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DO GYMNOSPERM NEEDLES PULL WATER THROUGH THE XYLEM PRODUCED IN THE SAME YEAR AS THE NEEDLE?[1]

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This research investigated the longevity of functional connections between leaf traces and stem xylem in 16 species of conifers to better understand the spatial use of sapwood for water transport. The first question was which ring(s) stained when a vacuum was applied to the distal end of the cut surface of a needle attached to a short stem segment. The vacuum was applied to either 1- or 2-yr-old foliage taken from 4–6-yr-old saplings. The 16 species were then categorized based on the growth ring that most consistently stained when the vacuum was applied to the 2-yr-old needles. There were three distinct stain patterns for the evergreen conifers and one pattern for the deciduous conifers. Three evergreen species could not be categorized. The second question used needle fall data to ask whether the leaf trace appeared to break because of age or stem diameter in 5-yr-old *Pseudotsuga menziesii* saplings. An apparent threshold stem diameter at which needles tended to be shed was more related to diameter than age. These xylem connection patterns could affect leaf cohort physiology and the spatial pattern of water flux in sapwood.

Key words: dye ascent; foliage retention; leaf age; leaf trace; water transport.

We know that foliage can remain alive for many years in evergreen species, but we have little knowledge of how the leaves maintain a connection with the xylem and phloem as the stem expands. This information is important for understanding the processes that occur at different depths within the sapwood and the phloem. For example, the radial patterns of sap flow and phloem activity should depend on how long the original xylem and phloem connections of the leaf trace remain active. Additionally, these patterns may give new insights into understanding the age-dependent changes in leaf physiology.

Limited research suggests that in conifers, the xylem and phloem of the leaf trace break as the stem increases in diameter (Tison, 1902, 1903; Elliot, 1937). Each year, a cambium at the base of the leaf trace produces new cells that connect the leaf trace to the current year's xylem and phloem. Tison (1903) reported in detail on the anatomical changes in cells that occur in *Picea excelsa* as the leaf trace is stretched: new tracheids are produced, and the old, stretched tracheids become nonfunctional. As the leaf trace stretches, new tracheids are made below the stretched region and become functional before the original tracheids have broken. Later in the same year, new tracheids are intercalated in the region where the leaf trace thinned as it stretched out. The leaf trace maintains the same diameter from year to year because the stretched and dead tracheids are not perpetuated in the new tissue, and their area is compensated by production of the new tracheids at the base (Elliott, 1937). The proximal ends of these new tracheids are attached to the current year's growth ring, and the distal ends are within the leaf trace at the abaxial surface. An interesting exception occurs in the coniferous genus *Araucaria*, in which the cambia of the leaf traces continue to produce tissues for years after the leaves have fallen (Markfeldt, 1885; Tison, 1903).

In evergreen angiosperms, there is no evidence that either the leaf trace xylem or the leaf trace phloem of the second and subsequent years is connected to the stem xylem or phloem (Elliott, 1937). This feature was investigated in 11 species (*Ficus repens* Hort, *Griselinia littoralis* Raoul, *Hedera helix* L., *Hypericum androsaemum* L., *Ilex aquifolium* L., *Myrtus communis* D.C., *Olearia haastii* Hook. f., *Pieris floribunda* Bth., *Pyracantha coccinea* Roem., *Veronica andersonii* Ldl., and *Viburnum tinus* L.). In contrast to the evergreen conifers, in deciduous angiosperms (Tison, 1902), the leaf trace can stretch very little before its xylem cells break. The leaf falls, and the cambium at the base of the leaf trace becomes inactive soon afterwards. The gap in the leaf-trace xylem is quickly filled with a small parenchymatous scar, and the vascular cambium re-establishes and produces continuous axial phloem and xylem exterior to the leaf trace scar.

The trigger is unknown for the development of a new connection with one ring and the subsequent breakage of the needle trace with the older ring. In *Picea excelsa*, the only species for which we found data on longevity of the connection, the original connection remains for only one year (Tison, 1902). Elliott (1937) states that a new connection is made annually for all species that he examined (*Araucaria imbricata* Pav., *Cedrus libani* Barrelier., *Cryptomeria japonica* Don., *Picea excelsa* Link., *Pinus sylvestris* L., *Taxus baccata* L., and *Taxus baccata* var. *fastigiata* Loudon.), but he does not discuss how long the older connections remain active. Studies with stain may reveal functional connections that anatomical studies alone cannot show.

The trigger for the needle trace to break with one ring and to connect with another growth ring is also unknown. One trigger could be needle age, with a scheduled breakage from the previous year's ring (Fig. 1, f) and new connection with a

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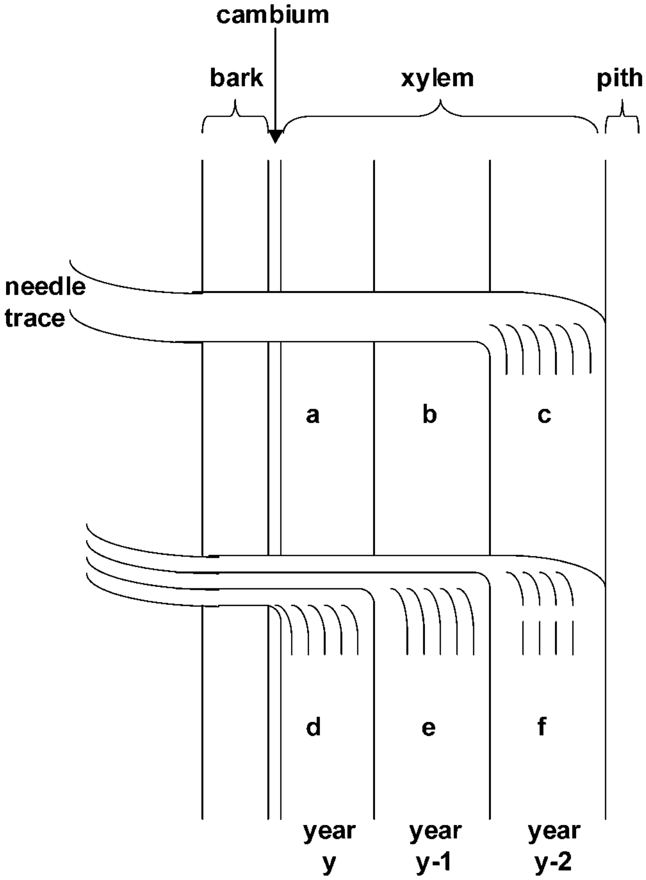

