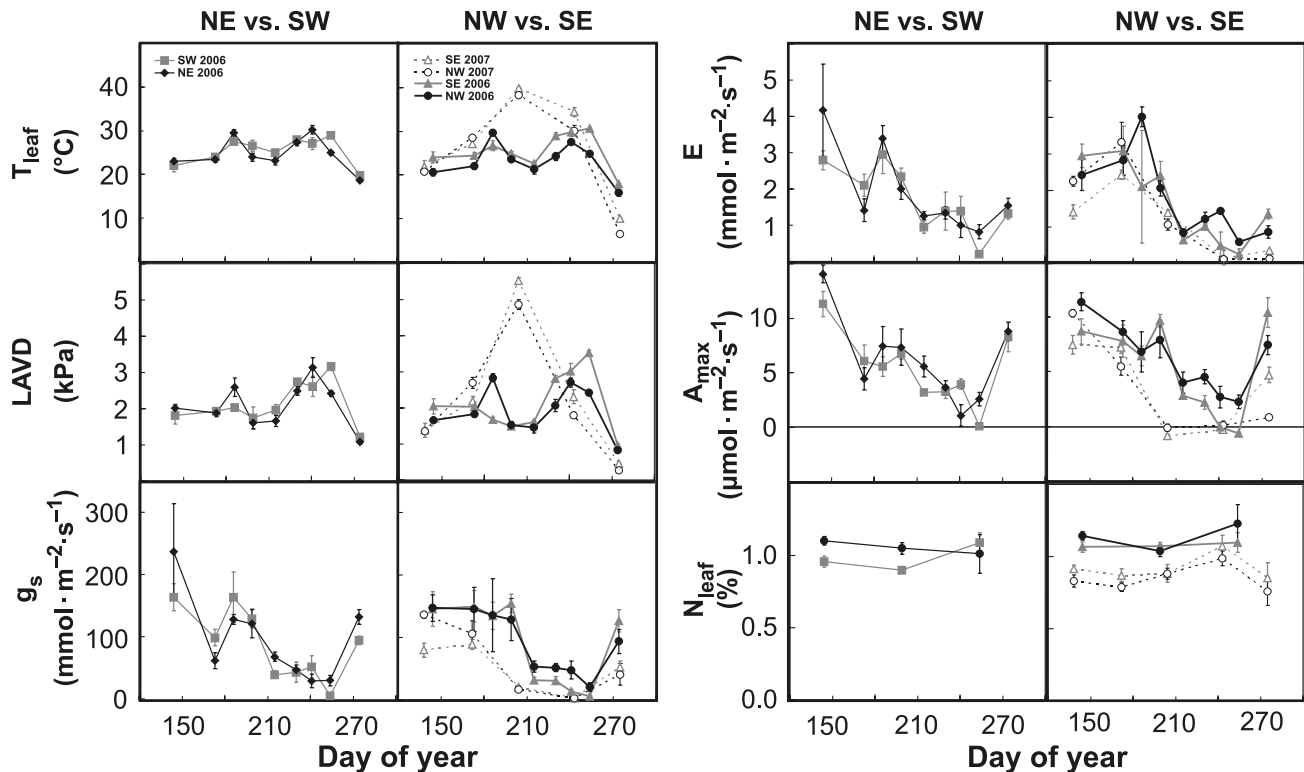
ing chinook conditions on day of year 325. Despite moist soils and above-freezing T_a, all trees were dormant in terms of photosynthetic activity by this date.

Between-year differences in photosynthetic gas exchange

A wider range of T_{leaf} and LAVD conditions was observed in 2007 than 2006 (Fig. 4). In response to higher VPD and lower rainfall, g_s was lower in 2007 than 2006. Consequently, A_{max} decreased earlier in 2007 and was negative by day of year 204 (Fig. 4). N_{leaf} content was also lower in 2007 than in 2006. The interannual decrease in E was proportionally smaller than that of g_s or A_{max} because of higher LAVD during measurements. With data pooled for both years, A_{max} and WUE (A_{max}/E) were negatively correlated with LAVD (Fig. 5), the latter as a consequence of the positive logarithmic relationship between g_s and A_{max}. The low A_{max} observed on day of year 275, 2007 (point A), could indicate that (*i*) T_{leaf} was suboptimal, (*ii*) cold hardening had begun in response to frost events, or (*iii*) photochemical capacity was reduced by drought stress. Severe drought caused low A_{max} on day of year 204 and 243, 2007, despite low LAVD (points B and C). Foliar $\delta^{13}C$ ratios were higher in 2007 than in 2006, especially in July (Fig. 6), though the 2006 measurements were taken on y2 needles, which were formed during the growth season of 2005, when 592.6 mm of rain fell from May to September at Park Gate, WLNP. A smaller difference was noted between y3 needles of 2007 (–24.74‰ ± 0.11‰) and y2 needles of 2006 (–25.10‰ ± 0.16‰).

