Water-related Phenomena in Winter Buds and Twigs of Picea abies L. (Karst.) until Bud-burst: A Biological, Histological and NMR Study

ELISABETH de FAY*‡, VALERIE VACHER‡ and FRANÇOIS HUMBERT‡§
‡Laboratoire de Biologie Forestière associé INRA, Université Henri Poincaré, Nancy I, BP 239, 54506 Vandœuvre-lès-Nancy Cedex, France and §Laboratoire de Méthodologie RMN, UPRESA 7042, Université Henri Poincaré, Nancy I, BP 239, 54506 Vandœuvre-lès-Nancy Cedex, France

Received: 25 February 2000 Returned for revision: 7 May 2000 Accepted: 12 August 2000 Published electronically: 24 October 2000

This paper investigates the uptake, transport, state and self-diffusion of water in twigs and buds of Picea abies L. (Karst.) trees in winter until reactivation in spring. The presence or absence of xylem in embryonic shoots, as well as the intensity and type of bud dormancy were also studied. Three clones of P. abies were grown in a forest in northeastern France. The clones differed in their timing of bud-burst, with the two clones from the Vosges showing different degrees of early bud-burst and the clone from Poland showing late bud-burst. One-bud cuttings grown in standard forcing conditions showed a great difference in dormancy between the two provenances, but only a small difference between the two Vosges clones. Earliness of bud-burst was not strongly linked to the intensity of dormancy. A dye experiment combined with light microscopy indicated increased velocity of water transport in stems up to a maximum in April, initial entry of water into embryonic shoots, protoxylem differentiation in embryonic shoots from April, and then, shortly before bud-burst, water transport through the newly-formed protoxylem almost up to the meristem. Nuclear magnetic resonance measurements of the transverse relaxation time (T2) and the self-diffusion coefficient of water confirmed these observations and showed water availability in embryonic shoots. The sequence of water-related phenomena occurring in early spring was the same in the three clones, but was earliest in the Vosges clone with the earliest bud-burst and latest in the Polish clone with late bud-burst. The results imply that this sequence of water-related phenomena leads to bud-burst.

Key words: Bud, bud-burst, dormancy, embryonic shoot, growth capacity, nuclear magnetic resonance (NMR), Norway spruce, Picea abies L (Karst.), self-diffusion, stem, water, xylem.

INTRODUCTION

At the start of spring plants require water for optimal hydrolysis of food storage macromolecules and for increased enzyme activity in order to resume activities that ceased through the winter. If they are to burst, buds must also have broken their dormancy. Dormancy is a temporary suspension of visible growth of any plant structure containing a meristem (Lang et al., 1987). It is a complex phenomenon which presents three phases through which buds of temperate deciduous trees pass between formation and bud-burst (Champagnat, 1989, 1992). The successive phases are known as: paradormancy, endodormancy and ecodormancy (Lang et al., 1987) and can be defined according to Chouard (1951), Lang et al. (1987) and Champagnat (1989, 1992) as follows. Paradormancy is regulated by physiological factors within the plant, outside the dormant structure: it is an inability to grow imposed by either the leader apex (apical dominance), adult leaves or bearing stem (other kinds of correlative inhibition); it generally starts in summer. Endodormancy is regulated by physiological factors within the dormant structure: it is an inability to grow when all environmental conditions are favourable and when correlated inhibitions are removed; it is generally induced in November and is still poorly understood, but its release requires chilling in many cases and takes place naturally at the end of winter. Ecodormancy, occurring in early spring after the release of endodormancy, is an inability to grow imposed by environmental factors; mild temperatures can lead ecodormant buds to burst.