Fig. 1. Representation of the junctions of y-2 needle traces with the stem xylem. The upper needle is connected with the y-2 growth ring (zone c): the needle trace is unbroken through zones a and b. The original connection below is broken in zone f, but new connections have been formed with the y-2 zone e) and y-3 (zone d) growth rings.

different annual ring (Fig. 1, d and e). Another trigger could be either the rate or magnitude of stem enlargement, in which the radial growth exceeds the ability of the leaf trace to either stretch or produce intercalary growth with sufficient speed. Stems that enlarge the fastest or to the greatest extent (possibly, beyond a threshold rate or distance) would have shorter-lived leaf connections. Similarly, needle insertion angle and leaf connection longevity may be related: Tison (1903) noted that species with needles that insert at small angles to the stem's axis will be subjected to more stretching force on the leaf trace with radial growth than will species with needles perpendicular to the stem.

We asked two questions in the current research. (1) Using stain and vacuum experiments on excised branches, through which growth ring does water flow to reach the 1-yr-old and the 2-yr-old needles? We focused on evergreen conifers because of their leaf longevity and their availability at the nursery, but included two deciduous conifers for comparison. We did not study current-year needles because their water can only flow through current-year wood. (2) In *Pseudotsuga menziesii* (Mirb.) Franco saplings, is needle fall related to needle age or diameter of the stem to which the needles were attached? This question is related to the first question in that it seeks the cause for the apparent xylem disconnection that occurs at needle fall. We looked for evidence to answer whether needles were dropped after a programmed time period or after a threshold of radial growth had occurred.

MATERIALS AND METHODS

Plant material—Coniferous species that were available to us, either at a local wholesale nursery (thirteen species), at a research site (one species), in raised beds near our laboratory (one species), or on campus (one species) were used. All trees were vigorous and young, except for the *Larix occidentalis* Nutt. trees (which were older; see later), and all evergreen trees had at least three years of needles. Fourteen species were evergreen, and two were deciduous: *Gingko biloba* 'Autumn Gold' or 'Magyar', and *Larix occidentalis*.

We sampled the following species from the Blue Heron Nursery, near Corvallis, Oregon, USA (44°34′ N, 123°17′ W, elevation 90 m): *Abies concolor* (Gord. & Glend.) Hildbr., *Calocedrus decurrens* Torr., *Cedrus deodora* D. Don, *Chamaecyparis nootkatensis* 'Pendula', *Ginkgo biloba*, *Juniperus virginiana* 'Skyrocket', *Picea abies* 'Pendula', *Picea pungens* 'Blue Select', *Pinus contorta* Dougl. ex. Loud., *Pinus flexilis* 'Vanderwolf', *Pinus nigra* Arnold, *Sequoia sempervirens* 'Soquel', and *Tsuga heterophylla* (Raf.) Sarg. This nursery was used because trees were plentiful enough that we could sample a branch from many different individuals and because all individuals within a species had been grown in similar conditions. The trees were generally 4–5-yr old, and 2–3 m tall. They were growing directly in the ground and were irrigated periodically throughout the summer and fertilized in early spring. They were also sprayed with three different herbicides each year.

We sampled *Pinus ponderosa* P. and C. Lawson from Crown Pacific land (43°32′ N, 121°41′ W, elevation 1355 m) near Crescent, Oregon, on the east side of the Cascades 170 km east of Corvallis. We used these *P. ponderosa* trees because they had more leaf age classes than did the *P. ponderosa* available to us at the nursery in Corvallis. Trees were self-sown and grew in a mixed-age-class stand. Individuals sampled were about 2–3-m tall and 5–6 yr old.

We sampled 5-yr-old *Pseudotsuga menziesii* saplings from raised beds near the laboratory in Corvallis. They had been irrigated in the summer for the first two years, but not thereafter, except for the month before this research was undertaken. They were never fertilized. The seed source was from the Hebo district of the Siuslaw National Forest.

Larix occidentalis samples came from three trees growing in Corvallis within 3 km of the laboratory. They came from lower branches of large trees, which were part of the landscaping on campus.

Staining experiments—*Experimental techniques*—These experiments were designed to learn the patterns of leaf/stem xylem connections and the consistency of the patterns among and within species. Most sampling was done between 10 July and 4 September 2003. Our goal was to pull stain through a conifer needle from each of two age classes (produced in 2001 or 2002) and quantify through which xylem growth ring (produced in 2001, 2002, or 2003) the stain passed. For clarity, we refer to the needles and wood produced in 2003, 2002, and 2001 as produced in year y, y-1, and y-2, respectively. We sampled one or two branches from each of five trees from the nursery, the *Pinus ponderosa* site, and the raised beds; and two to three branches from each of three *Larix occidentalis* trees. Most cut branches were second-order branches, although some were third order (where first order is defined as the leader), and all branches had at least two-year-old foliage (meaning three years' of needles: current year, one year old, and two year old) unless they came from deciduous species. Branches were cut early in the day, and their bases were placed immediately into water. They remained in water until they were used, a maximum of 4 days after harvest. Four samples were then made from each branch: two for the one-year-old needles and two for the two-year-old needles.