Lower g_s and c_i/c_a but higher WUE (A_{max}/g_s and A_{max}/E;

Table 4) were observed on the southeast slope in 2007, indicating greater physiological acclimation to drought stress. Maximum Photosystem II efficiency (F_v/F_m) was also slightly lower on the southeast slope (0.77 ± 0.02) than on the northwest slope (0.81 ± 0.04) on day of the year 172, 2007 ($P = 0.06$), despite the fact that measurements were taken before the onset of severe drought conditions. However, no between-aspect difference was observed in either A_{max} or $\delta^{13}C_p$, and no visible dieback or foliar damage resulted from the dry and warm conditions. The vegetation indices NDVI, CI, PRI, and WBI did not vary with day of year or between slope aspects (Table 5). Photosynthetic activity resumed by early October in 2007, but A_{max} and E increased by less than in 2006, especially on the northwest slope. Cooler T_{leaf} was observed on the October 2007 measurement date.
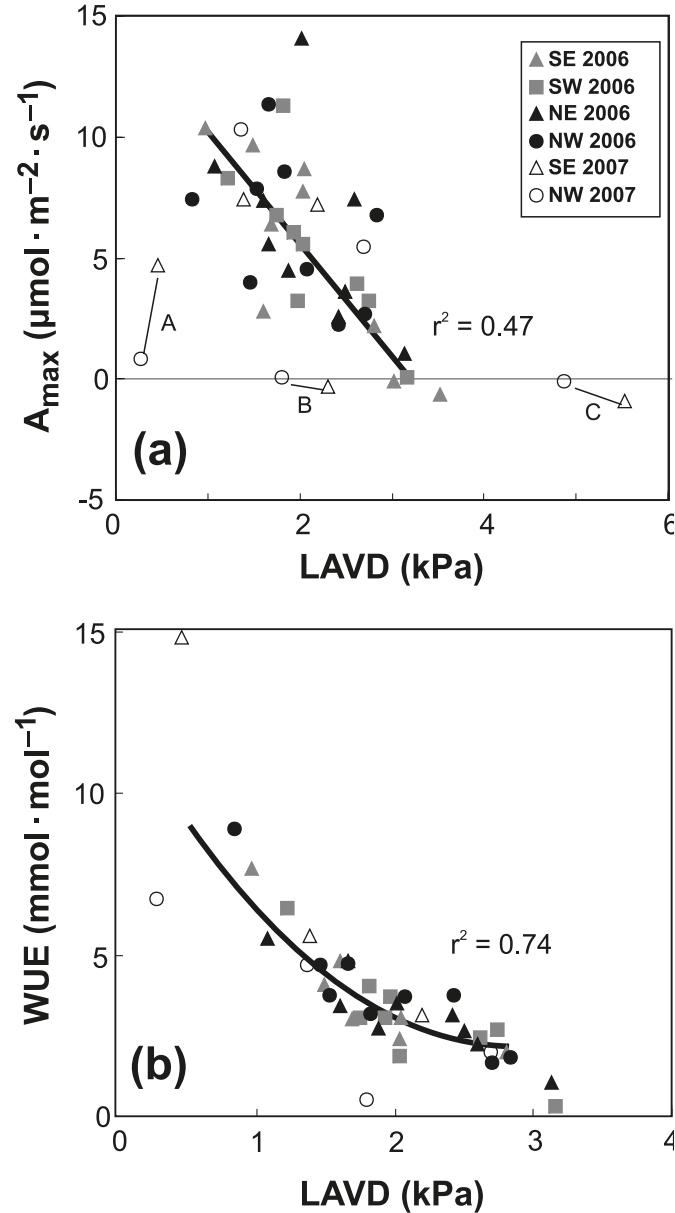
Needle–year effects

Foliar nitrogen content (N_{leaf}) was highest in y1 needles ($P < 0.01$; Fig. 6) in 2007, but this did not facilitate significantly higher A_{max}, g_s, or E ($P > 0.05$). SLA did not differ by needle year in 2007, but $\delta^{13}C_p$ ratios were higher in y1 than in either y2 or y3 needles. The rank order of these differences was inversely correlated with interannual variation in precipitation, which was highest in 2005 and lowest in 2007.

