XYLEM SAP FLOW AND PRESSURISATION IN *ACER PSEUDOPLATANUS*. L.

by

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ABBREVIATIONS, SYMBOLS AND UNITS

Abbreviations

The following abbreviations are used throughout the text:

dw	dry weight	min	minimum
fw	fresh weight	N.M.R	nuclear magnetic resonance
f.s.d	full scale deflection	O.D	outside diameter
G.T.R	Grants Temperature Recorder	rev.	revolutions
max	maximum	L.D.Tr	linear displacement transducer

Symbols

Ψ	water potential	Ψ_s	solute pressure potential
Ψ_x	xylem water potential	Ψ_p	pressure potential

Units

It is now accepted as standard to adopt Systeme International (S.I.) units, for which the unit of volume is the m^3. However, in an attempt to aid comprehension, the litre has been expressed not as $10^{-3}m^3$ but as dm^3 (cubic decimetre).

ie. 1 litre = $1dm^3$

($\therefore$ 1 M = 1 mole.dm^{-3})

SUMMARY

This thesis presents the results of an investigation into the enigmatic phenomenon of xylem sap pressurisation (or exudation) from dormant trees of Acer pseudoplatanus. L. The characteristics of the system were established, through initial experimentation, and found to relate to previous studies of exudation from trees of Acer saccharum (Marsh). More detailed investigations were then undertaken. Studies were performed both in the field, using mature trees, and in the laboratory, using stem sections. Previous hypotheses appeared inadequate to account for the new observations and an alternative mechanism was conceived. This new hypothesis, based on sap priming through internal frosting, is proposed.

Experimentation revealed that sap uptake, caused by sub-atmospheric stem pressures during cooling, was biphasic. The first phase of uptake was shown to be the result of thermal effects prior to freezing. The second phase was associated with freezing of sap within the tissue. Experiments already described suggest that uptake of sap (or water) before ice formation is a consequence of contraction of gas and liquid and dissolution of gases. The contribution of this phase to subsequent exudation is significantly less than responses induced by freezing of sap (eg. sap absorption before freezing, for change in stem temperature of $10^{o}C$, is $\sim 1/6$ that absorbed during freezing). Sap absorption during freezing is considered to be the response fundamental to 'conditioning' ie. priming of the system during cooling prior to exudation on warming. This event is somewhat paradoxical however, as liquid water expands during its change of state to ice. Conditioning was characteristically rapid and reversible. Sap pressurisation (or exudation) was triggered by melting of ice within the tissue. In the temperature regimes imposed, freezing seems to be extracellular.

Investigations indicated that the freeze-absorption mechanism was a purely physical process based in the sap wood core (ie. the conducting xylem). The presence or absence of sucrose within the xylem sap did not materially affect the system. However, water content of the tissue appeared crucial for optimal conditioning. This is probably a consequence of interaction between water content and gas content of the wood. Experiments monitoring stem density indicate that freezing may produce

growth of ice within gas spaces, compressing the entrapped gas. It is proposed that the gaseous sites may be gas-filled fibres. Anatomical studies revealed these were abundant and located near vascular tissue. Further work should determine the quantitative aspects of the hypothesis proposed.

Correlation of the time sequence of freezing, detected within twigs of a mature tree, with the phase of rapid sap uptake, indicated that ice formation may be of significance within the extremeties (twigs) of a tree, even though the main trunk is the region usually tapped. Ice formation in the remainder of the tree trunks seems to be by invasive 'seeding' of crystallisation from ice formed initially in the twigs. Internal redistribution of sap to the upper regions is envisaged as a consequence of this sequence of freezing, inducing water uptake from the soil by the roots. This is in keeping with the concept of 'conditioning', resulting in the familiar sap pressurisation on thawing which can cause the sap exudation from wounds, so essential to the sugar-maple industry.

GENERAL INTRODUCTION.

1. GENERAL INTRODUCTION.

The fact that species of the genus Acer exhibit transitory pressurisation of xylem sap during dormancy has been known for centuries. Natives of New England and Canada were collecting the sap for its sugar content long before Europeans arrived. Modern maple syrup production in the U.S.A. is a descendant industry of this procedure. Here sap is collected from a wound (borehole) in the maple trunk, and then reduced by boiling. The end product is a sweet, palatable syrup. Xylem sap exudation is not however restricted to Acer. Betula (birch), Juglans (walnut) and Vitis (grape) are but a few examples of other genera that will exude from wounds in the stem. The driving force in these cases though is root pressure, whereas it must be stressed that this is not the case for maple. Exudation is possible from excised stem segments when appropriately treated, (Marvin & Greene 1951).

Sap flow in maples is characteristically temperature dependant, (see later). Exudation will only result during a rise in air temperature subsequent to prior cooling of the tree below 0°C. This oscillation of temperature is fundamental to the system, and certainly a principal factor in restricting exudation to the dormant period. Maple tappers stress that best exudation (both for sugar content and volume) occurs when cold, frosty nights alternate with warm sunny days. Consequently, tapping is usually undertaken in late winter to early spring, before bud break, (ie. late February-April).

These observations make the phenomenon as yet unique. However, considerable confusion and controversy exists regarding explanation of maple exudation. Nearly all investigations have been performed using the sugar maple (Acer saccharum Marsh) as plant material, due to its economic value in the production of maple syrup. However, all Acer species exhibit similar properties. It has therefore been assumed that knowledge accumulated for A. saccharum. is generally applicable to the sycamore (Acer pseudoplatanus. L.), although the limitations of such an assumption have been realised.