Immediately before testing a needle, we cut a branch under water with clippers or a razor blade to produce a stem segment about 2 cm long. The cuts were then resurfaced with a new razor blade. The segment was free of branches and nodes and carried only one year of needles. (Note that we use the term 'node' according to a common forestry definition, to describe the

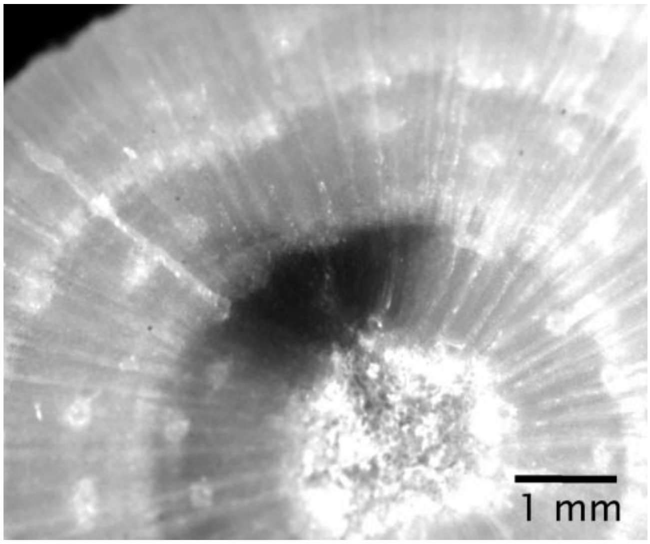

Fig. 2. *Pinus ponderosa* stem section with staining in the y-2 growth ring, after a vacuum was applied to 2-yr-old foliage for 5 min.

location on the stem between adjacent years' longitudinal growth.) Next, all needles were removed except for the needle about 1 cm from the branch sample's base. The tip of the retained needle was then removed under water with a razor blade to create a location through which to apply tension. For *Gingko biloba*, the leaf lamina was removed, leaving only the petiole.

In preliminary experiments, we had tried using different sample lengths, wrapping the stem surface in a paraffin sheet after removing all but one needle, or leaving all the foliage in place, but these preparations did not work as well. With longer samples and longer sampling times, the dye spread out more and it was harder to locate the reaction. In these cases it was unclear whether the dye locations represented mass transport (the focus of the study) or simply dye diffusion. Results were more consistent with branches of small diameter, so we confined sample diameters to 1–15 mm under the bark, depending on the species.

The base of each stem sample was placed in a miniature beaker, which was filled with 1% acid fuchsin in distilled water, then passed through a 0.22-μm filter. The beaker was a segment of latex tubing (10-mm inner-diameter) that was closed at the bottom with paraffin film. The cut needle was attached to a vacuum (67 kPa) for 5.0 min. To attach the vacuum, we used a clear latex tube, slashed it radially through about one third of the circumference, then flexed the tube to open the slash. The needle stub (with its tip removed) was pushed into the slash. When the tube was straightened, the open tip of the needle was inside the tube. We adjusted the curvature of the tube to avoid crushing the needle and to avoid air leakage. The downstream end of the tube was folded and clamped shut. The upstream end of the tube was attached to the building's vacuum system.

As a control for the tension, two samples of each species were also prepared with y-2 or y-1 needles in the same manner, but were not subjected to the vacuum.

Localization of the stain—Each sample was assessed immediately after the end of the 5-minute vacuum application. We removed the basal portion of the stem with a razor blade, shaving it carefully. At the very base, the entire cross-section was stained, but usually within 3 mm we reached an area in which only distinct spots of stain remained (Fig. 2). We then recorded the growth ring number (outward from the pith) in which each spot of stain was observed.

Data analysis—Results were somewhat variable within a species. Because we thought the nature of the variation was important, we give a tabulation showing all observations, rather than a mean with an estimate of variance. For each species, we showed the following. For vacuum applied through the y-2 needles, we tabulated the presence or absence of stain in the y-2, y-1, and/or y growth rings. For vacuum applied through the y-1 needles, we tabulated the presence or absence of stain in the y-1 and/or y growth rings. We then grouped the species based on which growth ring most consistently was stained when the vacuum was applied through the y-2 needles.

Leaf-fall experiments—*Retention of y-2 needles on three orders of stems*—These experiments were designed to help distinguish among two causes for needles drop: loss of the original connection and then drop at a pre-programmed time, or loss of connection and then drop when the stem reaches a threshold size beyond which the leaf trace cannot remain functional. Our first goal was to sample the same aged needles (produced in 2001, year y-2) from three branching orders (first order, second order, or third order) while tracking stem diameter, to learn whether there was an effect of branch order or stem diameter on the growth ring through which stain passed.

We chose the stem segment for the sample, then counted the number of attached needles and the number of missing needles (as indicated by scars) on the sample. The bottom of the sample was delimited 5 cm above the node because we observed that needles are often missing at the very bottom of the internode. The sample was 4 cm long so that it contained a relatively high number of needles or needle scars, which was essential for calculating percentages.

Three samples were made per tree: one on the first-order stem holding y-2 needles, one on a second-order stem holding y-2 needles, and one on a third-order stem holding y-2 needles. The samples were then excised from the tree with clippers. The under-bark and over-bark diameters at the bottom of each sample were measured with calipers, and recorded.