Hydraulic conductivity of stems during winter, and the associated problems of cavitation, xylem embolism (which blocks water transport) and xylem refilling after embolism have been largely studied in temperate deciduous trees (Tyye, 1983; Cochard and Tyye, 1990; Just and Sauter, 1991; Améglio and Cruziat, 1992; Améglio and Cruziat, 1992; Sperry et al., 1994; Améglio et al., 1995; Magnani and Borghetti, 1995; Hacke and Sauter, 1996; Lemoine et al., 1999). In contrast, little attention has been paid to water availability in buds of these trees. Water content of buds increases during spring in maple, birch, alder, beech and ash (Essiamah and Esrich, 1986), and is correlated with the phenological stage of buds in walnut (Améglio, 1994). In ash trees, the increase in osmotic and water potentials of buds at the end of the dormant period suggests that some water is available more than 1 week prior to the increase in bud water content and growth (Cottignies, 1983, 1990). Water availability in buds has been assessed in fruit trees using magnetic resonance imaging (MRI). A change in relaxation times was detected...
in flower and leaf buds of apple and blueberry during winter (Faust et al., 1991, 1995; Rowland et al., 1992); the change takes place regardless of the low or high chilling requirement of the cultivar, but after a shorter or longer time of chilling, respectively. This change was interpreted as meaning that water is in a bound and/or structured form in dormant buds, and in a free form in buds after the chilling requirement has been satisfied. These few investigations have shown that water availability changes suddenly in ash, apple and blueberry buds, but the time of change varies with the species studied: the change was detected shortly before bud-burst (i.e. at the end of ecodormancy) in ash and when the chilling requirement was satisfied (i.e. at endodormancy release) in fruit trees.

In conifers, there have been few studies on winter xylem embolism (Sucoff, 1969; Cochar, 1992; Sperry and Sullivan, 1992; Sperry, 1993; Sperry et al., 1994), spring recovery (Borghetti et al., 1991; Sperry, 1993; Sperry et al., 1994) and bud dormancy release and bud-burst (Dormling, 1989; Jacques et al., 1989). No literature deals with the water status of conifer buds in relation to bud-burst. The relationships between water, dormancy and bud-burst are thus very poorly documented in conifers. To address this problem we chose to focus on the water-related phenomena at short distances from the shoot apical meristem, notably within the bud in the embryonic shoot, which grows out at bud-burst, and to analyse them by nuclear magnetic resonance (NMR).

NMR is a powerful tool for investigating properties of water in biological systems because it is a non-invasive technique that provides information on both molecular structure and dynamics (Callaghan, 1991). In particular, two parameter types are of interest: NMR relaxation times and molecular self-diffusion coefficients. The longitudinal (T1) and transverse (T2) relaxation times correspond to the time constants associated with the rate laws which describe the return of equilibrium of nuclei (e.g. 1H, 13C) excited in a NMR experiment. As the rate of relaxation is very sensitive to molecular motion, the relaxation times for water protons depend on the water state. Thus, the T2 for water protons becomes shorter as water is more strongly bound to macromolecules. As far as molecular diffusion is concerned, it is important to note that there are two different types of diffusion: driven diffusion and self-diffusion. In the first, a gradient provides a driving force which results in net changes in the local concentration of the diffusing species. In the second, molecules diffuse randomly with respect to one another under equilibrium conditions without any net changes in the local concentration. Here, we are only concerned with self-diffusion. While relaxation times reflect complicated rotational molecular motions, self-diffusion is caused solely by random molecular translational motions. Because of the complexity of diffusion processes in biological systems the term ‘apparent self-diffusion’ is commonly used. For water protons diffusing within a tissue matrix, the observed diffusion rate and direction reflect the molecular and macromolecular barriers, or hindrances, that the protons experience during their translational displacement, and consequently also reflect the size and permeability of cells.

The objective of this paper is to investigate the water-related phenomena in vegetative buds and underlying stems of conifers, as well as the time of changes with regard to the physiological stage, i.e. endodormant, ecodormant and bursting buds. The biology and proton nuclear magnetic resonance (1H-NMR) of winter and spring buds and twigs were investigated in *Picea abies* L. (Karst.), Norway spruce, a widely distributed conifer abundant in north-eastern France, using three clones which differ in their timing of bud-burst. In addition, differentiation of xylem within buds was examined and related to changes in water availability determined by the other methods.

