We studied the effect of scion donor-tree age on the physiology and growth of 6- to 7-year-old grafted Scots pine (Pinus sylvestris L.) trees (4 and 5 years after grafting). Physiological measurements included photosynthetic rate, stomatal conductance, transpiration, whole plant hydraulic conductance, needle nitrogen concentration and carbon isotope composition. Growth measurements included total and component biomasses, relative growth rates and growth efficiency. Scion donor trees ranged in age from 36 to 269 years at the time of grafting. Hydraulic conductance was measured gravimetrically, applying the Ohm’s law analogy, and directly, with a high-pressure flow meter. We found no effect of scion donor-tree age on any of the variables measured. There was, however, great variation within scion donor-tree age groups, which was related to the size of the grafted trees. Differences in size may have been caused by variable initial grafting success, but there was no indication that grafting success and age were related. At the stem level, hydraulic conductance scaled with total leaf area so that total conductance per unit leaf area did not vary with crown size. However, leaf specific hydraulic conductance (gravimetric), transpiration, photosynthesis and stomatal conductance declined with increasing total tree leaf area and needle width. We hypothesize that needle width is inversely related to mesophyll conductance. We conclude that canopy and needle size and not scion donor-tree age determined gas exchange in our grafted trees.

Keywords: aging, Caledonian pine, crown size, hydraulic limitation, leaf-level adjustment, leaf specific conductance, Pinus sylvestris.

Introduction
Forest productivity and tree growth tend to decrease with age (cf. Gower et al. 1996, Ryan et al. 1997), although the reason for this is not fully understood. Increased respiratory load and increased resistance in the hydraulic pathway with increasing tree age have been invoked as an explanation (for a complete overview, see Ryan and Yoder 1997). In recent years, evidence has accumulated suggesting that height affects growth either by lowering leaf specific hydraulic conductance (Mencuccini and Grace 1996, Schäfer et al. 2000, Hubbard et al. 2002, McDowell et al. 2002a, Koch et al. 2004), with a consequent reduction in photosynthetic rate (Hubbard et al. 2001), or by shifting biomass allocation from leaf to root (Magnani et al. 2000). However, not all assumptions of the hydraulic limitation theory may hold. For example, leaf area to sapwood area ratio frequently decreases with increasing tree height (McDowell et al. 2002b), thus increasing the conductive capacity of the stem per unit leaf area with height. However, Köstner et al. (2002) found an increase in leaf area to sapwood area ratio with increasing age and McDowell et al. (2002b) noted an increase in some species with height. McDowell et al. (2002a) reported that leaf specific conductance was reduced in taller Douglas-firs (Pseudotsuga menziesii Mirb. Franco), although photosynthetic rates were not. In contrast, Phillips et al. (2002) found no consistent effect of height on hydraulic conductance in Douglas-fir trees over a wide range of heights. Mostly, though, these findings do not allow the effects of age and height to be distinguished (Niinemets 2002).

The purpose of our study was to assess the effect of age on the growth and physiology of Scots pine (Pinus sylvestris L). Our working hypothesis was that increased tree age would lead to decreased leaf specific conductance and gas exchange. Hubbard et al. (2002) tried to separate the effect of age from height by comparing the leaf specific conductivity of lower and upper branches of tall trees, but found no substantial differences. However, their approach confounded branch age with height. To investigate the effect of age, while controlling for the effects of height on leaf specific conductance, growth and gas exchange, we grafted scions from different-aged donor trees onto seedling rootstocks, thereby creating grafted trees of uniform height but with shoots of different physiological ages.

Our study was based on two assumptions. First, that age-related properties of the scion donor trees would be expressed in...
the grafted trees. Consistent with this assumption, it is a common and effective practice to graft scions from mature trees onto seedling rootstocks to increase seed production in seed orchards. It has also been shown that many morphological and physiological characteristics of the scion donor tree persist in the grafted plant (Greenwood 1984, Greenwood et al. 1989, Ritchie and Keeley 1994). Consistent with these reports, scions of our grafted trees were closely comparable to the scion donor trees in leaf nitrogen content, specific leaf area and leaf $^{13}$C isotope content (Martínez-Vilalta et al. unpublished results). Second, we assumed that the trauma of grafting did not mask size or age factors. As our study did not begin until 4 years after grafting, it is reasonable to suppose that any transient effects of grafting were by then negligible.

Materials and methods

Plant material

For this study, we worked with grafted trees with scions from 36-, 49-, 85-, 151-, 201-, 214- and 269-year-old naturally established Caledonian (Scots) pine ($\text{Pinus sylvestris}$ L.) donor trees growing in Guisachan Forest in the Fort Augustus Forest District, Morayshire, Scotland, U.K. (57°15′850″ N, 4°50.175′W). Scions were collected from the top third of each donor tree and grafted onto 2-year-old seedling rootstocks of Scots pine (stem diameter at grafting height was 7–10 mm) in January 1998. Grafting was conducted at the Tree Improvement Branch of the Northern Research Station (NRS, Forest Research, Bush Campus, Roslin, Scotland, U.K.). Scions (6–7 cm in length) were attached by the “apical side veneer graft” technique. Grafting tape was removed 6–8 weeks after grafting and all rootstock stems above the graft were removed after one year. About 6 months after grafting, the grafted trees were transferred to 4 dm$^3$ polyethylene bags in which they were maintained until our measurements were conducted. While at NRS, the seedlings were watered and fertilized following standard protocols.