Discussion
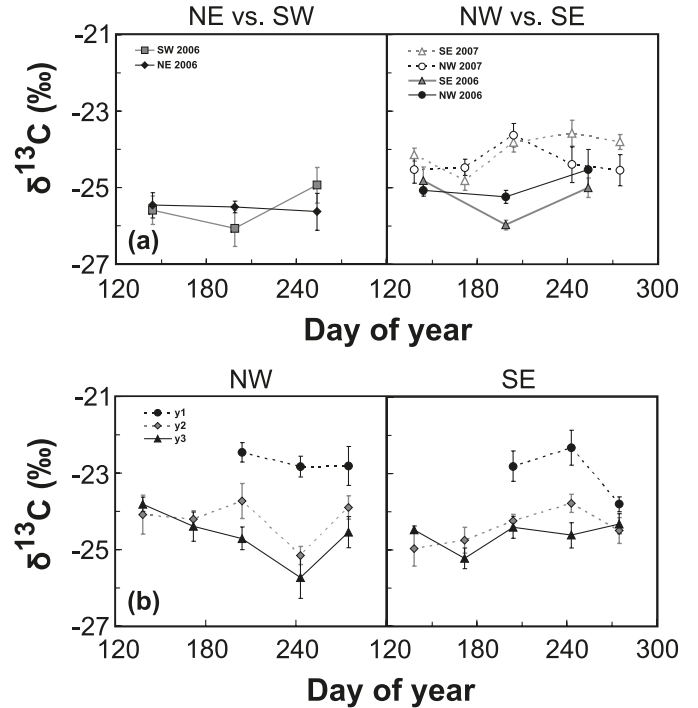
Conservative water-use strategy

Limber pine exhibited a variety of characteristics indica-

Fig. 5. (*a*) Relationship between leaf-to-air vapour pressure deficit (LAVD) and net light-saturated photosynthesis (A_{max}) of *Pinus flexilis* needles in 2006 and 2007. Points A to C indicate outliers not used to determine the coefficient of determination. (*b*) Relationship between LAVD and water-use efficiency (WUE; A_{max}/E). Negative and near-zero (lowest three shown) WUE values were not included in the analysis.

Fig. 6. (*a*) Stable carbon isotope composition ($\delta^{13}C_p$) of *Pinus flexilis* needles of northwest- (NW), southeast- (SE), northeast- (NE), and southwest- (SW) facing slopes vs. day of year in 2006 and 2007. (*b*) Seasonal pattern of $\delta^{13}C_p$ in year 1 (y1; 2007), year 2 (y2; 2006), and year 3 (y3; 2005) needles of *P. flexilis* on NW and SE slopes in 2007.

season WUE (A_{max}/E) was maximized by concentrating photosynthetic activity during periods of low atmospheric demand.

The results of this study corroborate those of previous studies on the whole-plant hydraulics of *P. flexilis*. Under conditions of low to moderate VPD and high θ_v, sapflow (J_s) and whole-plant hydraulic conductance were similar in *P. flexilis* and *Abies lasiocarpa* and higher in *P. flexilis* than in *P. contorta* (Pataki et al. 2000). However, limber pine exhibited the largest proportional reduction in J_s in response to soil moisture depletion on days with high VPD. Fischer et al. (2002) demonstrated that *P. flexilis* had lower canopy conductance (g_c) and higher stomatal sensitivity to high VPD than did *P. ponderosa*. Midday leaf water potential was also higher (–1.4 vs. –1.8 MPa) and showed little seasonal variation despite declining θ_v. Collectively, these results show that *P. flexilis* gas exchange rates are sensitive to high atmospheric demand.