**MATERIALS AND METHODS**

**Plant material**

The Norway spruce plants studied were grown in a clone plot within the INRA-Nancy research station, located in the national forest of Amance, north-eastern France (48°47′ N, 6°18′ E, 240 m elevation). They were grafted trees belonging to three clones of two provenances and features. Two clones were of the Vosges ecotype (clones Ger 15 and Ger 19, named respectively V15 and V19) from Kerkoff in the Vosges mountains (48°06′ N, 6°51′ E, 700–900 m elevation). This ecotype presents a particular phenotype (columnar form) at the adult stage and has early bud-burst; it is thus sensitive to late frosts when planted at low elevation. The other clone was of Polish, low elevation, origin (Polish clone T125), with late bud-burst and is thus insensitive to spring frosts. The Vosges trees were approx. 30 years old, the Polish trees 20 years old; all were vegetatively mature. Preliminary observations at the site showed that V19 had somewhat earlier bud-burst than V15 and confirmed that the Polish clone had late bud-burst.

Four trees of each Vosges clone and three trees of the Polish clone were sampled from November 1994 to May 1995. Lateral twigs from the end of healthy branches were taken from the lower or the median crown zone of the trees for biological, histological and NMR investigations.

**Biological methods**

**One-bud cutting test.** The test was adapted from the one-node cutting test commonly performed in broad-leaved trees. It consists of isolating buds as one-node cuttings to remove most extravascular influences, then culturing the cuttings in forcing conditions (Champagnat, 1992; Crabbé and Barnola, 1996). This testing method is based on the definition of Chouard (1951) according to which dormancy (endodormancy) is an inability to grow when all environmental conditions are favourable and when correlative inhibitions are removed. It allows the determination of the intensity and duration of endodormancy as well as the physiological stage (endodormant, ecodormant) of the buds at a given time. Twenty 6-cm-long cuttings of the end part of current year shoots, bearing only the terminal bud plus needles (lateral buds at the top and needles at the base were removed) were made from November to May at monthly intervals (bimonthly in April). The base of the cuttings was
inserted through a polystyrene sheet which was floated on water in a transparent plastic box, which remained half-opened, placed in a growth chamber. Cuttings were grown in controlled conditions (photoperiod: 16 h, photon flux density: 250 µmol m⁻² s⁻¹, temperature: 25°C ± 2°C). Two millimetres of the base of cuttings was cut away weekly in order to prevent embolism caused by a possible proliferation of microorganisms, and the water was changed every 3 d. The number of buds showing a green tip (appearance of microorganisms, and the water was changed every necessary according to the chromatic axial symmetry, using the software Graphic Convexor V 3.1.

**NMR methods**

One current year shoot of each clone was harvested at monthly intervals from March to May (bimonthly in April) and prepared for ¹H-NMR spectroscopy. Scales were removed from the bud, and the bud heart (embryonic shoot) was cut off, coated with melted paraffin to prevent dehydration and placed into a 5 mm NMR tube. A small piece of the underlying stem was excised and prepared in the same way as the embryonic shoot. All proton NMR measurements were carried out at 26°C on a home-built spectrometer equipped with an electromagnet and operating at 90 MHz. The radio-frequency probe used was made up to two coils: (1) a Helmholtz coil receiving the NMR signal and producing homogeneous radio-frequency pulses; and (2) a flat concentric two-turn coil generating a radio-frequency field gradient (up to 80 Gauss cm⁻¹) along its axis, to measure self-diffusion coefficients (Humbert et al., 1996).

\[ T_2 = 1/\sum_i^n \int_0^t \frac{1}{2} e^{-t/T_{2i}} \]

where the fit parameters were the populations \( f_i \) and the transverse relaxation times \( T_{2i} \) corresponding to the different water states, \( i \) ranging from 1 to 3. For the results reported here, a good fit was obtained with \( i \) equal to 2 revealing two proton populations.