Retention of three years of needles on first-order stems—Our second goal was to sample first-order stems only (the leader) while tracking needle class age (produced in years y-1, y-2, and y-3; 2002, 2001, 2000, respectively) and stem diameter to learn whether there was an effect of stem diameter on the growth ring through which stain passed. The harvest and sample preparation were the same as described for the previous experiment. Three samples were made from the first-order stem of each tree: one on stem holding y-1 needles, one on stem holding y-2 needles (the same sample used in the previous experiment), and one on stem holding y-3 needles.

Data analysis—Retention of y-2 needles and retention of needles on the leader were both analyzed as a function of stem diameter. Stem diameter was taken separately as either diameter under the bark or over the bark. Because we had no a priori model, we fitted the data with linear and nonlinear models using SAS software (SAS 8.2, SAS Institute, Cary, North Carolina, U.S.A.).

RESULTS

Staining experiments—When stain was pulled through the y-2 needles of *Pseudotsuga menziesii*, six of the 10 samples had only their y-2 growth ring stained, two samples had stain in both the y-2 and y-1 growth rings, and two samples had stain in the y-2, y-1, and y growth rings (Fig. 3). We concluded that the y-2 needles of *Pseudotsuga menziesii* pulled mostly through the y-2 growth ring. This type of conclusion was drawn for each species.

When samples were not subjected to tension and used as controls, stain was visible at their bases, but upon cutting, we never encountered any spots of stain remaining within a growth ring. These spots indicated the water pathways only when a vacuum was applied.

When a vacuum was applied through the y-2 needles, stain moved most frequently through the y-2 growth ring for four of the species (Fig. 3). Likewise, when a vacuum was applied through the y-2 needles, stain moved most frequently through the y-1 growth ring for four species (Fig. 4) and most frequently through the y growth ring for three species (Fig. 5).

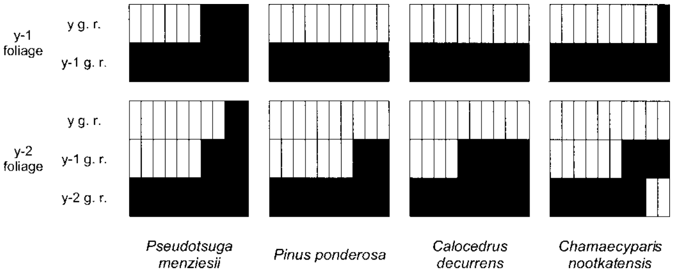

Fig. 3. Xylem staining pattern for species for which stain moved most frequently through the y-2 growth ring (g. r.), when a vacuum was applied through the y-2 needles. Each column ($N = 10$ for each needle class age and species) represents the results of one sample. A cell in the graph is filled (black) if that growth ring had stain in it and is open (white) if that growth ring had no stain in it. Stain was pulled through either a needle formed in year y-2 (bottom panel) or y-1 (top panel), and its presence was assessed in the y-2, y-1, and y growth rings (bottom panel) or y-1 and y growth rings (top panel).

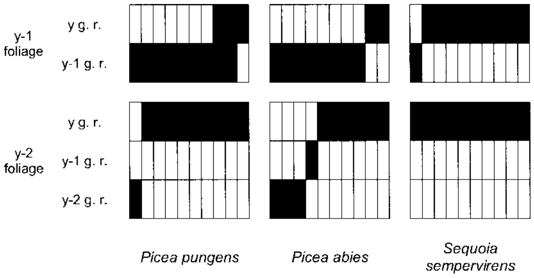

Fig. 5. Xylem staining pattern for species for which stain moved most frequently through the y growth ring (g. r.), when a vacuum was applied through the y-2 needles. Graph is as described in Fig. 3.

The staining patterns for another three species were so variable that we could not classify them in this manner (Fig. 6). In the deciduous species, almost all the staining occurred in the current-year xylem when the vacuum was applied through the current-year foliage (Fig. 7).

The first group is species for which the needles pulled mostly through the xylem that was made in the same year as the needles (Fig. 3). This observation was true both for the y-2 needles and xylem and the y-1 needles and xylem. The four species in this group had similar patterns of staining, with the main exception that stain occurred in the y growth ring in a few samples of *Pseudotsuga menziesii*, one sample of *Chamaecyparis nootkatensis*, and none of the samples of *Pinus ponderosa* or *Calocedrus decurrens*.

The second group is species for which the y-2 needles pulled most frequently through the y-1 xylem (Fig. 4). This group was quite diverse in its staining patterns. *Pinus contorta*, *Abies concolor*, and *Cedrus deodora* stained in all three rings, depending on the sample. *Pinus nigra* stained only in the outer two growth rings. This group was also variable in which ring(s) stained when the vacuum was applied through the y-1 needles. Had they followed the same pattern as the y-2 needles, most of the stain would have been located in the y xylem for the y-2 needles. That pattern was observed only in *Cedrus deodora*: the other species had the majority of their stain in the y-1 xylem (the same ring that stained for the y-2 needles) when the vacuum was applied through y-1 needles.

The third group is species for which the y-2 needles pulled most frequently through the y xylem (Fig. 5). Like the second group, this group was quite diverse in its staining patterns. For example, two of the species (*Picea pungens* and *Picea abies*) also had staining in the y-2 growth ring. Had they followed the same pattern as the y-2 needles, most of the stain would have been located in the y xylem for the y-1 needles. However, that pattern was only observed in *Sequoia sempervirens*: in the two *Picea* species, the y-1 needles pulled most frequently through the y-1 growth ring.

Three of the species did not fit into any of the these groups because of their high variability from sample to sample: *Pinus flexilis*, *Tsuga heterophylla*, and *Juniperus virginiana* (Fig. 6).

In the two deciduous species, *Ginkgo biloba* and *Larix occidentalis*, the y foliage stained mostly in the y growth ring, regardless off whether the foliage was attached to 2- or 3-yr old branches (Fig. 7).