Leaf-level and whole-plant physiology

Five grafted trees per scion donor tree were measured in late summer 2002 in a greenhouse at the University of Edinburgh (Edinburgh, Scotland), at which time, the trees were 6 years old (4 years post-grafting) and all had both current- and previous-year needles. In two-thirds of the grafted trees, a third needle age class was present and made up a small proportion (range: 2–36%; median: 20%) of the total leaf area, but these needles were not subject to physiological measurements, although they were included in the measurement of leaf area for the calculation of transpiration ($E$) and leaf specific hydraulic conductance ($K_{\text{s,l}}$).

We measured predawn ($\Psi_{\text{PD}}$) and near-midday ($\Psi_{\text{MD}}$) leaf water potential, net photosynthesis on a leaf area basis ($A_{\text{net}}$), stomatal conductance ($g_{s}$), $E$ and $K_{\text{s,l}}$ on September 11, 2002 (Day 1), and September 12, 2002 (Day 2), on current- and previous-year needles, respectively. In the evening preceding each measurement day, the grafted trees were well watered and allowed to drain. Pots were then wrapped in plastic bags and sealed at the root collar to prevent evaporative water loss. To minimize transpiration, the crowns of the plants were loosely covered overnight by large black plastic bags, which were removed before $\Psi_{\text{PD}}$ (0500–0730 h) was determined with a Scholander type pressure chamber (Skye Instruments, Llandrindod Wells, U.K.). Leaf water potential was measured again around midday (1030–1630 h) ($\Psi_{\text{MD}}$) on both days. We assumed that the difference between $\Psi_{\text{PD}}$ and $\Psi_{\text{MD}}$ ($\Psi_{\text{DIFF}}$) reflected the soil-to-leaf water potential difference.

Net photosynthesis ($A_{\text{net}}$) and $g_{s}$ were measured with an LCPro infrared gas analyzer with a conifer cuvette (ADC, Hoddesdon, U.K.) on both measurement days between 1130 and 1630 h. The needles were aligned side-by-side in the cuvette to avoid mutual shading. Leaf areas of the exposed portions of the needles were measured with an LI-3100C leaf area meter (Li-Cor, Lincoln, NE). Irradiance in the cuvette was maintained at 1500 µmol m$^{-2}$ s$^{-1}$ at all times. Cuvette temperature was unregulated, but varied little (36.2–37.4 °C and 34.1–36.1 °C on Days 1 and 2, respectively).

Daytime transpiration was determined by weighing plants at 1000 and 1600 h. Leaf specific conductance was calculated by the Ohm’s Law analogy ($K_{\text{s,l}} = E/\Psi_{\text{DIFF}}$) (cf. Meinzer and Grantz 1990). Transpiration and $K_{\text{s,l}}$ were calculated for both days of measurements.

Total leaf area per plant ($A_{\text{L}}$) was estimated by multiplying total foliated stem length for each age cohort by mean needle density and mean individual needle area for that cohort. Mean needle density per plant was estimated by counting the needles ($n = 15–45$) on 5 cm sections of each foliated stem segment (i.e., age cohort) of the main stem and two branches. Mean individual needle area was estimated by measuring length and width of needles comprising a subsample of needles within each stem segment ($n = 27$ per tree). The measured needles were subsequently dried and weighed. Specific leaf area (SLA) was calculated as the leaf area of the subsample needles divided by their dry mass.

A subsample of the current- and previous-year needles from each tree was dried and ground to a fine powder with a liquid nitrogen freezer mill (6750 Freezer/Mill, Spex CertiPrep, Metuchen, NJ). These samples were sent to the Cornell University Stable Isotope Laboratory (COIL, Ithaca, NY) for analysis of nitrogen concentration ([N]$_{L}$, % mass basis) and carbon isotope composition (carbon-13 ($^{13}$C)). Stable isotope ratios were expressed in relation to a PeeDee Belemnite standard. Carbon isotope composition was used as a seasonally integrated measure of the stomatal limitation to carbon assimilation (Farquhar et al. 1998).

Hydraulic conductance measurements

Hydraulic conductance was measured with a high pressure flow meter (HPFM, Dynamax, Fallstone, TX) on a subset of the trees ($n = 3–4$ per scion age). Measurements took place during the winter of 2003–2004. The plants measured were
those used previously for leaf physiology measurements plus one plant with a scion from the 269-year-old donor tree, which was added to increase sample size for that age group.

Hydraulic conductance was measured in the root system, the grafted section of the stem and a section of stem above the graft region. The night before measurement, the grafted trees were watered and covered with black plastic bags. In the morning, the shoots were cut under water about 4 cm above the soil surface. The aboveground part was kept under water while the root system was carefully removed from the substrate and attached to an HPFM. After the root system had been measured, the section of stem immediately above the graft union was cut underwater and its hydraulic conductance measured, after removal of all needles. Finally, the hydraulic conductance of the graft region of the stem was measured.