The first careful studies of maple exudation were by Clark (1875). He tested 26 different tree genera, observing the presence or absence of sap flow on wounding. Investigating maple exudation further, he used mercurial gauges to record detailed changes in sap pressure during 24 h. periods. This revealed the characteristic oscillation of maple sap pressure for the first time. A sudden rise to a positive value near

noon was followed by a decline to a value below atmospheric, (ie. development of a sap tension), during the night. These extremes of pressure, when measured in the basal part of a sugar maple (A. saccharum) are commonly +0.075MPa and -0.075MPa, values supported by later investigation (Jones 1903, Milburn & Zimmermann unpublished). Pressures recorded decrease with height of the tapping point, due to hydrostatic pressure (10m ≡-0.1MPa).

Jones et al. (1903) showed that pressure fluctuations in maple correlated well with air temperature, although not apparently quantitatively. A positive pressure was produced when the air temperature rose above $0^{o}C$, but sap tensions prevailed below $0^{o}C$. This influence of temperature stimulated proposals for possible mechanisms. Some were depend nt upon a physical system, others involved temperature effects in living cells. A general discussion of these suggestions was published by Wiegand (1906) based largely on quantitative analysis of data from Clark and Jones, (loc. cit.) From calculations, it was concluded that simple expansion or contraction of gas, liquid and wood, or combinations of these, was inadequate to account for the values of pressure observed, or volumes of exudate collected. (Sap flow, of course, only occurs from a wound when the pressure within is above atmospheric, and volumes so collected may frequently be as large as 5 to 6 litres per day, amounting to approx. 60 litres in a season.) Effects due to change of state were also dismissed as inadequate. These conclusions were later supported by Marvin (1958) and Sauter (1974). Wiegand preferred a system involving thermal effects on living cells. He proposed that a temperature gradient existed radially within the tree, peripheral cells being warmer than those of deeper tissue during a temperature rise. This assumedly affected membrane permeability and associated osmotic properties of the cells. Radial movement of water resulted from the inner parts of the tree to the outer, and so produced exudation. Although not fully investigated, this mechanism is thought unlikely as flow can be achieved with no detectable radial temperature gradient, (Marvin & Greene 1951).

Extensive investigation of maple sap flow was undertaken by Johnson et al. (1945). Results were published relating exudation from whole trees, cut stumps (ie. the basal part of stems with roots embedded in soil) and stems (ie. excised trees.) The volume of sap collected from severed stumps compared to that from stems was strongly emphasised, the former being approx. 5 times the latter. Consequently they concluded, falsely, that root pressure was a vital part of the exudation phenomenon.

Regarding the flow mechanism, they suggested that oxygen availability was the key factor, affecting cellular activity and sap hydrostatic pressure in some way. However, no supporting data were presented. Experiments dismissing the involvement of root pressure were performed by Stevens and Eggert (1945). They too used severed trees, with a variety of treatments. Most importantly, stem bases were either stood in water or sealed and left dry. Those with a water supply gave near normal sap yield (compared to controls), whereas dry stems did not yield sufficient sap to produce a sample. If however, the latter were stood in water (after removal of the wax sealing the cut surface) subsequent exudation was possible. Water supply was obviously an important factor. In agreement with the earlier suggestion by Jones et al (1903), they concluded that the cooling period, (during which a sap tension is detected) was responsible for uptake of water and replenishment of sap. This view was further supported by Marvin and Erickson (1956) who statistically analysed data accrued from experimentation. It was emphasised that the phase of crucial influence on exudation was the previous cold period rather than the rise in temperature associated with exudation. This cooling phase with sap replenishment was termed the 'conditioning period' by Marvin (1958). Subsequent warming is considered more as a trigger mechanism producing sap exudation.

Stevens & Eggert (loc. cit.) suggested a physical mechanism was responsible for sap absorption during cooling. They proposed the following:-

> 'The water in the cambium and outer layers of the trunk freezes first, leaving a concentrated sugar solution in the cells. As it freezes, the warmer sap or water moves towards the ice crystals as they form, from the areas of normal sugar content in the inner layers of the xylem. This results in a higher concentration of sugar in the latter cells producing a strong osmotic pull along the length of the trunk. The water, as it moves towards the outside of the trunk, is replaced from the lower parts of the trees. Thus the direction of flow is through the roots and up the trunk to the upper parts of the tree where it is freezing. As the air becomes colder, the sap freezes deeper in the wood; this continues until all the sap is frozen, from the cambium into the xylem to the heart wood and from the top of the tree to the ground or a little below it. Such a condition usually occurs during the night, the time depending on how cold it gets and the rate of temperature fall.'

This attractive hypothesis implies the presence of sucrose (or similar solute) in the xylem sap is critical for exudation which, as yet, is not proven satisfactorily (see later). Freezing of extracellular sap is a real phenomenon, although a few degrees of supercooling may occur (eg. ~6-8°C) (Levitt 1972, George et al 1974a). However whether freezing or partial freezing, of sap is a necessary part of the maple exudation system is a matter of controversy.

Experimentation described to date had been in the field and so subjected to natural environmental fluctuations. This made quantitative interpretation of results difficult. Marvin & Greene (1951) adopted a laboratory approach using excised stems of sugar maple. A special chamber was constructed with accurate temperature and humidity controls. Exudation collected after temperature treatments indicated no apparent patterns of a) duration of flow b) sap flow rate or c) volume of sap exuded. Variations of these were found not only between seperate stem samples, but also different samples of the same stem. Experiments were therefore of a qualitative nature- ie. whether or not sap flow occured after treatment.