Self-diffusion coefficients were measured using the radio-frequency field gradient technique described by Humbert et al. (1998). In comparison with the conventional static field gradient technique, this method has the advantage of being simpler instrumentally and almost insensitive to magnetic susceptibility inhomogeneities caused by tissue heterogeneity in the sample and, especially, by the air–water interfaces. Self-diffusion coefficients parallel (\( D_// \)) and perpendicular (\( D_\perp \)) to the longitudinal axis of buds or stems were measured by orienting the samples parallel or perpendicular to the direction of the field gradient, respectively.

**Histological method**

The presence of xylem in buds was determined histologically. In each clone, two terminal buds were fixed in spring at monthly or bimonthly intervals, and preserved in chromic-acetic-formalin fixative, CRAF 1 (Sass, 1958). Buds with scales removed were then cut longitudinally with a freezing microtome. The 40-µm-thick median sections were treated with the Wiesner reagents (Sass, 1958) to stain lignins: fresh sections were left for 3 min in 1% phloroglucin in 95% ethanol and mounted in 50% HCl, and

\[ V_i = 1/t_i \]

If buds do not burst after a given time, the velocity is considered to be equal to zero. The average velocity of bud-burst can be then calculated:

\[ V_m = 1/n \sum_{i=1}^n V_i \]

where \( n \) is the total number of buds, and subsequently the mean time required for bud-burst:

\[ MTB = 1/V_m \]

**Dye uptake and transport.** The method was adapted from that of Essiamah and Eschrich (1986) for measuring the ascent of a dye solution in broad-leaved trees. The use of azosulfamide, a water-soluble dye translocated through xylem and intercellular spaces in peach (Ashworth, 1982) permitted measurement of the velocity of water transport in the stem through the xylem and showed water entry into the bud and the embryonic shoot. Six 30-cm-long twigs of each Norway spruce clone were harvested between 0900 and 1100 h at monthly intervals from December to May, (bimonthly in April). In the laboratory, the twig bases were re-cut under water in order to avoid cavitation, quickly transferred to a 1% (w/v) solution of azosulfamide (4-sulfamylphenyl-2-azo-7-acetamido-1-hydroxynaphthalene 3,6-disulfonic acid, disodium salt, Sigma France, A-2759), and incubated in the growth chamber. After a measured period of time (approx. 60 min or 30 min at the end of the experiment), the height to which the dye had risen was determined in three of the twigs as follows: a few needles were taken from along the axis and the leaf traces were observed to find the uppermost trace stained, then the stem was cut transversely in the insertion zone to find the front of the dye solution with the help of a stereomicroscope. The distance of the dye front from the base of the twigs was measured. The other three twigs were left in the dye solution for longer periods to determine how far the dye solution moved up. After 1, 2 or 3 d following application of the dye, buds were cut longitudinally and the presence of the red dye determined under the stereomicroscope. Colours of photographs reported here were changed as
observed by standard light microscopy. The combination of oxidative polymerization and formaldehyde condensation reactions, taking place in the fixating liquid CRAF 1, produced insoluble red-brown phenolic derivatives (tannins) also visible in the tissues.

To study the seasonal variations of water-related phenomena it was necessary to sample several times in succession and thus to take a limited number of specimens at each date of sampling. At the end of the experiments, a substantial number of buds and stems (approx. 200 per clone) was nevertheless examined. Because of the small number of samples at each sampling time, results are generally expressed as means, exceptionally as means and 95% confidence limits. It is well-known that, within a tree or a clone, all buds do not develop at exactly the same time and a certain desynchronization exists at the time of bud-burst. However, as we collected all the twigs and shoots from the same zone of the tree crown, chose the most vigorous ones and sampled at approximately the same time of day (between 0900 and 1100 h), it can be assumed that development and water-related phenomena did not differ strongly within a clone from one bud (or one twig) to another in the crown zone studied.