Limber pine favours south-facing slope aspects when in competition with spruce and fir, and growth rates are often highest in warm and dry years (Bigler et al. 2007). At Lakeview Ridge, however, A_{max} responded negatively to T_{leaf} above 22 °C and to LAVD above 1 kPa. These observations establish the ecophysiological basis to explain why annual tree-ring growth increment is positively correlated with September and October T_a but negatively correlated with June T_a (Villalba et al. 1994). Similarly, Chhin et al. (2008) found radial growth of *P. contorta* to be positively correlated with

tive of conservative water usage, including low SLA and mass-based N_{leaf}, but high needle longevity, WUE, and foliar $\delta^{13}C$ composition. However, this did not facilitate positive A_{max} during the warm and dry episodes of July and August. Instead, A_{max} was highest in the spring and fall, when T_a and VPD were low and θ_v was high relative to that of late summer. The invariability of the canopy reflectance indices (NDVI, CI, PRI, and WBI) and the strong photosynthetic response to September rainfall suggest that the thick needles of limber pine are able to withstand long episodes of drought, with little impact on subsequent photosynthetic capacity. At a xeric, subalpine krummholz site, whole-

Table 4. Summary of repeated measures analysis of variance (ANOVA) on light-saturated net photosynthesis (A_{max}), stomatal conductance (g_s), transpiration (E), water-use efficiency (WUE; A_{max}/g_s and A_{max}/E), internal to atmospheric CO_2 concentration ratio (c_i/c_a), leaf nitrogen content (N_{leaf}), stable carbon isotope composition ($\delta^{13}C_p$), leaf temperature (T_{leaf}), and leaf-to-air vapour pressure deficit (LAVD) from day of year 138–275 (2007), including (i) between-aspect differences, (ii) among-needle year differences, and (iii) aspect × day of year interaction.

Variable	Between-aspect differences (least-square mean ± standard error)				Aspect × day of year			Among-needle year differences (least-square mean ± standard error)				
	Northwest	Southeast	F ratio	P value*	df	F ratio	P value*	Year 1	Year 2	Year 3	F ratio	P value*
A_{max} (µmol·m⁻²·s⁻¹)	3.4±0.2	3.4±0.2	0.00	0.97	4	9.95	<0.01	3.8±0.3	3.5±0.2	3.0±0.2	2.21	0.12
g_s (mmol·m⁻²·s⁻¹)	61±4	46±4	7.24	<0.01	4	3.36	0.01	59±6	50±4	52±4	0.82	0.44
E (mmol·m⁻²·s⁻¹)	1.41±0.11	1.15±0.10	3.25	0.07	4	3.17	0.02	1.45±0.16	1.16±0.11	1.05±0.12	1.13	0.33
WUE (mmol·mol⁻¹)												
A_{max}/g_s	0.058±0.006	0.092±0.006	17.80	<0.01	4	3.78	0.03	0.072±0.011	0.083±0.006	0.070±0.006	1.29	0.28
A_{max}/E	5.2±0.8	7.8±0.8	6.63	0.01	4	3.04	0.06	6.4±1.4	7.8±0.8	5.5±0.7	2.48	0.09
c_i/c_a	0.72±0.03	0.59±0.02	18.00	<0.01	4	4.59	0.01	0.67±0.04	0.63±0.02	0.68±0.02	1.60	0.21
N_{leaf} (%)	0.87±0.02	0.92±0.02	2.40	0.12	4	0.69	0.60	0.98±0.03	0.89±0.02	0.81±0.02	9.53	<0.01
$\delta^{13}C_p$ (‰)	−23.97±0.11	−23.90±0.10	0.22	0.64	4	3.52	0.01	−22.89±0.15	−24.17±0.11	−24.74±0.11	48.99	<0.01
T_{leaf} (°C)	24.7±0.4	26.3±0.4	10.30	<0.01	4	3.45	0.01	24.9±0.5	26.0±0.4	25.6±0.4	1.49	0.23
LAVD (kPa)	2.18±0.07	2.34±0.07	2.98	0.09	4	5.17	<0.01	2.16±0.10	2.29±0.07	2.27±0.08	1.00	0.37

*Differences were considered significant at $P \leq 0.05$.