The HPFM technique measures flow through the sample segment under a known external pressure difference (Tyree et al. 1995). In all cases, hydraulic conductance was measured using the transient mode of the HPFM (Tyree et al. 1995). The conductance measurements were not made until water flowed from the distal end of the test segment, which typically took 15–30 min of flushing at constant pressure (~0.1 MPa). Hydraulic conductance was estimated as the slope of the linear portion of the curve linking flow rate and applied pressure ($r^2 > 0.99$), usually between 0.1 and 0.4 MPa. Removing the substrate from the root system and defoliating the shoot substantially improved the linearity of the relationship between flow and pressure. Hydraulic conductivity ($K_g$) of the graft region was calculated by multiplying $K_g$ by the total length of the graft region. Total hydraulic conductance ($K_T$) was calculated as the inverse of the sum of the component resistances. The relative contribution of graft resistance to total hydraulic resistance in the tree (%$R_{gr}$) was calculated as graft hydraulic resistance divided by total hydraulic resistance. Total leaf area of these trees (AL) was determined directly with an LI-3100C leaf area meter. Hydraulic conductance per unit leaf area ($K_T^{AL}$) was calculated by dividing $K_T$ by the corresponding AL value.

**Growth measurements**

Growth and hydraulic measurements were made on the same set of grafted trees. Root and leaf dry mass ($M_R$ and $M_L$, respectively) were measured directly, whereas stem dry mass ($M_S$) was estimated from stem volume and density. The stem was assumed to approximate a cylinder with radius equal to the cross-sectional radius at the base of the stem, and length equal to stem length above the grafting point. Total wood volume was estimated as half the volume of this cylinder, based on data from two grafted trees for which the diameter and length of all individual branches were measured. Wood density was measured on 2-cm stem sections from each tree (cf. Oliveras et al. 2003). Total grafted tree biomass ($M_T$) was calculated as the sum of the component dry masses measured after oven-drying at 70 °C for one week. Growth allocation to roots and shoots (R:S ratio) was calculated by dividing root mass by the sum of graft, stem and leaf masses. Scion relative growth rate (RGR) was calculated as the difference between the logarithm of current aboveground biomass and the logarithm of initial scion mass divided by five, the number of years since grafting. Growth efficiency ($E_{g,AL}$) was calculated by dividing aboveground biomass increment (stem and leaves) growth by mean total leaf area (AL) and the number of years (5) since grafting.

**Experimental design and data analysis**

The grafted trees ($n = 35$) were positioned in a $5 \times 7$ grid in a greenhouse, yielding a randomized complete block design. Five blocks, each comprising one row containing one tree from each scion donor tree ($n = 7$), were measured. Trees were sampled in the same sequence within each row and a new row was sampled only after completing measurements on all trees in the previous row. Day 1 was sunny and warm, whereas Day 2 was overcast and cool.

Data were analyzed by regression analysis with donor-tree age, graft conductivity ($K_g$), total leaf area (AL and $A_L$) and needle width as the independent variables. Analysis of variance tests for a randomized complete block design were conducted on $A_{net}$, $g_s$, $Ψ_{diff}$, leaf nitrogen concentration ($[N]_L$) and δ13C with parent tree age as the independent variable. Where ANOVA indicated significant differences between means ($α = 0.05$), differences among means were assessed with Tukey’s HSD test (JMP-IN, SAS Cary, NC). Because $E$ and $K_{S\cdotL}$ were measured both days on the same plants, we analyzed these variables using a repeated measures ANOVA for a randomized complete block design.

**Results**

**Physiological measurements**

At both the leaf (current and previous-year needles) and the whole-crown level, neither $A_{net}$, nor $g_s$, nor $Ψ_{diff}$ was related to scion parent-tree age (Table 1). Neither $E$, nor $K_{S\cdotL}$, nor $[N]_L$, nor δ13C showed a trend with scion donor-tree age (Table 1), and with few exceptions, mean responses per donor tree were similar. For example, mean $A_{net}$ of current-year needles ranged from 8.12 µmol m$^{-2}$ s$^{-1}$ for scions from 151-year-old trees to 13.95 µmol m$^{-2}$ s$^{-1}$ for scions from 85-year-old trees (Table 1). Mean $K_{S\cdotL}$ on Day 1 ranged from 2.55 to 4.56 mmol m$^{-2}$ s$^{-1}$ MPa$^{-1}$ for trees grafted from 269- and 201-year-old donor trees, respectively. Additionally, on Day 1, $E$ ranged from a mean of 2.68 to 3.70 mmol m$^{-2}$ s$^{-1}$ for trees grafted from 151- and 49-year-old donor trees, respectively (Table 1). In two cases, ANOVA indicated significant differences between means ($Ψ_{diff}$, Day 1; and $A_{net}$, Day 2), but when all means were compared simultaneously, there was only one significant difference ($Ψ_{diff}$, Day 1; Table 1). Furthermore, there were no significant trends with age in either case (linear regression; $Ψ_{diff}$: $P = 0.21$, $r^2 = 0.05$, $n = 35$; and $A_{net}$: $P = 0.51$, $r^2 = 0.01$, $n = 35$). Response patterns for Day 2 measurements resembled those for Day 1, but overall values were lower, due
Table 1. Mean (± SE) physiology parameters for grafted Scots pine trees by donor-tree age and day of measurement. Abbreviations: \( A_{\text{net}} \) = net photosynthesis (µmol m\(^{-2}\) s\(^{-1}\)); \( g_s \) = stomatal conductance (mmol m\(^{-2}\) s\(^{-1}\)); \( \Psi_{\text{DIFF}} \) = water potential difference between leaf and soil at midday (\( \Psi_{\text{PD}} - \Psi_{\text{MD}} \); MPa); \( E \) = whole-crown transpiration rate (mmol m\(^{-2}\) s\(^{-1}\)); \( K_SL \) = leaf specific hydraulic conductance measured gravimetrically (mmol m\(^{-2}\) s\(^{-1}\) MPa\(^{-1}\)); \( \delta^{13}\text{C} \) = leaf \( ^{13}\text{C} \) carbon isotope composition (%); \( [N]_L \) = leaf nitrogen concentration (%); \( P \) = \( P \) values for ANOVA test of differences between mean response per scion donor-tree age group; and letters indicate differences between means based on Tukey’s HSD (see text for explanation of different tests used). Net photosynthesis, \( g_s \), \( \Psi_{\text{PD}} \) and \( \Psi_{\text{MD}} \) were measured on current (Day 1) and non-current (Day 2) needles specifically. Transpiration rate and \( K_SL \) were measured on the whole crown on both days of measurements, whereas \( \delta^{13}\text{C} \) and \( [N]_L \) were measured on a sample of needles taken from the whole crown after measurements were completed.