Needle fall experiments*—Retention of y-2 needles on three orders of stems*—In the experiment with *Pseudotsuga menziesii* saplings in which needle retention was studied on stems of three orders, as expected, the first order stems were larger in diameter than the second order stems, which were larger than the third order stems. The first order stems had a wider range of diameters than did the second order stems, which had a wider range of diameters than did the third order stems. The diameters of the three stem orders did not overlap (Table 1, Fig. 8).

Retention of y-2 needles was highly variable among trees. The variation was higher going toward the right on the graph, which corresponds to both higher stem diameters and lower order branches (Table 1, Fig. 8). From these data, one cannot distinguish, for example, whether the third order stems have higher needle retention because they are third order stems or because they have small diameter.

There is a weak but highly significant negative linear rela-

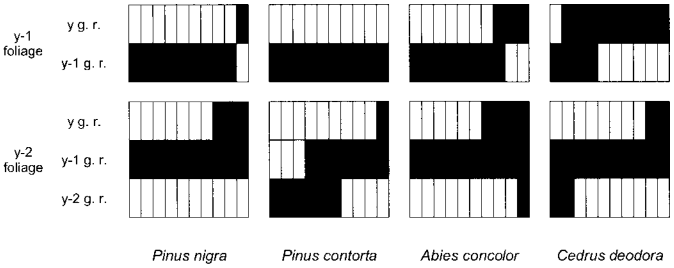

Fig. 4. Xylem staining pattern for species for which stain moved most frequently through the y-1 growth ring (g. r.), when a vacuum was applied through the y-2 needles. Graph is as described in Fig. 3.

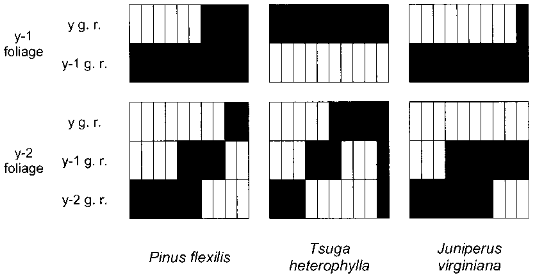

Fig. 6. Xylem staining pattern for species for which staining pattern was highly variable among samples when a vacuum was applied through the y-2 needles. Graph is as described in Fig. 3. g. r., growth ring.

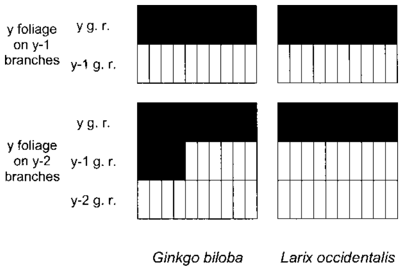

Fig. 7. Xylem staining pattern of deciduous species when a vacuum was applied through the year y (current year) foliage. Graph is as described in Fig. 3. g. r., growth ring.

tionship between needle retention and under-bark diameter ($R^2 = 0.290$, Fig. 8). The linear analyses were then run using three other estimates for stem size: diameter over bark, diameter under bark squared (an index of xylem area), and bark thickness. Diameter over bark gave the same value of R^2 and similar coefficients as did diameter under bark ($a = 95.9$, $b = -1.56$). Diameter under bark squared gave a slightly higher R^2 (0.321), and bark thickness gave a lower R^2 (0.148). In all four cases, the intercepts and slopes were significantly different from 0 ($P < 0.001$).

A three-parameter sigmoidal model was also applied to the data. This model is statistically significant and the R^2 is higher (0.381, Fig. 8). According to this sigmoid model, the retention of y-2 needles reaches 50% for an under-bark diameter of 22 mm. Where the diameter is less than 18 mm, almost all needles are retained, whereas where the diameter is greater than 25 mm, almost all needles are lost.

Retention of three years of needles on first-order stems—In the second experiment with *Pseudotsuga menziesii* saplings, we looked at retention of three age classes of needles on first-order stems (leaders). The youngest class of needles had the highest retention (from 77 to 100%), but the needles were also located on the smallest diameter stems (7–17 mm under bark). In contrast, the older class of needles had the lowest retention (from 0 to 39%), but they were located on the largest stems (17–32 mm under bark). There was a large overlap in stem diameters of the three needle age classes. Most of the y-1 needles were retained and most of the y-3 needles were lost, but the y-2 needles had a very large range of retentions, from 6 to 100% (Table 2, Fig. 9).

When all needle classes were pooled, the under-bark diameter was highly and significantly correlated with needle retention on the primary stem. The best fit was a sigmoidal relationship ($R^2 = 0.648$, Fig. 9), although both linear and quadratic relationships were also significant, with R^2 of 0.627 and 0.624, respectively. For a diameter under bark of 19 mm, 50% of the needles are retained.

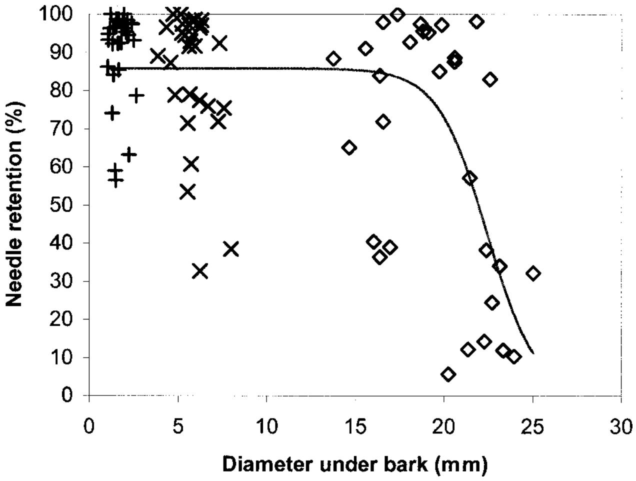

Fig. 8. Relationship of stem diameter (under bark) and the retention of y-2 needles assessed in year y, for first-order stems (the leader) (◇), second-order stems (×) and third-order stems (+) of *Pseudotsuga menziesii* ($N = 30$ observations per stem order). The best-fit sigmoidal curve is shown.