Stem samples collected in August (while the tree was in leaf) gave no exudation upon freezing and warming. Material that was killed by steam, but considered undamaged otherwise, also failed to exude. This latter observation was considered as strong evidence for the involvement of living cells in sap flow. However it was concluded that the cooling (conditioning) phase was at least in part a physical phenomenon. Freezing of sap was considered unnecessary for sap flow on warming. Exudation was possible by warming stems previously unfrozen, although volumes obtained were smaller than from stems that were first frozen and then thawed.

The precise site of pressurisation is not known. It has been shown by Jones et al (1903) that most of the sap flow into a hole made in a maple trunk occurs from above. Presumably the site(s) is(are) therefore in the upper regions of the stem. Frequently sap flow may be obtained from one side of a tree in sunlight while no flow is obtained from the shaded side of the trunk. Accordingly yields tend to be greatest form boreholes made in the south side of the tree, (Kramer 1949). Recently Milburn & Zimmermann (unpublished) have suggested these and similar enigmatic observations are due to ice blockage of xylem conduits. In a sunlit environment of subzero air temperatures, shaded parts of the tree may be frozen while regions in

direct sunlight have thawed. Hence vascular conduction and flow may be established for only part of the tree. This may be complicated further by spiralling of vascular tissue within the tree. As thawing proceeds, pressurisation of the tree as a whole may then result. In this state, lower or no +ve. pressure is detected in the higher branches, due to hydrostatic effects. Thawing rates also differ between different parts of the tree due to differing heat capacities. Twigs and thin branches warm (and conversely cool) more rapidly than thick ones. Liquid continuity may therefore exist in the extremities of the tree during early thawing, before more basal parts thaw. This delay is reversed for the freezing process.

As already stated, the sap pressurisation system is stem based. In the field, however, replenishment of sap is presumed to be accomplished by absorption of water through the roots. Resistance to this movement would therefore influence subsequent exudation. Milburn & Zimmmermann (unpublished) have shown that sap can be sucked out of root stumps (using a vacuum pump) with ease during the exudation season. A pressure of 0.03 MPa below atmospheric pressure is sufficient. Late in the season however, (but before 'bud-break') little sap was obtained even with a pressure nearly 0.1 MPa below atmospheric. They concluded that the permeability of the root system may be an additional factor determining sap exudation - mediated through effects on water status of the stem wood.

Although no satisfactory explanation of maple sap exudation exists the recent and most acceptable mechanism was suggested by Sauter (1974). It is generally upheld that sap flow requires living cells and additionally a physical system. Sauter suggested that living cells were of consequence in the generation of CO_2, which gave rise to a temperature-dependant physical system. He conceived three main components to the mechanism:-

1) 'Living System' - of the wood, (ie. all the living cells). Aerobic and anaerobic respiration generates CO_2 for the physical system, the efficacy of which is dependent upon the CO_2 level. The living system is therfore termed the 'charging unit'

2) 'Air System' - of the wood, (ie. the region of importance is considered to be the fibrous tissue around the vessels). CO_2 available is dissolved in sap generally throughout the wood, but has particular consequence within the fibre walls. CO_2 solubility changes dramatically with temperature (eg. at atmospheric pressure,

100cm^3 of water dissolves 171cm^3 of CO_2 at 0°C, but only 88cm^3 at 20°C). Pressure resulting from CO_2 coming out of solution due to temperature rise would be accentuated by the fibre walls - in theory. Conversely, cooling of sap would result in reduced pressure (ie. a tension). This system he termed the 'power unit'.

3) 'Water System' - of the wood (ie. the vessels). Being embedded in the fibrous tissue, pressure changes round the vessels would either 'squeeze' and pressurise them or give 'relaxation', producing a sap tension. When severed, outflow would only occur when the hydrostatic pressure in the vessels was originally approx. that of atmospheric pressure.

In this system then, the rate of flow is dependent on compression of the vessels, which is itself dependent on the CO_2 accumulated (determined by temperature and cell activity). The hypothesis is supported by experimentation on stem sections, (Sauter, 1974), which showed that CO_2 production was sufficient to load the air system within one to a few days, dependent on temperature. Moreover, the presence of abundant gas-filled fibres in close association with the xylem vessels is characteristic of maple wood, and has been observed frequently. (eg. Jones 1903, Wiegand 1906, Sauter 1974). In addition, many other workers (cited earlier) have observed that volumes of sap exuded are higher than can be accounted for by simple thermal expansion of gas, water or cell walls. Volume changes from differential gas dissolution are considerably greater than changes from thermal expansion, and were considered large enough to account for volumes exuded from stems subjected to controlled temperature change, (Sauter, personal communication). All temperature treatment however, was in a range above the freezing point of the sap (4-22°C). Although sound in principle, compression of xylem vessels is most unlikely, as large forces are required, (eg. Molz and Klepper (1973) showed that for a cotton xylem cylinder of 5.07mm. diameter, a load of 1MPa produced no change in diameter, measured to within 0.008mm.). It may be, as claimed by Sauter, that only very small changes in vessel diameter are required to account for observed exudations, but the difficulty of substantiating such changes is enormous. Marvin (1949) noted that xylem diameter changes for sugar maple were minute. An increase in diameter of 55 μm was observed for a xylem cylinder of 46cm. Hence the change was merely 1.2 μm cm^{-1} wood. (ie. 0.0012%). Simultaneous changes in bark thickness were detected however, and these were far larger - a thickness of 1cm. exhibiting a

a change of 82 μm (ie. 0.8%). That diameter changes are greater in the bark is a general observation, (Marvin 1949, Molz & Klepper 1973, Pook & Hall 1976). Differences in xylem and bark cell wall elasticity obviously contribute, and also the fact that living cells readily exhibit turgid and flaccid states. Contraction of bark during cooling, (reversed by thawing), is considered a consequence of extracellular freezing. Cells shrink as water diffuses out to ice loci. (Small & Monk 1959, Levitt 1972, Pook & Hall 1976). Responses of the xylem cylinder during freezing may be expansion. (eg. Snowgum - Pook & Hall 1976) or contraction (sugar maple - Marvin 1949). The nature of the response however, is thought to be determined principally by the water content of the tissue, (Levitt 1972, Pook & Hall 1976). That of high % water exhibits increased diameter on freezing and even 'frost splitting'