<table>
<thead>
<tr>
<th>Age</th>
<th>n</th>
<th>Day</th>
<th>( A_{\text{net}} )</th>
<th>( g_s )</th>
<th>( \Psi_{\text{DIFF}} )</th>
<th>( E )</th>
<th>( K_SL )</th>
<th>( \delta^{13}\text{C} )</th>
<th>( [N]_L )</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>5</td>
<td>1</td>
<td>10.36 ± 1.49</td>
<td>84.43 ± 12.70</td>
<td>0.84 ± 0.04b</td>
<td>3.31 ± 0.67</td>
<td>3.92 ± 0.70</td>
<td>–29.93 ± 0.32</td>
<td>1.08 ± 0.04</td>
</tr>
<tr>
<td>49</td>
<td>5</td>
<td>1</td>
<td>12.15 ± 1.01</td>
<td>98.81 ± 9.22</td>
<td>0.98 ± 0.09ab</td>
<td>3.70 ± 0.47</td>
<td>3.94 ± 0.62</td>
<td>–29.67 ± 0.19</td>
<td>1.22 ± 0.06</td>
</tr>
<tr>
<td>85</td>
<td>5</td>
<td>1</td>
<td>13.95 ± 2.56</td>
<td>107.21 ± 22.27</td>
<td>1.13 ± 0.09a</td>
<td>3.65 ± 0.15</td>
<td>3.28 ± 0.26</td>
<td>–30.13 ± 0.31</td>
<td>1.13 ± 0.06</td>
</tr>
<tr>
<td>151</td>
<td>5</td>
<td>1</td>
<td>8.12 ± 1.22</td>
<td>65.16 ± 12.36</td>
<td>0.83 ± 0.05b</td>
<td>2.68 ± 0.25</td>
<td>3.35 ± 0.55</td>
<td>–29.57 ± 0.27</td>
<td>1.16 ± 0.05</td>
</tr>
<tr>
<td>201</td>
<td>5</td>
<td>1</td>
<td>10.59 ± 1.09</td>
<td>87.90 ± 10.26</td>
<td>0.86 ± 0.10b</td>
<td>3.82 ± 0.45</td>
<td>4.56 ± 0.42</td>
<td>–29.91 ± 0.07</td>
<td>1.07 ± 0.05</td>
</tr>
<tr>
<td>214</td>
<td>5</td>
<td>1</td>
<td>10.96 ± 2.75</td>
<td>84.76 ± 21.75</td>
<td>1.15 ± 0.04a</td>
<td>3.44 ± 0.29</td>
<td>3.02 ± 0.30</td>
<td>–29.99 ± 0.19</td>
<td>1.22 ± 0.06</td>
</tr>
<tr>
<td>269</td>
<td>5</td>
<td>1</td>
<td>8.22 ± 1.80</td>
<td>69.03 ± 14.59</td>
<td>1.11 ± 0.10a</td>
<td>2.76 ± 0.22</td>
<td>2.55 ± 0.23</td>
<td>–30.41 ± 0.28</td>
<td>1.07 ± 0.05</td>
</tr>
</tbody>
</table>

\(^1\) The same value is reported for Days 1 and 2 because Day was the repeated measure.

\(^2\) \( n = 33 \).

in part to lower solar irradiance on Day 2.