T_a in spring but not late summer. The benefit of the warmer and drier years likely occurs during the shoulder seasons, when low temperatures would otherwise be more limiting to growth. At WLNP, T_a and VPD were supraoptimal during July and August, resulting in a lower A_{max}. Given that an extended period of higher than optimal T_a and VPD was observed in limber pine near the upper limit of its local range, these climatic constraints do not appear to be limiting to upward migration.

Climate change and the shoulder seasons

Mean annual temperatures at WLNP rose by approximately 0.6 °C during the twentieth century (Hamilton et al. 2001), following a sustained period of cold summers in the nineteenth century (Luckman 2000). The bimodal seasonal pattern of gas exchange observed in limber pine during the warm and dry growth seasons of 2006 and 2007 would likely not occur during cooler and wetter summers. In the absence of stress related to drought and VPD, photosynthetic capacity would be expected to increase early in the growth season, with rates maintained until Photosystem II reaction centres are lost, chlorophyll content decreases, and the capacity for nonphotochemical quenching increases during cold hardening (Savitch et al. 2002).

Downscaled output from the Canadian Coupled Global Circulation Model (CGCM1-A) indicates that a doubling of c_a (2 × CO_2) might result in a T_a increase of 2 °C in southwestern Alberta by 2050, with little change in precipitation compared with a 1960–1989 base period (Shepherd and McGinn 2003). Mean T_a was 2.0 °C above the historic climate mean during the growth seasons of 2006 and 2007. Micrometeorological conditions were, therefore, representative of those that might be encountered during drier than normal years in a 2 × CO_2 climate. The bimodality of the seasonal pattern of photosynthetic activity under these conditions indicates that the shoulder seasons (spring and fall) may become increasingly important to limber pine growth and survival in the event of climate warming.

Drought stress and survivorship in limber pine

Despite the record heat and dryness of July and August 2007, it is apparent that the threshold limits for widespread mortality were not reached. All study specimens survived and only two dead *P. flexilis* specimens over 0.5 m, representing about 1% of individuals, were found at the study site in the spring of 2008. One of these trees was on the southeast slope and one was on the northwest slope. No mortality was observed in the spring of 2007. This is consistent with observations of drought-induced mortality in northern Colorado, where both early- and late-season drought led to spruce and fir mortality, but had no effect on pines from 1910 to 2004 (Bigler et al. 2007). However, the twentieth century was likely the wettest of the past two millennia in continental regions of North America (Schindler and Donahue 2006), and the palaeoclimatic record indicates that more severe and prolonged drought conditions have occurred in the past, in both Colorado (Woodhouse and Overpeck 1998; Meko et al. 2007) and Alberta (Laird et al. 2003). Furthermore, mortality events were reported in dense, young, high-altitude stands of *P. flexilis* in the Sierra Nevada of California, in response to a series of very dry years from 1985 to

Table 5. Summary of repeated measures analysis of variance (ANOVA) on canopy reflectance indices of *Pinus flexilis* trees (day of year 138, 172, 204, and 243, 2007), including (*i*) between-aspect differences and (*ii*) aspect × day of year interaction.

Index	Between-aspect comparison (least-square mean ± standard error)				Aspect × day of year		
	Northwest	Southeast	F ratio	P value	df	F ratio	P value
NDVI	0.80±0.01	0.82±0.01	1.56	0.22	3	0.61	0.61
CI	0.49±0.01	0.51±0.01	1.31	0.26	3	0.63	0.60
PRI	−0.065±0.005	−0.067±0.007	0.05	0.82	3	0.13	0.95
WBI	1.22±0.02	1.24±0.02	0.43	0.52	3	1.10	0.36

Note: NDVI, normalized difference vegetation index; CI, chlorophyll index; PRI, photochemical reflectance index; and WBI, water band index.

1995 (Millar et al. 2007). A subsequent drought had no effect on survivorship. Thus, observations of low mortality may indicate that selection for drought-resistant individuals occurred during an earlier stage of stand establishment.

With regard to the seasonal timing of drought, the Sierra Nevada mortality event occurred during a period of low precipitation during the wet seasons, which occur before and during the early portion of the growth season. Although limber pine survives at xeric, wind-exposed sites with shallow soil and frequent summer drought, their distribution suggests that they may be more susceptible to the severe moisture deficits that occur in the spring. Limber pine is found along the eastern front ranges of the Rocky Mountains, where there is a late spring maximum precipitation regime; in high altitude ecosystems in California with a winter precipitation maximum; and in interior montane regions of Idaho, Nevada, Utah, and northern Arizona. Snowfall is common throughout its range and provides additional spring moisture. Its range ends in the dry monsoonal montane regions of southern New Mexico and Arizona, where the wet season occurs later in the summer.