Considerable investigation of the water - and gas - content of maple wood was undertaken by Jones et al (1903). Variations between different parts of individual trees (eg. root, trunk, twigs) were so high that generalisations were considered impossible. Comparison of % water content of the inner and outer xylem tissues of the trunk was more profitable. The outer sap wood (depth 3 inches) maintained a relatively steady value between December and April, (eg. approx. 38%), whereas that of the inner wood (depth 6 inches) increased dramatically, (eg. Dec. 30%, April 58.8%). Water content for both samples dropped in the ensueing months to similar values (eg.approx. 27%). Jones commented that the outer younger tissues were well known to be most actively concerned in the general phenomenon of the ascent of sap. In comparing the values for summer and winter, he stressed that accordingly young sap wood is the first to be highly charged with water. Although much data has been amassed on this general topic, (Spaulding 1900, Jones (loc. cit.) , Gibbs 1935) understanding of maple exudation has not been dramatically enhanced. Interpretation of results seems impaired by the considerable enigmatic variation found. It appears, therefore that further understanding of the maple system is required before fuller interpretation is possible of the data so far collected.

The ability of a maple tree to exude would have attracted little interest if the sap did not contain sugar. Its presence is only detected during dormancy, and simultaneous manifestation of positive sap pressure makes collection a simple and economic procedure. Exudate from the basal part of a sugar maple trunk may contain 0.5 - 7% sucrose (Bryan

et al 1937). Such variation is present between stands of trees, individuals, and also throughout the tapping season, (Taylor 1956). Usually the value is nearer 2 - 3%, and this is approximately twice that recently shown for sycamore (Milburn, unpublished). Exudates from higher up the tree, particularly from the branches, are more concentrated, (Jones et al 1903). This may simply be an effect from the source of sucrose, which is derived principally from carbohydrates stored as starch during the summer months. It was clearly shown that starch is deposited in the outer ray cells, progressing inwards. No starch was detected in cells of the pith unless the ray cells were full. Hence more starch may be stored in the twigs and branches than may be stored in the trunk. Jones et al (loc. cit.) also showed that defoliation during summer greatly reduced the sugar yield in the following spring. Tapping of the tree however, appears not seriously injurious, as trees have been tapped for many years without visible damage. It was estimated that tapping removes less than 10% of the total sugar in the tree.

Although sucrose is by far the main constituent of the sap, other trace compounds are also present. These include organic acids, nitrogen compounds (including ammonia, peptides and amino acids - Pollard & Sproston 1954) inorganic salts, and various other substances (Taylor 1956). Conversion of starch to sucrose commences during dormancy, when temperatures are low. This is not considered to be the classic frost hardening response (Scarth 1944, Levitt 1972) where increased solute content is associated with acclimation to low temperatures during several weeks. This usually occurs within living cells, and xylem conduits are dead. Work by Marvin and Morselli (1971) has detected the presence of rapid low temperature hydrolysis of starch to sugars in maple stems and tissue cultures. They infer that a precise temperature control mechanism is operative at 4-6°C, its influence mediated by effects of temperature on pH. However further investigation is necessary to clarify this.

The selective abundance of sucrose, as opposed to other sugars, in the xylem sap has been noted by various workers (eg. Morse & Wood 1895, Jones et al 1903, Taylor 1956). It was not until recently however, that explanation of this was published (Sauter et al 1973). Studies of the release of sucrose into the vessel lumens of sugar maple indicated the intimate involvement of specialised cells - called 'contact cells'. These are located in the xylem rays and have large elliptical pits of 3-5 μm diameter abutting the xylem vessels. Biochemical and enzyme-cytochemical investigation showed a greatly increased activity

of respiratory enzymes (eg. succinate, NAD - dependant isocitrate, and alcohol dehydrogenases) and acid phosphatases in these cells. Acid phosphatase activity - responsible for the conversion of starch to sucrose - was demonstrated to be concentrated at the large pits previously described. Increased enzyme activity was also shown to be limited to the time when sucrose appeared in the vessels (generally November - April), disappearing when leaf expansion occured. As the contact cells were the only cells showing such associations, Sauter concluded them to be the specific sites of metabolically controlled sucrose release to the xylem vessels.

It is not surprising that the degree of exposure and the size of a tree and its crown, influence the sap sugar content, (Morrow 1955). Photosynthesis during the previous growing season undoubtedly influences the amount of starch that may be stored for conversion to sucrose. Heredity, soil condition and tree age are also influential. It is interesting that not only do these factors influence % sugar content of the sap, but also volume yield, (and pressure as trees of high pressure give high yield). The considerable variation found here between trees is indeed attributed in part to these factors. However, above these variations, many workers have noted a degree of consistency. Trees that produce high yields (volume) tend to retain their yearly status relative to other trees. Moreover, higher yielding trees also tend to give higher % sugar content of the sap (Morrow 1952). This observation was supported by findings from an 18 year study by Marvin et al (1967). Further investigation of this by Morselli et al (1978) used image-analysis by computer. It was revealed that more and larger vascular rays were detected in sugar maples of higher sap and sugar yield. This accords with findings by Sauter (loc. cit.) regarding the specific loading of sucrose, as contact cells are located in the vascular rays.