The linear analyses of needle retention vs. stem size were then run using the three other estimates of stem size. Diameter over bark gave a similar value of R^2 (0.624) and similar coefficients as did diameter under bark ($a = 158.4$, $b = -4.88$). Under-bark diameter squared gave a slightly lower R^2 (0.610), and bark thickness gave a much lower R^2 (0.358). In all four cases, the intercepts and slopes were significantly different from 0 ($P < 0.001$).

When each needle class was examined separately, there was a statistically meaningful correlation between needle retention and stem diameter only for the y-2 needles (Table 3). Its R^2 was 0.22, and both the intercept (a) and the slope (b) were significantly different than 0. As in the experiment investigating the effect of branch order on needle retention in same-aged needles, this experiment showed a negative relationship between needle retention and stem diameter for the y-2 needles.

DISCUSSION

Staining experiments—The present study shows that different-aged needles are attached to different water supplies in the stem. Within a species, the y-2 and y-1 foliage generally pulled through different growth rings, with only four exceptions. Both the y-2 and y-1 foliage pulled mostly through the y-1 growth ring in *Cedrus deodora* and mostly through the y growth ring in *Sequoia sempervirens*. The current-year foliage

TABLE 1. Stem diameters and retention of y-2 needles assessed in year y, for first order, second order, and third order stems of *Pseudotsuga menziesii*.

	Diameter under bark (mm)			Needle retention (%)		
Value	1st order stem	2nd order stem	3rd order stem	1st order stem	2nd order stem	3rd order stem
Mean	19.6	5.8	1.7	62.5	84.1	90.1
SE	3.1	0.9	0.5	33.5	17.9	12.1
Minimum	13.8	3.9	1.0	5.7	32.7	56.5
Maximum	25.3	8.0	2.7	100.0	100.0	100.0

TABLE 2. Stem diameters and retention of y-3, y-2, and y-1 needles assessed in year y, for first order stems of *Pseudotsuga menziesii*.

	Diameter under bark (mm)			Needle retention (%)		
Value	y-3 needles	y-2 needles	y-1 needles	y-3 needles	y-2 needles	y-1 needles
Mean	24.6	19.6	11.3	3.0	62.5	94.1
SE	4.1	3.1	2.2	7.4	33.5	7.3
Minimum	16.6	13.8	7.3	0.0	5.7	76.7
Maximum	31.8	25.3	16.5	38.8	100.0	100.0

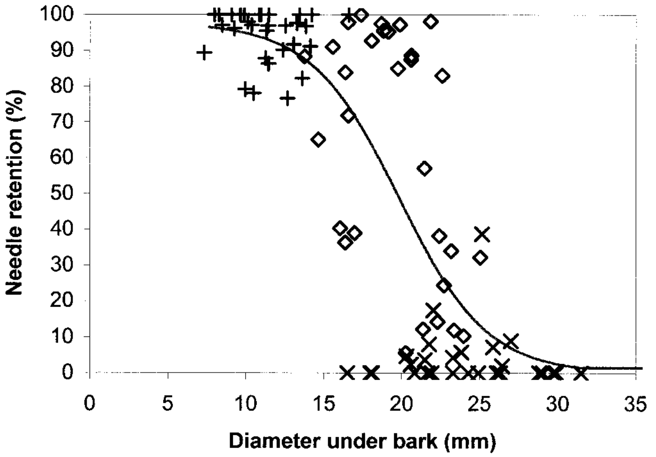

Fig. 9. Relationship of stem diameter (under bark) and the retention of y-2 (×), year y-1 (◇), and y (+) needles assessed in 2003 on first order stems (the leader) of *Pseudotsuga menziesii* ($N = 30$ observations per needle class). The best-fit sigmoidal curve is shown.

of the two deciduous species, *Gingko biloba* and *Larix occidentalis*, pulled stain mostly through the y growth ring.

The first group is species for which the needles pulled mostly through the xylem that was made in the same year as the needles (Fig. 1, zone a). Apparently, and in contradiction to the observations of Tison (1902) and Elliott (1937) on evergreen conifers, these species do not have functional breaks in the leaf trace, and if they do make new connections in the new xylem, they are not functional under the conditions we tested.

The second group is species for which the y-2 needles pulled most frequently through the y-1 xylem. This pattern would result from a breakage of the original leaf trace-stem xylem connection (Fig. 1, zone f) and production of a new active connection (zone e). For *Pinus nigra*, *Pinus contorta*, and *Abies concolor*, the original connection of the leaf trace with the stem xylem appears to remain functional for 2 yr, but in the third year, it reconnects, not with the outer growth ring, but with the ring that is 1 yr old. For *Cedrus deodora*, the process looks different. One-year-old foliage (produced in year y-1) is linked to current year xylem (year y), which implies that the needle trace has already been broken and reconnected. Then this new connection remains, and the 2-yr-old foliage is consequently attached to the 1-yr-old xylem.

In *Picea excelsa*, apparently the needle is connected physiologically with only the current year xylem (Tison, 1902). This result contrasts with our findings. In the present study, individuals of both species of *Picea* had y-2 foliage connected to the current year's stem xylem (y), but the y-1 foliage remained attached to the y-1 stem xylem. This result implies that the leaf trace remains connected to the xylem produced in the same year as the needle for one year beyond the year the needles are produced, and then the connection breaks and a new connection is made between the trace and the outer growth ring.