RESULTS

Growth potential of buds

The latent period and the final rate of bud-burst varied during the winter and spring in the three clones, the latent period tending to decrease and the final rate to increase (Fig. 1). However, the mean time required for bud-burst (MTB) strongly differed in November, December and January between the Polish and Vosges clones; it was high in the Polish clone (varying between 16 000 and 19 000 h) and low in both Vosges clones (less than 3000 h), but lower in V19 than in V15 (Fig. 2A). From 15 February, MTB was relatively low in the three clones and it decreased continuously until bud-burst.

Dye uptake, transport and location

The dye solution moved up the twigs of the three clones in winter and spring, but the velocity of the ascent was slow in winter (approx. 20 cm h\(^{-1}\)), increasing gradually from 15 January in V19, or dramatically at the end of March in the two other clones, finally reaching a maximum of 70 cm h\(^{-1}\) (Fig. 2B) when the growth capacity was maximal (compare Fig. 2B and Fig. 1). After staining the central cylinder of stems (xylem and pith), the red dye entered buds (Fig. 3). In winter, it stained only the bud base i.e. pith, xylem and cortex beneath the nodal diaphragm where it accumulated. (The nodal diaphragm, also called crown, is a structure observed in vegetative buds of some gymnosperms, that extends across the pith at the base of the embryonic shoot.) In early spring, faint staining appeared in the basal part of the embryonic shoots (Fig. 3A and B). Finally, from 6 April in V19, 26 April in V15 and 10 May in the Polish clone, stain occurred throughout the length of the embryonic axis. However, in the central part it was very restricted (Fig. 3C and D). The ascent of dye to the embryonic needles (Fig. 3E) and almost to the shoot apical meristem occurred shortly before or at the start of bud outgrowth when the velocity of dye transport was greatest (see Fig. 2B).

Proton NMR transverse relaxation times and self-diffusion coefficients of water in embryonic shoots within buds and in underlying stems

At the end of March, the self-diffusion of water in stems of the three clones was anisotropic, the apparent self-diffusion coefficients being higher along the longitudinal axis (Table 1). In contrast, at that time, the self-diffusion of water in the embryonic shoots was isotropic, becoming anisotropic during April, a little earlier in the Vosges clones than in the Polish clone (Table 1 and Fig. 4A–C).

For all samples, multiexponential analysis of T2 data indicated two water populations with specific relaxation times. For both populations, a strong increase in T2 was observed in embryonic shoots from April; for example, the
short $T_2$ varied from 4 to 30 ms in a fortnight or a month (Fig. 4D–F). However, the high proportion (about 85 %) of water with a long $T_2$ found at the end of March decreased in early April in the V19 clone, with the earliest bud-burst, but not until early May in the Polish and V15 clones (Fig. 4D–F). At that time the relative sizes of the two populations of water (free and bound) were approximately equal and got closer to that found in the stems of the three clones. This change occurred when (or a little after) longitudinal strands of red staining were observed in the embryonic axes.

**Xylem in buds**

In all the buds examined, phloroglucinol-HCl positively reactive helical tracheids and bordered pit tracheids were always found beneath the nodal diaphragm, forming a few layers of xylem around the pith (Fig. 5A and D). In contrast, above the nodal diaphragm, i.e. in the embryonic shoots within the buds, xylem was initially absent, appearing during early spring (Table 2). In the three clones sampled on 20 March and in the Polish clone sampled on 6 April (Fig. 5A and B), no tracheids were seen in the embryonic shoots, which were approx. 5 mm long. In the Vosges clone V15 sampled on 6 April, the situation was almost the same (Fig. 5C) except that one elongated cell with helically-thickened but Wiesner-negative walls (not stained with phloroglucinol-HCl) was observed at the base of one, still small, embryonic axis (not shown). In the Vosges clone V19 sampled on 6 April and in the three clones...
clones sampled on 24 April (Fig. 5D–G) and in May, numerous tracheids with helical wall thickenings were observed, arranged in one strand, along the very elongated embryonic axes, almost up to the shoot apical meristems (last-formed tracheids approx. 0.5 mm away from the top of the apical dome in an embryonic axis 15 mm long). Tracheid walls in these embryonic axes were stained with phloroglucinol-HCl; however, the staining was faint or negative at the end of the embryonic axes.