The presence of sugar in xylem sap and the detection of positive sap pressure are only observed in dormant maples. This has led to some degree of association. As stated above, a distinct correlation has been found between volume yield (and pressure) from a tree and sap sugar content. However, evidence for direct influence of one on the other is rare and difficult to interpret. Marvin & Greene (1951) studied sap flow, in response to cooling and warming, from excised stems previously eluted with various solutions. Stems that had sap displaced by distilled water showed an absorption on warming rather than exudation. Replacement of

the water with precollected sap gave exudation, after appropriate treatment. More experimentation with other solutions caused Marvin to conclude that the presence of 'osmotically active' solute was crucial, but this need not be sucrose (eg. raffinose, maltose, lactose were all effective.) However, more recent work by Sauter (unpublished) indicates that stem sections from sugar maples in full leaf will exude if cooled and warmed simulating typical 'late winter' temperatures. Sap from trees in leaf contains no detectable sucrose, and often approximates to distilled water with regard to its solute content. Obviously further investigation of this topic would be of use.

Consideration of reasons for the presence of sucrose in maple xylem sap during dormancy, and the manifestation of positive sap pressure, reveals various speculative possibilities. It has been suggested that it may be a readily available substrate for future growth. As dormancy breaks, transpiration would cause nutrients in the xylem sap to be transported to the growing leaves - much needed during an initial period of rapid growth, (Milburn 1979). Alternatively the sucrose may be involved in the pressurisation system - directly (Marvin & Greene 1951) or indirectly. The role played by sap pressurisation is also unclear. A credible suggestion is that positive hydrostatic pressures would help 'repair' - ie. refill - gas filled vessels that had previously embolised. Hence non-conducting vessels may be made available for future water transport, a system of obvious advantage to a diffuse porous plant.

In summary, then, the phenomenon of maple sap exudation is a unique, complex system as yet inadequately explained. Though temperature-dependent, a clear quantitative relationship with sap flow has not been demonstrated. Considered at least in part to be a physical process, living cells are thought to be involved either directly or indirectly. The critical phase determining subsequent sap flow (and pressure) on warming is the cooling or conditioning period. During this, water is absorbed by the stem, through the roots, from the soil. Throughout this phase a tension is detected within the tree. The mechanism responsible for induction of this sap tension/ absorption is the issue of mystery, and although proposals have been made, none fully account for observations recorded to date.

Reasearch undertaken for this thesis aimed to clarify certain issues concerning maple exudation that appeared crucial for fuller understanding of the phenomenon. Acer pseudoplatanus was studied, as mature trees were readily available and hence results from laboratory and field experimentation could be compared. This species has also been shown to respond in a similar way to A. saccharum, (Milburn unpublished). Clearly some aspects of the topic are of greater importance than others - as evident from the introduction - and concentrated investigation of these was considered profitable. Whether sucrose was involved directly in the system and whether living cells were intimately involved seemed questions with, as yet, ill-defined answers. Greatest priority, however, was given to investigation of the conditioning (cooling) phase. Knowledge of processes operating during this will undoubtedly further general understanding of exudation, and hopefully reconcile the numerous enigmatic, sometimes conflicting observations.

MATERIALS & METHODS.

2. MATERIALS AND METHODS.

2.1 Plant Material & Growth Conditions

The experimental plant used in this research was Acer pseudoplatanus. L. (the sycamore). Field investigations utilised the fact that mature specimens were available, growing openly in a field in Garscube Estate, Switchback Road, Glasgow. Three trees were chosen, appearing healthy, approximately 13m. tall and with trunk diameters of 50 - 60 cm. measured at 1m. height. They were positioned 20m. equidistant from each other, the nearest neighbouring tree of any kind being at 10m. Each of the three trees was assigned a letter - 'A', 'B' or 'C'. Tree 'A' (plate 2.1) was used for intensive investigation, and a small, wooden hut was positioned 2m. from the base on the north side for storage of experimental equipment. The trees were isolated enough to be untroubled by passers-by, and individually fenced to prevent damage by farm animals.

Laboratory work was performed using seedlings, supplied by Ben Reid Nurseries Ltd., Aberdeen, U.K. These were 3-4 years old when purchased in April 1977. (Plate 2.2) In total 600 were individually planted in soil (proportions, sand:loam:peat - 1:1:1)using whale-hide pots, and positioned on a 10 x 10m. plot in the Botany Laboratory Garden, Garscube Estate. With subsequent growth, the roots of most seedlings penetrated the pots and entered the soil beneath. Shoot growth was also substantial, many seedlings doubling their height, (eg. from 1m. to 2m.), during the project. To ensure more uniform stem segments for experimentation (see later) pruning of any side shoots on the lower 40cm. of stem was performed soon after purchase. The only other interference with the seedlings - apart from sampling - was the control of weeds by the application of 'Paraquat' (Scottish Agricultural Industries Ltd., Edinburgh, U.K.) during the summer months. This was considered to have no adverse effects.

2.2 Experimental Conditions.

The most important parameter affecting xylem sap exudation in Acer appeared to be that of temperature. Hence, experiments throughout

Plate 2.1
Tree 'A', used for intensive investigation of sap pressure potential, (height of tree is approx. 13m.)