**Growth**

Neither total biomass, nor \( A_{\text{L}} \) nor R:S allocation was affected by scion donor-tree age (Table 2). Furthermore, SLA showed no change with increasing donor-tree age (Table 2). Consistent with the lack of donor-tree-age effects on \( M_T \) and \( A_{\text{L}} \), neither RGR nor \( E_{g,AL} \) was affected by scion donor-tree age (Table 2). Mean responses generally varied more for these variables than for the physiological variables. For example, \( M_T \) ranged from a mean of 0.04 to 0.10 kg per tree for scions from 201- and 151-year-old donor trees, respectively (Table 2), whereas \( A_{\text{L}} \) was 0.05–0.12 m\(^2\) per tree for scions from 201- and 269-year-old donor trees, respectively. However, \( E_{g,AL} \) was similar in most scions, with scions from the oldest and next to oldest donor trees recording the lowest (0.34 kg m\(^{-2}\) year\(^{-1}\)) and highest (0.52 kg m\(^{-2}\) year\(^{-1}\)) mean growth efficiencies, respectively (Table 2).

**Hydraulic properties**

Values of \( K_R \), \( K_{GR} \), \( K_T \) and \( K_{TAL} \) showed no relationship with scion donor-tree age (Table 3). Significant differences between means were noted for \( K_SL \), but no single pair was significantly different according to Tukey’s HSD test (Table 3), and

Table 2. Mean (± SE) growth parameters for grafted Scots pine seedlings by scion donor-tree age. Abbreviations: \( M_T \) = total mass (kg); \( AL \) = total leaf area (m\(^2\)); R:S = root to shoot ratio (kg kg\(^{-1}\)); \( SLA \) = specific leaf area (m\(^2\) kg\(^{-1}\)); RGR = relative growth rate (kg kg\(^{-1}\) year\(^{-1}\)); \( E_{g,AL} \) = growth efficiency (kg shoot mass m\(^{-2}\) total leaf area year\(^{-1}\)); and \( P \) = \( P \) value for ANOVA test of differences between mean responses per scion donor-tree age group (sample size is given in parentheses).

<table>
<thead>
<tr>
<th>AGE</th>
<th>n</th>
<th>( M_T )</th>
<th>( AL )</th>
<th>R:S</th>
<th>( SLA )</th>
<th>RGR</th>
<th>( E_{g,AL} )</th>
</tr>
</thead>
<tbody>
<tr>
<td>36</td>
<td>3</td>
<td>0.08 ± 0.01</td>
<td>0.09 ± 0.01</td>
<td>0.52 ± 0.06</td>
<td>3.16 ± 0.12</td>
<td>0.78 ± 0.02</td>
<td>0.41 ± 0.02</td>
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<tr>
<td>49</td>
<td>3</td>
<td>0.06 ± 0.01</td>
<td>0.06 ± 0.01</td>
<td>0.56 ± 0.09</td>
<td>2.83 ± 0.15</td>
<td>0.74 ± 0.06</td>
<td>0.47 ± 0.09</td>
</tr>
<tr>
<td>85</td>
<td>3</td>
<td>0.06 ± 0.02</td>
<td>0.06 ± 0.02</td>
<td>0.49 ± 0.05</td>
<td>2.68 ± 0.04</td>
<td>0.71 ± 0.07</td>
<td>0.43 ± 0.05</td>
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<tr>
<td>151</td>
<td>4</td>
<td>0.10 ± 0.01</td>
<td>0.11 ± 0.01</td>
<td>0.42 ± 0.01</td>
<td>2.97 ± 0.07</td>
<td>0.86 ± 0.01</td>
<td>0.49 ± 0.01</td>
</tr>
<tr>
<td>201</td>
<td>3</td>
<td>0.04 ± 0.01</td>
<td>0.05 ± 0.01</td>
<td>0.40 ± 0.08</td>
<td>3.01 ± 0.30</td>
<td>0.67 ± 0.03</td>
<td>0.40 ± 0.03</td>
</tr>
<tr>
<td>214</td>
<td>4</td>
<td>0.08 ± 0.01</td>
<td>0.08 ± 0.01</td>
<td>0.43 ± 0.06</td>
<td>2.95 ± 0.07</td>
<td>0.81 ± 0.04</td>
<td>0.52 ± 0.02</td>
</tr>
<tr>
<td>269</td>
<td>3</td>
<td>0.09 ± 0.03</td>
<td>0.12 ± 0.05</td>
<td>0.63 ± 0.15</td>
<td>3.23 ± 0.19</td>
<td>0.78 ± 0.11</td>
<td>0.34 ± 0.01</td>
</tr>
</tbody>
</table>

\(| P \) = \( P \) value for ANOVA test of differences between mean responses per scion donor-tree age group (sample size is given in parentheses).
Table 3. Mean (± SE) hydraulic conductance values (kg s⁻¹ MPa⁻¹ × 10³) for grafted Scots pine trees measured with a high pressure flow meter. Values are reported by measured section and scion-donor-tree age. Abbreviations: $K_R$ = hydraulic conductance of root section plus 4 cm of stem; $K_S$ = hydraulic conductance of stem section above grafted region; $K_{GR}$ = hydraulic conductance of grafted section of stem only; $K_T$ = total hydraulic conductance of roots, stem and grafted region combined; $K_{T/AL}$ = leaf specific conductance based on $L$ (kg s⁻¹ MPa⁻¹ m⁻² × 10³); $\%R_{GR}$ = percent contribution of graft hydraulic resistance to total hydraulic resistance; and $P = P$ value for ANOVA test of differences between means of donor-tree age groups (sample size is given in parentheses).