Sequoia sempervirens is the only species that behaved as described by Tison (1902) for *Picea excelsa*. The needle trace appears to break each year (at least functionally) and reconnect with the current year's xylem. As expected, the deciduous species, *Gingko biloba* and *Larix occidentalis*, also were connected primarily with the current year's xylem.

For the 13 species just discussed, we saw patterns that tended to occur within the species. For *Juniperus virginiana*, *Pinus flexilis*, and *Tsuga heterophylla*, the strategy of needle connection varied so much from individual to individual that we could not extract a species strategy with the sample size employed. Perhaps further study of the manner in which the pattern varied within species (as was done for *Pseudotsuga menziesii*, reported here) and even within individuals would give more insight into causes of functional leaf trace breakage and connection with the stem.

We found no publications with tabulations of conifer needle longevity, although information on individual species was often available. Conifers range from having needles that live less than 1 yr up to around 40 yr (*Pinus longaeva*, Ewers and Schmid, 1981). We had no information for most of the species in the current study, except for *Pseudotsuga menziesii* (6–8 yr, Balster and Marshall, 2000; and more commonly 6–7 yr, Weiskittel, 2003), and *Pinus ponderosa* (2–9 yr, Ewers and Schmid, 1981; and 3–4 yr in our study area, personal observation). The species differed in needle longevity by a factor of about two, but had the same needle connection patterns.

It would be interesting to learn whether species with long-lived needles are more capable of producing new functional connections with current year's xylem than are species with short-lived needles. It would also be interesting to conduct the same study on needles older than 2 yr to learn whether their connection patterns are consistent with the 2-yr-old foliage. Ewers (1982) studied the relationship of new xylem and phloem development in needles and found that none of the 11 species studied produced new xylem in the vascular bundle, but that they all produced a small quantity of new phloem (which appeared to be replacing phloem that died annually). There was an inverse relationship between the maximum leaf longevity of species and their rates of secondary phloem production, leading Ewers to state that it was improbable that secondary growth in the vascular cambium of leaves controlled leaf longevity.

Further work could also seek a relationship between longevity of needle trace connection and sapwood depth, angle

TABLE 3. Correlation of needle retention by *Pseudotsuga menziesii* and under-bark stem diameter for each needle class separately. y represents needle retention; x represents diameter under bark, $N = 30$ for each needle age class.

Equation	By needle age	Coefficients (P values)		R^2
		a	b	
$y = a + bx$	Three-year old	4.99 ($P = 0.570$)	−0.08 ($P = 0.815$)	0.002
	Two-year old	163.66 ($P < 0.001$)	−5.17 ($P = 0.008$)	0.223
	One-year old	93.23 ($P < 0.001$)	−0.27 ($P = 0.670$)	0.001

of leaf insertion, and phylogenetic relationships. There was no relationship between sapwood depth and pattern of needle trace connection for the two species for which we have very good sapwood depth information. *Pseudotsuga menziesii* has relatively narrow sapwood (typically about 5 cm, Megraw, 1986; Gartner, 2002). It had the same pattern of needle trace connection as did *Pinus ponderosa*, which has extremely wide sapwood (typically 20 cm in 225-yr-old trees, Domec and Gartner, 2003, possibly more in older trees). There were too few species in this project to look at phylogeny rigorously, but we did observe that the two species of *Picea* (*P. abies* and *P. pungens*) shared the same pattern of needle trace connections, whereas the three *Pinus* species (*P. ponderosa*, *P. nigra*, and *P. contorta*) had two different patterns. These observations neither strongly support nor contest the hypothesis that the strategy is deeply canalized phylogenetically for either of the species.

This study also aimed at learning more about how deep sapwood is used for water transport in trees. We know that most intertracheid pits are on the radial walls (Panshin and DeZeeuw, 1980, P. J. Schulte, University of Nevada, Reno, personal communication), facilitating water movement within a growth ring but not between growth rings. Therefore, when water moves up a tree, the highest sap flux will occur in the growth ring that has the highest driving force (caused by transpiration from needles attached to that ring) assuming that all rings have similar specific conductivity. Currently, it is unclear whether the higher sap flux several cm inward from the cambium now reported for many conifers (e.g., Nadezhdina et al., 2002, and reviewed in Phillips et al., 1996; Gartner and Meinzer, 2005) results from needles having the highest transpiration in xylem that is several years old, from several-year-old xylem having higher specific conductivity than younger xylem (Mark and Crews, 1973) or some other not-yet understood phenomenon. However, a simplistic calculation sheds doubt on the probable importance of deep functional needle attachment on the observed sap flux patterns. To explain the peak in sap flux 2 cm from the cambium in trees growing at the modest growth rate of 4 mm/year necessitates the highest total transpiration in needles that are five years old. This outcome is not consistent with our knowledge of water use by different needle cohorts (see later). However, more refinement is needed in many areas to fully understand the role of needle connections. For example, we need a more spatially precise radial pattern of sap flux and of specific conductivity than is usually provided.

One more implication of leaf trace longevity is that the old leaf traces may function as pathways for radial movement of sap (MacDougal et al., 1929). There are no direct pathways, and yet it is known that some water does move in the radial direction (otherwise, only the outer growth rings would have sap flow).

Many authors have reported physiological, chemical, and microorganismal variations in different-aged needles. To some extent, these differences may reflect differences in hydraulic and/or phloem supply to these needles. Moreover, the consequences of disturbances to different needle age classes (such as by ice storms, herbivores, and pathogens) could differ in species that have different leaf connection strategies.