Plate 2.2

Example of sycamore seedlings (total population ~600) used in laboratory experimentation. Growth and cultivation was performed in the open (see text). Height of each ~ 1.5m. Note lower 40cm. stem lacks side branches.

the project revolved around manipulation of this, though field work was of course subject to natural environmental changes. An integral part of laboratory experimentation was a controlled temperature chamber, (see diag. 2.5), built around the cooling unit of an incubator (Gallenkamp Ltd., London, U.K.). The principle aim of its construction was to provide a relatively easy means by which plant material (ie. 30cm. stem segments) could be repeatedly subjected to standard temperature treatments. Sheets of polystyrene (thickness 5cm.) were cut and positioned to seal off the upper half of the incubator. A polystyrene box (inner dimension 24 x 38cm.) was placed inside this to form an inner compartment, which was used for treatment of segments. The net result of this arrangement was for the temperature of the inner compartment to lag behind that of the outer during cooling until the whole equilibrated to a stable minimum temperature. This was $-5^{o}C$ in early experimentation, but later reduced to about $-10^{o}C$ with improved insulation, (the actual value depended upon the room temperature). A system of double chambers was necessary to produce a low final minimum temperature, yet with a slow enough rate of cooling. Its use was simple. A cool phase was induced by turning the incubator thermostat setting to minimum, and a warm phase by alteration to $+15^{o}C$. Each temperature phase involved an initial period of rapid change (approx. 1 hour) when the compartment air temperature altered by 75% of its total change. This was followed by a longer period (approx. 3 hours) of increasingly slower equilibration of temperature to its final value. Some degree of automation was possible using a time switch at the mains socket. With the incubator thermostat set to a minimum, successive switching on and off produced cyclical temperature changes of predetermined duration. The only inaccuracy here was the maximum temperature attained during the warm phase of $+17^{o}C$ ($\pm$ $1^{o}C$). This was considered acceptable.

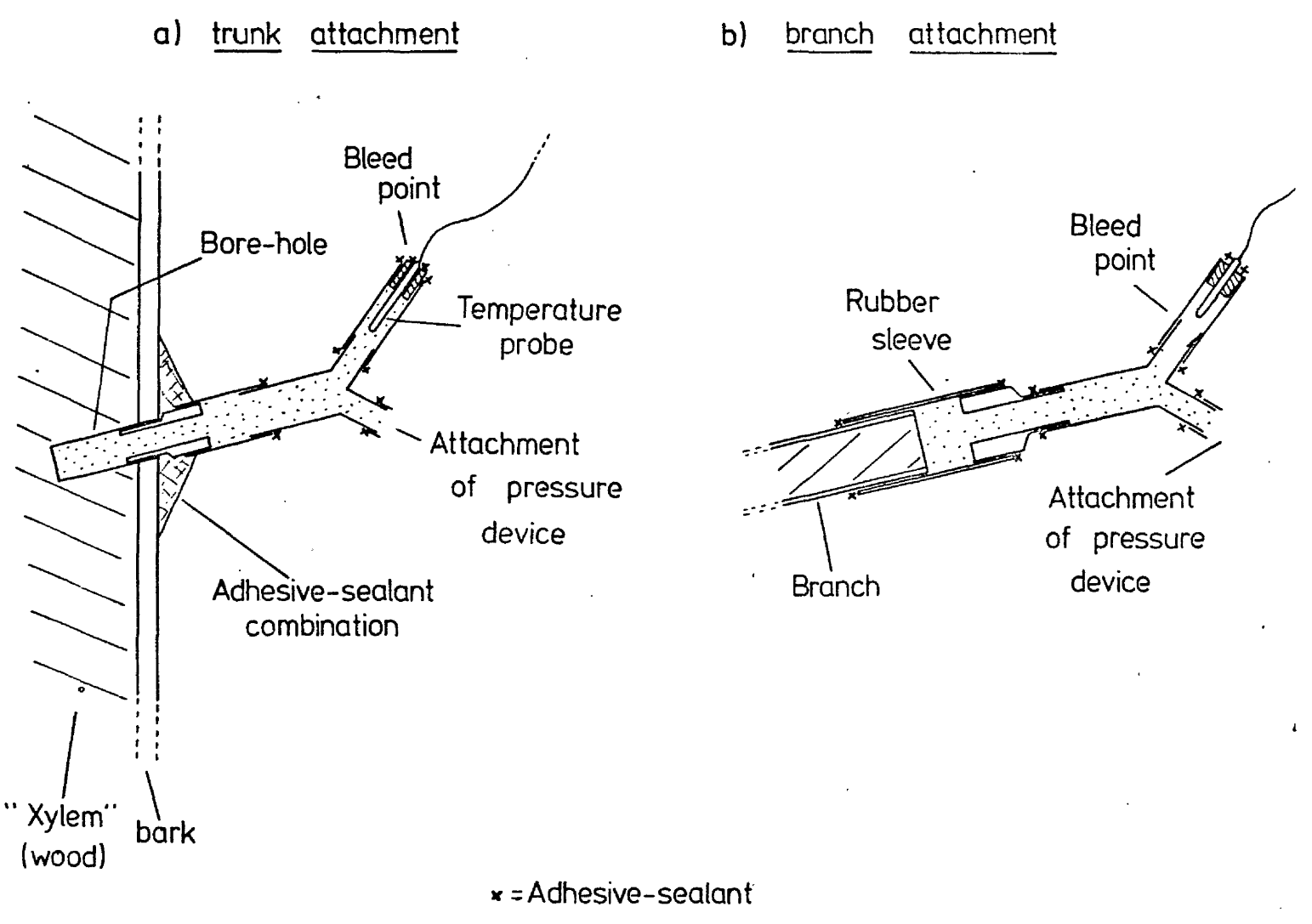
Stem segments for treatment were positioned in the chamber angled downwards at approx. $30^{o}C$ to the horizontal, apical ends flush with the outside surface of the compartment insulation. The holes in the latter, through which the stems were placed, were well packed with insulation. Stem segments were not illuminated during experimentation. Temperature gradients within the chamber were monitored with appropriately positioned temperature probes. Little difference was found on a vertical plane, but horizontally air in the front 5cm. of the chamber was found to be approx. $2^{o}C$ ($\pm$ $1^{o}C$) higher in value than