<table>
<thead>
<tr>
<th>Age</th>
<th>n</th>
<th>$K_R$</th>
<th>$K_S$</th>
<th>$K_{GR}$</th>
<th>$K_T$</th>
<th>$K_{T/AL}$</th>
<th>$%R_{GR}$</th>
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</thead>
<tbody>
<tr>
<td>36</td>
<td>3</td>
<td>0.23 ± 0.05</td>
<td>0.06 ± 0.01</td>
<td>0.57 ± 0.08</td>
<td>0.04 ± 0.004</td>
<td>0.50 ± 0.04</td>
<td>7.8 ± 1.1</td>
</tr>
<tr>
<td>49</td>
<td>3</td>
<td>0.11 ± 0.01</td>
<td>0.04 ± 0.01</td>
<td>0.51 ± 0.09</td>
<td>0.02 ± 0.01</td>
<td>0.38 ± 0.08</td>
<td>4.5 ± 1.1</td>
</tr>
<tr>
<td>85</td>
<td>3</td>
<td>0.13 ± 0.06</td>
<td>0.04 ± 0.01</td>
<td>0.22 ± 0.05</td>
<td>0.03 ± 0.01</td>
<td>0.50 ± 0.07</td>
<td>10.9 ± 2.3</td>
</tr>
<tr>
<td>151</td>
<td>4</td>
<td>0.27 ± 0.06</td>
<td>0.11 ± 0.02</td>
<td>0.91 ± 0.15</td>
<td>0.07 ± 0.01</td>
<td>0.65 ± 0.13</td>
<td>8.2 ± 2.1</td>
</tr>
<tr>
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<td>0.10 ± 0.05</td>
<td>0.03 ± 0.00</td>
<td>0.24 ± 0.07</td>
<td>0.02 ± 0.003</td>
<td>0.40 ± 0.05</td>
<td>8.9 ± 1.4</td>
</tr>
<tr>
<td>214</td>
<td>4</td>
<td>0.15 ± 0.05</td>
<td>0.05 ± 0.01</td>
<td>0.70 ± 0.15</td>
<td>0.03 ± 0.01</td>
<td>0.47 ± 0.02</td>
<td>5.8 ± 0.5</td>
</tr>
<tr>
<td>269</td>
<td>3</td>
<td>0.28 ± 0.09</td>
<td>0.09 ± 0.03</td>
<td>0.78 ± 0.40</td>
<td>0.06 ± 0.02</td>
<td>0.56 ± 0.09</td>
<td>9.4 ± 1.4</td>
</tr>
</tbody>
</table>

$P^1 = n = 3.$  
$P^2 = n = 2.$

there were no significant trends with age (linear regression; $P = 0.29, n = 23$). In general, grafted trees with scions from 201-year-old donor trees had the lowest conductances, whereas grafted trees with scions from 151-year-old donor trees had the highest (Table 3). The relative contribution of the grafted region to hydraulic conductance ranged from a mean (± SE) of 10.9% ± 2.3% for plants with scions from 85-year-old donor trees to 4.5% ± 1.1% for plants with scions from 49-year-old donor trees (Table 3), indicating that the graft union did not limit stem hydraulic conductance. In addition, $K_{GR}$ was significantly greater than shoot or root conductivity (ANOVA: $P < 0.01, n = 68$; data not shown). The results for $K_{T/AL}$ were qualitatively consistent with our estimate of $K_{S-L}$ obtained gravimetrically. However, when both measurements were converted to common units, $K_{T/AL}$ was consistently higher. This can be explained on the basis of lower leaf areas and higher $K_T$ for the HPFM measured grafted trees. The higher $K_T$ is the result of removing the leaves and the loss of fine roots. In contrast, $K_{S-L}$ was measured in vivo with leaves and fine roots attached. Since leaf area measurements of the HPFM measured trees were made in winter, it is likely that AL was reduced by needle loss during the fall.

**Variability within scions**

Although there was little difference in response between scions of different ages, there was variation in response between scions within donor-tree age groups (Tables 1–3). For example, standard errors of the means of the physiological measurements ranged from about 4 to 30% among scions from a donor-tree age group. With only two exceptions, we found no relationship between scion-donor-tree age and variance of the mean (data not shown). There was a significant effect of age on AL and $M_R$ variance, but only in the case of AL variance was there a consistent trend with age (data not shown). However, given that mean AL did not vary significantly among trees (Table 2), we conclude that there was no consistent effect of scion donor-tree age on AL.

There was no evidence that measurement variability was a result of pot size effects on growth and physiology. Root-to-shoot ratio, $[N]_L$ and δ¹³C did not vary with leaf area (AL or $A_L$) despite a fivefold difference between grafts with the smallest and largest crowns (data not shown). This suggests that the plants were not root-bound. Further investigation, however, showed that AL was related to $K_{gr}$ ($P < 0.01, r^2 = 0.78, n = 22$; Figure 1). Consequently, trees with lower $K_{gr}$ had less leaf area (Figure 1).