The foliar nitrogen concentration (Naidu et al., 1993; Ryan et al., 1996) and photosynthetic performance (Freeland, 1952; Hom and Oechel, 1983; Wang et al., 1995) both decline with increasing the needle age. Chabot and Hicks (1982) observed a rapid increase in the apparent photosynthetic rate to a maximum and then a gradual decrease until senescence and death. In evergreen conifers, they found that the maximum photosynthetic rate declined at 30–50% per year. Other foliar chemistry can change as well. For example, the current-year foliage of *Pinus sylvestris* had higher capacity of ribulose biphosphate carboxylase and higher chlorophyll concentration but lower carbon concentration and sugar soluble concentration than 1-yr-old needles (Jach and Ceulemans, 2000). Aging of needles has an effect on stomatal conductance as well (Watts et al., 1976; Teskey et al., 1984). Respiration rates also decrease with needle age (Wang et al., 1995; Zha et al., 2002). In addition to physiological and chemical changes, microorganismal concentrations also vary with needle age. For example, frequency of infection by *Rhabdocline parkeri* on *Pseudotsuga menziesii* increased logarithmically with needle age (Stone, 1987). It would be interesting to learn to what extent these age-related trends are related to the water connection of the foliage vs. the foliage microenvironment or other factors.

Leaf-fall experiments—In the second series of experiments, we wanted to learn whether needle drop was related to needle age or diameter growth of the stem. We are assuming that needle drop occurs when the connection between the leaf trace and the stem breaks, without new tracheid growth to maintain a connection. The research by both Tison (1902) and Elliott (1937) implied that the leaf trace breakage occurred as an age-related phenomenon, although in both cases the authors discussed the stretching and breaking of leaf trace tracheids by force of the new radial growth. Obviously, needle aging and growth diameter go co-vary.

The experiment in which we compared needle retention of the y-2 needles on three orders of stems was inconclusive because there was no overlap among the branch orders in stem diameters. In the experiment in which we compared needle retention on first-order stems, we found that for the y-2 needles especially, there was a negative relationship between needle retention and stem diameter. The relationship was not affected by including the bark diameter (either as over-bark diameter, or bark thickness), which suggests that it is the xylem growth that causes the mechanical pressure, not the phloem growth. These experiments suggest that xylem diameter growth is a cause of needle drop on the leader. Consequently, ecological factors such as competition and suppression that affect xylem diameter growth may have an impact on needle retention.

The sigmoidal models provide a threshold value of diameter at which there is 50% needle retention, highlighting the fact that diameter is also a major factor in the needle drop. This threshold value is about 22.5 mm in the experiment in which the y-2 leaves were studied on three different orders of stem (Fig. 8), which is larger than the mean value of stem diameter for the first order stems studied (Table 1). In other words, large first order stems had lost half their needles; stems smaller in diameter had higher retention. This threshold value is about 20 mm for the three age classes of needles on first-order stems (Fig. 9), which corresponds to the diameter of first-order stems where needles are produced in y-2 (Table 2). On average, half the two-year old needles are retained, with more of the one-year-old and fewer of the three-year-old needles retained.

Only one species was chosen to tackle this issue. Further research will benefit from studying more species and more years' worth of needles. However, this will require older trees, which will most likely not be growing in the common-garden conditions of the saplings in this experiment.

This research suggests that the xylem radial position at which water is accessed can differ by needle age class in evergreen conifers. The variability appears to be related to species, but assessments in more environments and in older age classes are needed to evaluate the relative influence of environment in different species. Assuming there is much higher resistance to radial than axial water transport (by about a factor of 100 times, Milota et al., 1995), then it is likely that foliage of different ages will have different water relations, which could cascade to other effects on needle ecophysiology and needle response to disturbances. The research also suggests that the water potential gradient may vary from species to species within the outer several growth rings. If the same patterns of connection occur in the phloem, this research suggests that activity may be spatially heterogeneous in the phloem, as well.

Conclusions—This research found that in a common nursery, saplings of different species had different patterns of longevity of their functional foliage connections to the stem xylem. Previous studies suggested that in conifers, every year a needle will break its connection to the previous year's stem xylem after it establishes functional connections with the current year's stem xylem. This pattern was only seen in one species, *Sequoia sempervirens*, and in many of the individuals of *Tsuga heterophylla*. Far more common was that the stem xylem connection that was made the year of needle production would remain functional for two years (*Pseudotsuga menziesii*, *Pinus ponderosa*, *Calocedrus decurrens*, *Chamaecyparis nootkatensis*) or for one year beyond the current year. Of the species that maintained the connection for one year beyond the current year, four of the species made a subsequent connection from the two-year-old foliage to the one-year-old wood (*Pinus nigra*, *Pinus contorta*, *Abies concolor*, *Cedrus deodara*) and two of the species made a subsequent connection from the two-year-old foliage to the current year's wood (*Picea pungens*, *Picea abies*). As expected, the deciduous conifers (*Gingko biloba*, and *Larix occidentalis*) had functional connections almost solely with the current year's stem xylem.

In almost all the samples in the staining experiments, there was a high sample-to-sample variability with respect to the growth ring through which a given-aged needle pulled water. For three of the species (*Pinus flexilis*, *Tsuga heterophylla*, *Juniperus virginiana*) the variability was so high that we were unable to assign a leaf trace connection pattern to them. To try to better understand the causes of leaf trace connection patterns, we asked the simple question for one species, *Pseudotsuga menziesii*, whether there is a strong relationship of leaf retention to either needle age or stem diameter. There was evidence that stem diameter affected needle retention, such that larger stems had lower needle retention. The result suggested that the needle traces are physically broken by the radial increase in xylem (not phloem), and then the needles drop.

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