**DISCUSSION AND CONCLUSIONS**

From the values of the mean time required for bud-burst (MTB), which is used for comparisons of endodormancy in experimental and forcing conditions (Crabbé and Barnola, 1996), it can be deduced that the Polish clone of *Picea abies* was very endodormant and that endodormancy release occurred between 15 January and 15 February. From 15 February, ecodormancy occurred. Buds of the Polish
clone behaved similarly to those of several temperate broad-leaved trees (Champagnat, 1992), for instance walnut (Mauget, 1976, 1982) and Gleditsia triacanthos (Aillaud, 1986) which are generally very endodormant from November to February and then ecodormant until bud-burst. In contrast, the Vosges clones showed relatively little endodormancy. Their endodormancy release is more difficult to date in the experimental conditions but the buds of the Vosges clones were clearly ecodormant in April. Ultimately, the Polish and Vosges clones of *Picea abies* differed greatly in their degree of bud endodormancy while there was little difference between the two Vosges clones, V19 and V15. As the delay in bud-burst of the Polish clone compared to the V15 Vosges clone was small and of the same order as that of V15 compared to V19, the earliness of bud-burst appears to be relatively independent of the maximal intensity of endodormancy, as in walnut (Mauget, 1982). The difference between the clones disappeared in early spring when all the buds developed under favourable environmental conditions (ecodormant buds).

Results showed that ecodormancy is the time when changes in water availability occur in stems and embryonic shoots of buds of *Picea abies*. As changes are the same in the three clones, it appears that the water-related phenomena occurring during dormancy before bud-burst are general phenomena in *Picea abies* trees. The first change detected occurs in the stems and concerns the velocity of water transport. As the velocity of dye ascent in twigs was of the same order as the velocity of crude sap transport in gymnosperms (Robert and Catesson, 1990), it can be concluded that water transport in twigs was first slow in winter and then increased gradually or abruptly and was relatively fast in early spring. As a good correspondence was found between the patterns of sap velocity and cavitation rate in broad-leaved trees (Borghetti et al., 1993), and as winter embolism of xylem was shown to take place, if only to a small extent, in *Abies lasiocarpa* (Sperry and Sullivan, 1992), *Abies balsamea* and *Picea rubens* (Sperry, 1993), it can be surmised that the low water transport in *Picea abies* twigs in winter was due to xylem embolism. Spring recovery of xylem conductivity after winter embolism was described in *Picea abies* (Raschi et al., 1999). The late winter or early spring change in the velocity of water transport in *Picea abies* twigs could thus be explained by xylem refilling after winter embolism. Xylem refilling is hard to understand in conifers as water in the xylem of stem pieces of *Picea rubens* and *Abies balsamea* (Sperry, 1993), *Picea glauca*, *Larix laricina* and *Abies lasiocarpa* (Sperry et al., 1994) is always under tension, never at positive pressure, and xylem parenchyma in *Pinus sylvestris* seems to have no role in the refilling process (Borghetti et al., 1991), contrary to the situation in various broad-leaved trees (Sauter et al., 1973; Fromard, 1990; Améglio and Cruziat, 1992). However it may occur in Norway spruce, as in ash, beech, birch and maple (Essiamah and Eschrich, 1986) dye ascent in the stem through the xylem was slow in winter and increased sharply before bud-burst. Note that, in the case of the broad-leaved trees, the rise in dye ascent corresponded to a rise in transpiration rate (Essiamah and Eschrich, 1986).