Delayed cold hardening

Timely cold acclimation is important in the Rocky Mountains of Alberta because of the early development of very cold continental air masses. However, the high A_{max} observed in early October indicates that further research is warranted to determine whether limber pine has evolved relatively late photoperiod-related cold hardening, to extend the growth season and increase overall WUE. In years with available soil moisture and late hard frosts, delayed cold hardening may partially compensate for reduced growth during summer drought. The lower early October photosynthesis rates of 2007, compared with 2006, may indicate that T_a was suboptimal or that cold hardening had begun. The daily mean temperature was 1.9 °C warmer in September 2006 than September 2007 at Waterton Park Gate, with especially mild conditions near the end of the month and only four frost days. By contrast, eight frost days were observed in September 2007.

Seasonal trends in leaf traits

In both 2006 and 2007, SLA increased with day of year as drought conditions progressed, but a decrease was observed late in 2006, coincident with a renewed period of photosynthetic productivity (Fig. 3). Such a pattern could be explained by greater photoassimilate storage within needles during periods of high carbon gain. Leaf nitrogen decreased with day of year on an area basis (grams per square metre) as SLA increased, but N_{leaf} did not vary with day of year on a mass basis (Fig. 4). Given the high correlation between mass-based N_{leaf} content and mass-based A_{max} (Reich et al. 1999), the absence of a seasonal trend in N_{leaf} suggests that drought may not have impacted photosynthetic capacity. Furthermore, no seasonal variation was observed in any of the canopy reflectance indices that are typically correlated with photochemical capacity (CI, NDVI, and PRI) or xanthophyll cycle activity (PRI).

Slope aspect effects on physiological acclimation to water stress

In montane environments, wind exposure and canopy effects mediate the slope-aspect-related microclimatic differences that result from variable solar insolation (Ashcroft 2006). The Lakeview Ridge study site is located on a highly exposed mountain peak in an area with persistently strong winds. Growth season T_a was 1.1 °C higher on the southeast slope than on the northwest slope at 1 m and 2.0 °C higher near the surface. The differences resulted from higher southeast-facing temperatures in August and September, when the solar zenith angle increased. Near-surface VPD was also higher on the southeast slope. Whereas maximum T_a was higher on the southeast slope, minimum T_a was slightly lower, likely due to greater areal coverage of groundcover, as opposed to rock, beneath the sensor height. The southeast slope, therefore, represents a more extreme temperature environment than the northwest slope, with higher daytime solar radiation intensity and more effective radiational cooling.

During the severe drought year (2007), *P. flexilis* trees of the southeast slope exhibited lower g_s, E, and c_i/c_a, but higher WUE (A_{max}/g_s and A_{max}/E) and T_{leaf}. Although each of these characteristics is indicative of water stress, A_{max} did not differ with slope aspect (Table 4). Among-aspect differences were less prevalent during the moderate drought of 2006, despite higher T_{leaf} and LAVD on the southeast slope. Although some aspect × day of year interaction was observed, the lack of among-aspect differences in mean growth season A_{max} indicates that physiological acclimation to water stress prevented a negative impact on the net carbon gain. Furthermore, there was a tendency for higher branch-length increment on the southerly aspects (Table 2). Given the lack of among-aspect variation in A_{max}, possible causes of higher branch increment on south-facing slopes include longer photosynthetically active daylength, due to

stronger isolation, and higher daytime T_a during the cool and relatively moist shoulder seasons.

Summary

The results of this study indicate that limber pine possesses leaf structural and physiological traits that facilitate a highly conservative water-use strategy. However, rather than conserving water to maintain photosynthetic uptake during hot and dry periods, limber pine foliage exhibits a strong negative response to high VPD. Photosynthetic gas-exchange activity is concentrated within periods of low to moderate atmospheric demand, resulting in higher overall growth season WUE. In the context of a warming climate, limber pine is an example of a species that is capable of using the shoulder seasons to compensate for periods of water stress that tend to occur in late summer.

Acknowledgements

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