the rest. This was because of reduced quality of insulation in the region where stem segments were inserted.

2.3 General Methods.

i) Preparation of plant material for experimentation.

Field Apart from determination of xylem sap tensions using the pressure bomb, field work was restricted to those months of the year when trees were dormant. These investigations involved the monitoring of xylem sap pressures using manometric recording devices and/or Bourdon pressure gauges (pp. 24, 30). Attachment of these to trees (see diag. 2.1a) involved boring holes in the trunk (ht. 1m.) initially to a depth of 3cm. (ie. approx. 2cm. into the xylem.) Slight deepening of the holes was performed when necessary, (eg. every 5-10 days). This was because of xylem blockage in the region of the borehole presumably caused by natural wound responses, (Gilman & Steponkus 1972) and entrapped air (Kelso et al. 1963, Baxter 1977). Usually 2 holes were made per tree, each 1.25cm. in diameter, angled downwards at approx. 30° to the horizontal. This ensured that any gas could float free of the hole. A tapering plastic adaptor (dimension: one end, 1.2cm. O.D., 0.9cm. bore; other end, 1.1cm., O.D., 0.9cm. bore) was hammered firmly into place, and the whole washed out with distilled water. A Y-junction was attached, acting both as an attachment for the pressure recording device and a bleed point at which later all air could be excluded from within. The system was sealed by location of the temperature probe and/or bung. Impact adhesive (Evostik, Evode Ltd., Stafford, U.K.) was applied around the hole, to a radius of 5-7cm. followed by silicone rubber sealant and adhesive, (Dow Corning International, Brussels, Belgium.). The joint might be subjected to hydrostatic pressures of $\pm$0.075MPa, and after much experimentation with glues this combination was found to give a particularly good seal. Application had to be to a dry surface however. Drilling of the holes was often undertaken when the tree was pressurised, and here exudation was allowed to occur freely. When the glue had set sufficiently to withstand the pressure, the appropriate pressure device was attached and the system sealed. Alternatively, the tree was bored when the xylem sap pressure exhibited a transient tension (eg. when the air temperature prevailed below freezing point of the sap) prior to thawing and development of a positive hydrostatic pressure. Here, air blockage