After the increased water transport in stems, several other changes occurred in embryonic shoots within buds. Dye penetration into buds and embryonic shoots, and the histological study suggest that water entered embryonic shoots of ecodormant buds in early spring, first through the apoplasm of parenchyma cells and later mainly through the

---

**Fig. 4.** Variation of the apparent self-diffusion coefficient and of the transverse relaxation time of water in embryonic shoots of the three clones (Pol: Polish; V15: Vosges V15; V19: Vosges V19) of *Picea abies*. (Karst.) during spring. A–C, Apparent self-diffusion of water parallel (Δ) and perpendicular (●) to the longitudinal axis; D–F, variation of the (T2) transverse relaxation time of bound water (○), and of the rate of free water (●). The four parameters were obtained from the same sample at each date of sampling. Note that there is no data point corresponding to T2 for the bound water in the case of V19 sampled on 21 March, which could mean either the rate of the bound water was too low to be detected or the T2 of bound water was very short (tightly bound water).
newly-formed helical tracheids which make up the protoxylem of conifers (Esau, 1977). The initial time of water entry into the embryonic shoots could not be specified in relation to the sharp increase of water transport in the experimental conditions. A comprehensive study combining the dye ascent experiment with measurements of water content and water potential at regular and short intervals is necessary. However, it is likely that the initial time of water entry into the embryonic shoots was associated with the resumption of development of these embryonic shoots, as bud swelling in maple was associated with a sharp increase in water content in the buds (Essiamah and Eschrich, 1986). As for the mechanism of initial entry of water into the embryonic shoots in early spring, Coutinnes (1986) proposed that, in the case of ash, some water which was bound is liberated in the cells of the shoot apex and contributes to the hydrolysis of starch into soluble sugars, thus modifying the osmotic potential and therefore the requirement for water. It must be stressed that protoxylem was formed prior to bud-burst of Picea abies trees and was functional shortly before or at the start of bud outgrowth. This production and functioning of tracheids is compatible with a further elongation, since their coil type of secondary wall thickening and the primary wall can be extended passively. They appear in embryonic shoots prior to bud-burst in leaf buds, but xylem vessel elements void of cytoplasm and with secondary wall thickenings have also been observed in flower buds of peach that swelled visibly in spring (Ashworth, 1982).

Nuclear magnetic resonance measurements of transverse relaxation times (T₁) and self-diffusion coefficients of water confirmed these observations and described the water availability in embryonic shoots before bud-burst, indicating the bound or free state of water as well as the hindrances to water diffusion, which corroborates the data on absence, presence and functioning of xylem. Indeed, three situations corresponding to three successive periods with regard to protoxylem were detected in embryonic shoots of ecodormant buds. At first, when protoxylem was absent, the water diffusion in embryonic shoots was isotropic, while in underlying stems it was anisotropic, water diffusion along the direction parallel to the longitudinal axis being less hindered, as in wood of Douglas fir and sugar maple (MacGregor et al., 1983; Peemoeller et al., 1985). Then, while protoxylem was differentiating, a similar anisotropic molecular diffusion appeared in the embryonic shoots, indicating that the motion of water molecules became less hindered parallel to the longitudinal axis than perpendicularly. Shortly after the beginning of xylem differentiation, T₂ increased by up to an order of magnitude for bound water, indicating the conversion of water from a bound to a freer state, as in apple leaf buds (Faust et al., 1991, 1995), suggesting the appearance of intracellular free water according to Stout et al. (1978) and Callaghan (1991). However, it must be mentioned that the increase in T₂ occurred in apple at the release of endodormancy, whereas in Norway spruce the rise occurred during ecodormancy. Finally, when water ascended in embryonic needles and axes almost to shoot apical meristems, the high proportion of water with a long T₂ (usually assigned to the free water component) decreased in embryonic shoots, and approached that obtained in underlying stems. This decrease could be due, at least in part, to the formation of hydration water following the transport of water in the newly-functional xylem (here, the water associated with xylem walls).