Hydraulic conductance of the grafted trees increased with AL such that hydraulic conductance per unit leaf area ($K_{T/AL}$) remained the same regardless of AL (Figure 2A). However, leaf specific conductance ($K_{S-L}$) and, particularly, $E$ decreased with increasing AL (Figure 2B and Figure 3, respectively). In addition, $A_{net}$ and $g_{st}$ of current needles (measured on Day 1) also decreased with $A_L$ (data not shown), but showed an even stronger negative relationship with current needle width (Fig-

![Figure 1](https://heronpublishing.com)  
*Figure 1. The relationship between graft region conductivity ($K_{gr}$) and tree leaf area (AL). Trees with greater graft conductivity have more leaf area: $y = 0.03 + 5.23x$, $r^2 = 0.78$, $n = 22$, $P < 0.01$.***
Figure 4), which was positively related to \( A_L \) (Figure 5). We saw no relationship between \( A_{\text{net}} \) or \( g_s \) and previous-year needle width on Day 2 (Figure 4).

Discussion

Although several studies have provided strong evidence that trees above a certain height have reduced growth as a result of reduced gas exchange (Hubbard et al. 2002, McDowell et al. 2002a, Koch et al. 2004a), the evidence is inconclusive because of covariance in height and age. To test the separate effects of height and age on gas exchange and growth, we used grafted trees of similar sizes, but with scions from donor trees of different ages, in the assumption that the scions would retain the physiological properties of the donor trees (Day et al. 2001).

Our results show that there was no relationship between scion donor-tree age and any of the measured physiological or growth variables. For example, \( A_{\text{net}} \) was the same or higher in grafted trees with scions from 269-year-old donor trees as in grafted trees with scions from 39-year-old donor trees (Table 1). Furthermore, photosynthetic capacity indicated by [N]L and stomatal limitation to assimilation as indicated by \( \delta^{13}C \) were unaffected by scion age (Table 1). Whole-tree biomass, RGR and \( E_{g,AL} \) were similar regardless of scion donor tree age (Table 1). In a related field study, which included the scion donor trees, we found an effect of size on \( E_{g,AL} \) and \( K_{S-L} \) (Martínez-Vilalta et al. 2006). Thus, our results support the hypothesis that tree height or size, but not age, affects growth, hydraulic conductance and gas exchange (Hubbard et al. 2001, 2002, Koch et al. 2004, Mencuccini et al. 2005).

In a study of grafted red spruce (\( Picea rubens \) Sarg.) trees (Day et al. 2001), scion donor-tree age appeared to affect leaf-level physiology. In younger trees, there is evidence of age-related control on both leaf- and stem-level properties, which are epigenetically controlled and not readily reversible, unless by repeated sequential grafting (Greenwood 1984, 1995, Hutchison et al. 1990). Our results, however, suggest that, for grafted Scots pine trees, scion donor-tree age does not affect leaf- and plant-level physiology or growth. We, too, found some significant differences among grafted trees according to scion donor-tree age, but we found no consistent trend of increasing or decreasing growth, hydraulic conductance or physiology with increasing scion donor-tree age (Tables 1–3). The discrepancies between studies may reflect the difference in species and the greater age of scions in our study, but parallel studies with grafted sycamore (\( Acer pseudoplatanus \) L.) and ash (\( Fraxinus excelsior \) L.) trees, and cuttings of a poplar clone (\( Populus deltoides \times balsamifera \) sp. \( tri-chocarpa \)) show results consistent with our findings for Scots.
pine (Mencuccini et al. 2005).

Within scion donor-age cohorts, we found a great variation in growth rate and gas exchange, which was age independent (Table 1). This was most likely the result of variability in grafting success, although pot size may have been a contributing factor. Day et al. (2001, 2002) found that grafting success with *Picea rubens* was related to how closely scion diameters matched rootstock diameters, but that specific hydraulic con-

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**Figure 4.** The effect of needle width on net photosynthesis ($A_{\text{net}}$) (A, B) and stomatal conductance ($g_s$) (C, D). Figures A and C are for current-year needles and Figures B and D are for previous-year needles. Note that the $x$-axes have been truncated. (A) $y = 24.9 - 12.2x$, $r^2 = 0.34$, $n = 35$, $P < 0.01$; (B) $y = 7.8 + 1.7x$, $r^2 = 0.01$, $n = 35$, $P = 0.65$; (C) $y = 196.8 - 95.2x$, $r^2 = 0.31$, $n = 35$, $P < 0.01$; and (D) $y = 68.6 + 15.5x$, $r^2 = 0.01$, $n = 35$, $P = 0.68$.

**Figure 5.** The relationship between (A) current needle width and total leaf area ($A_L$); (B) non-current needle width and $A_L$; (C) current needle width and total current needle area; and (D) non-current needle width and total non-current needle area. (A) $y = 0.004 + 0.117x$, $r^2 = 0.22$, $n = 35$, $P < 0.01$; (B) $y = 0.019 + 0.120x$, $r^2 = 0.13$, $n = 35$, $P = 0.04$; (C) $y = -0.032 + 0.091x$, $r^2 = 0.30$, $n = 35$, $P < 0.01$; and (D) $y = -0.046 + 0.096x$, $r^2 = 0.31$, $n = 35$, $P < 0.01$. 