Our study highlights the sequence of events in water availability taking place during spring when buds were ecodormant. The chronology of the most important events preceding bud-burst are: (1) increase of the velocity of water transport in stems; (2) differentiation of protoxylem in embryonic shoots; (3) rapid supply of water to embryonic needles and axes, almost to the shoot apical meristem (Fig. 6). Note, moreover, that most changes of ¹H-NMR signals could be related to the differentiation of protoxylem. This sequence of events was the same in the three clones of P. abies, but was earlier in the V19 Vosges clone with the earliest bud-burst than in the V15 Vosges clone with the intermediate bud-burst, and latest in the Polish clone with late bud-burst. The important role of water in bud-burst previously proposed in some broad-leaved species is, thus, confirmed and clarified in the conifer Picea abies. Our results imply that differentiation of conducting elements in developing embryonic shoots is essential to bud-burst and to normal shoot growth of Norway spruce trees, and that bud-burst occurs as a result of the functioning of protoxylem which permits the mass hydration of embryonic shoots and therefore rapid elongation. Bud-burst corresponds to a dramatic change in shoot growth rate, but results from the qualitative development of shoot apices, which progressively resumes after the release

---

**FIG. 5.** Different views of buds (scales removed) showing the presence or absence of xylem in embryonic shoots on two sampling dates (6 and 24 Apr. 1995) in Polish and V15 Vosges clones of Picea abies L. (Karst.). Median longitudinal sections fixed in CRAF 1 which, in addition to fixing, stains tannins, and treated with phloroglucinol-HCl, which stains lignins. BB, Bud base; Co, cortex; Di, nodal diaphragm; Em N, embryonic needle; Em S, embryonic shoot; M, meristem; N, (embryonic) needle; Pi, pith; Pr, procambium; T, tannins; X, xylem. A, General view of an embryonic shoot and of part of the bud base in the Polish clone sampled on 6 Apr. 1995: phloroglucinol-HCl stained xylem is visible in the bud base, i.e. only beneath the nodal diaphragm. Note a gap due to the autolysis of pith cells under the nodal diaphragm. Bar = 250 µm. B, Detail of A showing the procambium bordering the pith: xylem is not yet differentiated in that part of the bud. Bar = 50 µm. C, Detail of an embryonic axis in the V15 Vosges clone sampled on 6 Apr. 1995, also showing several layers of procambium bordering the pith, but no xylem. Bar = 50 µm. D, General view of an embryonic shoot and of a part of the bud base in the Polish clone sampled on 24 Apr. 1995. In the elongated embryonic shoot, xylem is now present on each side of the pith as a narrow strand faintly stained with phloroglucinol-HCl. Under the nodal diaphragm, the gap due to the autolysis of pith cells is visible. Bar = 250 µm. E, Detail of the embryonic shoot of D showing the procambium and a few tracheids very close to the apical meristem (0·6 mm from the top of the apical dome): xylem is present but does not yet react to phloroglucinol-HCl here. Bar = 50 µm. F, Detail of the same embryonic shoot as in E, showing some tracheids which stain positive with phloroglucinol-HCl at the base of the embryonic axis. Bar = 50 µm. G, Detail of an embryonic axis in the V15 clone sampled on 24 Apr. 1995: tracheids with helical wall thickenings faintly stained with phloroglucinol-HCl are visible. Bar = 50 µm.
of endodormancy, together with the increase in size as spring progresses.

**ACKNOWLEDGEMENTS**

We thank Professor P. Barnola (University Henri Poincaré, Nancy I) who was the originator of the project of pluridisciplinary research, the station of INRA-Nancy for providing plant material, and Dr Anas Mansour for assisting with the cutting and staining of buds.

**LITERATURE CITED**


**FIG. 6.** Sequence of the water-related events occurring in *Picea abies* L. (Karst.) after the release of bud endodormancy and leading to bud-burst.