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ductivity was unrelated to scion donor-tree age. It is possible that, initially, grafting success may have been an important factor in the growth of our grafted trees. We found a significant positive relationship between $K_{GR}$ and AL (Figure 1). This suggests that grafting success initially limited crown growth. However, at the time of our study, $K_{GR}$ was considerably higher than $K_{s}$ or $K_{g}$ (Table 3) and graft conductivity ($K_{GR}$) was significantly greater than shoot or root conductivity (data not shown). In other words, resistance to flow through the graft region was considerably lower than in the roots or stem and therefore could not have been a limiting factor at the time of our study (see %$K_{GR}$, Table 3). This is consistent with findings reported by Atkinson et al. (2003) and Clearwater et al. (2004).

Furthermore, our grafted trees had at least four years of xylem growth post-grafting. We have evidence (Mencuccini et al., unpublished results) that, in two years post-grafting, there is no effect to be found of grafting on growth, which makes it even less likely that there was an effect of grafting at the time of our study.

We found no evidence that pot size limited grafted tree growth in our study. Root-to-shoot ratio, [%N], and $^{18}$C did not vary with tree leaf area (data not shown). A third and final possibility is that differences in scion growth are the result of differences in the vigor of the seedling rootstocks used. Atkinson et al. (2003) and Clearwater et al. (2004) found that scion performance was lower on low vigor rootstocks. However, we were unable to ascertain if that was a factor in our grafted trees.

Grafted-tree size appears to have affected growth and physiology in our study, but not in a manner expected. Crown-based measurements ($A_{net}$, $g_{s}$, $E$ and $K_{s,T}$) all decreased with increasing $A_{t}$ (Figures 2B, 3 and 4). A similar response to increased leaf area has been noted for a variety of plant species and types (Meinzer and Grantz 1990, Ren and Sucoff 1995, Mencuccini and Comstock 1999). All of these studies concluded that $g_{s}$, $E$ and $K_{s,L}$ decreased, thereby maintaining constant leaf water potentials across different leaf areas. We, too, found no change in $W_{SM}$ in response to leaf area (Day 1: $r^2 = 0.09$, $n = 35$, $P = 0.08$; Day 2: $r^2 = 0.05$, $n = 35$, $P = 0.21$; data not shown) and might conclude that the grafted trees altered their hydraulic properties in a way that avoided excessive water stress with larger crowns. However, stem-based measurements showed that grafted trees with larger crowns, and therefore greater AL, had higher $K_{T}$ such that $K_{T,S}$ was unchanged with leaf area (Figure 2A). We should, therefore, have expected $g_{s}$ (and $A_{net}$), $E$ and $K_{s,L}$ to remain unchanged with increasing AL, but they did not. This suggests that trees with greater leaf area had reduced gas exchange as a result of some leaf- or crown-level factor.

Whereas $E$ and $K_{s,L}$ were closely related to $A_{t}$ (Figures 2B and 3), $A_{net}$ and $g_{s}$ were more closely related to needle width (Figure 4), which suggests that larger or wider needles had lower stomatal densities. However, we find little support for this in the literature. Kouwenberg et al. (2004) found no relationship between needle size and stomatal density in Tsuga heterophylla (Rafn.) Sarg., and Pensu et al. (2004) suggest that, for Pinus sylvestris, stomatal density may increase, not decrease, with needle size. Additionally, a small, but significant, increase in stomatal density with increasing needle size was noted in Scots pines of different ages in a related study (E. Korakaki, University of Edinburgh, personal communication). One possibility is that wider needles have greater mesophyll resistance. In a model proposed by Zwieniecki et al. (2004), wider needles would have lower surface resistance, but higher mesophyll resistance to vapor flow of water. According to their model, the lower surface resistance would be insufficient to offset the higher mesophyll resistance and, thus, wider needles would have lower rates of $E$ and $g_{s}$, and, consequently, $K_{s,L}$ and $A_{net}$, respectively. This explanation is consistent with our findings for current-year needles, but not for previous-year needles. However, previous-year needle width was less well related to $A_{t}$ ($r^2 = 0.13$, $n = 35$, $P = 0.04$), than current-year needle width ($r^2 = 0.22$, $n = 35$, $P < 0.01$; Figure 5). Moreover, previous-year needles had lower gas-exchange rates than current-year needles (Figure 4; Table 1). Furthermore, non-current needles were near the end of their lifespan when gas exchange measurements were made. This indicates that $E$ and $K_{s,L}$ response patterns mainly reflected current-needle responses in our experimental plants. Thus, although tree height or size over the longer term may determine hydraulic properties of Scots pine trees (Mencuccini 2003, Mencuccini et al. 2005), in seedlings, leaf-level adjustments may regulate water use and gas exchange.

In conclusion, we found no evidence for scion donor-tree age effects on growth or physiology of grafted Scots pine trees. We found, instead, large variation in gas exchange across grafted trees independent of scion donor-tree age. Our results suggest that this variability was linked to differences in total leaf area or needle width.

Acknowledgments

NERC (U.K.) competitive grant NER/A/S/2001/01193 to Maurizio Mencuccini supported Jordi Martínez-Vilalta and made this study possible. Dirk Vanderklein was supported by funds from University of Edinburgh and Montclair State University while on sabbatical. This study would not have been possible without the support and supply of trees from the Tree Improvement Branch of the Northern Research Station (NRS, Bush Campus, Roslin, Scotland, U.K.). We also thank Johanna Pulli and Nick Weir for their help in the field and lab.

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