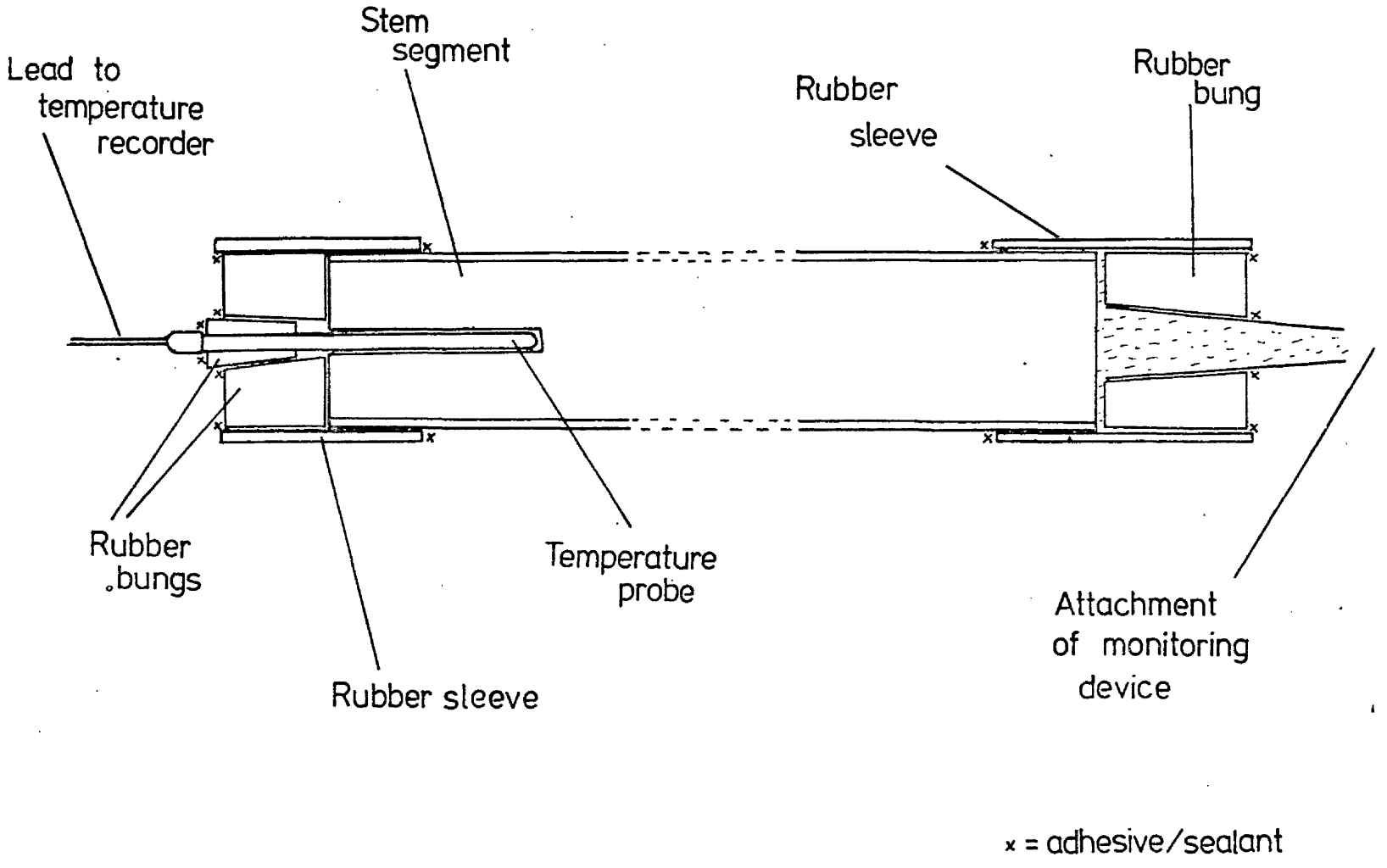


Diag. 2.1: Arrangement for attaching pressure monitoring devices to mature trees.
a) trunk attachment. b) branch attachment.

(embolism) of the exposed xylem vessels occured, preventing any response by the attached pressure device. In such instances results were disregarded until the tree had thawed (usually the following day) when production of a positive sap pressure displaced and dissolved the aforementioned air, producing liquid continuity.

The same principle of procedure was used for attachment to a branch, (diag 2.1b). After excision with a hacksaw, the stump attached to the tree was trimmed with a clean, sharp razor blade. A tight-fitting rubber sleeve and tapering plastic adaptor of appropriate sizes were located, and the joints sealed well with glue as before. The branch and surface were washed with distilled water before filling and sealing of the system. Branches selected were angled to the horizontal such that any gas could float free of the cut surface during experimentation.

Laboratory The principle plant material used for laboratory investigations was 30cm. stem segments sampled from the stock of seedlings. These were convenient to handle, allowed some degree of standardisation of experimental material, and gave well defined responses when subjected to a standard temperature treatment. Segments were chosen for uniformity for each experiment but fresh weights for each batch (with bark intact) varied (eg. 30 - 60g.) depending upon diameter of stems (eg. basal diameter 0.9 - 1.9cm.) The majority of experiments involved attachment of segments to a monitoring device (eg. manometric recorder, p. 28, pressure transducer, p. 31, simple pipettes p. 22). Preparation for this was as follows. Seedlings in the garden were selected for uniformity and details noted, (eg. height, age, relative yearly growths). Frequently in the summer state pressure potential (Ψ_p) was determined using the pressure bomb (see p. 23). They were then individually excised, 5cm. above ground level using secateurs or a hacksaw. Occasionally samples were diseased. This detection was simple and obvious, as the basal cut surface showed black necrotic regions. In such instances alternative samples were taken. After transportation to the laboratory, sap samples were taken (see p. 33) and less frequently bark samples (see p. 35). If barkless segments were required the bark was stripped from the basal 35 - 40cm. of stem, using a blunt knife to scrape the wood if necessary. The naked wood (xylem core) was then wrapped in polythene and self adhesive P.V.C tape (R.S. Components Ltd., U.K.) wound tightly around it so as



Diag 2.2 Illustration showing seedling stem segment prepared for attachment to pressure monitoring device.

to form a waterproof protective coating. All stems were then treated the same way. Namely a hole 3.5cm. x 0.3cm. was drilled centrally into the basal end of the stem. A tight fitting rubber sleeve with bungs locating a steel encased thermistor probe (5cm. x 0.32cm.) was positioned so as to embed the probe into the wood and seal the stem cut surface (see diag. 2.2). For ease of insertion, a small amount of soft, yellow paraffin was used as lubricant (B.D.H. Chemicals Ltd., Dorset, U.K.). Ensuring the region was dry, impact adhesive (Evostik) was applied to the joints, followed by silicone rubber sealant and adhesive, (Dow Corning). The stem was cut to 30cm. under distilled water using secateurs or a hacksaw, and the end trimmed with a clean, sharp razor-blade. This reduced the possibility of air blockage of xylem conduits at the exposed surface. A tight fitting rubber sleeve and tapering plastic adaptor were then attached before removal from the water. After blotting dry, adhesive was applied to the joints as above.

As standard procedure, stems were then usually 'pretreated'. That is, they were appropriately positioned in the controlled temperature chamber (p. 17) and supplied with distilled water at 15°C for 12 hours. Clean $10cm^3$ glass pipettes were used, attached by vinyl tubing. Each water meniscus was manipulated so as to be level with the basal end of the relevant stem segment. This treatment was adopted in an attempt to standardise tissue water status to same degree. It was particularly useful during summer months, as stems exhibited considerable sap tension (and associated water deficits) due to normal transpirational demands. However, it must be noted that results described in the following chapters could be obtained - in qualitative terms - from stem segments not 'pretreated'.

ii) Measurement of xylem sap pressure potential, (Ψ_p).

The energy status of water in plant tissue is commonly expressed as its water potential (Ψ), a value relating the chemical potential of pure, free water to that within the plant. It cannot as yet be directly measured, but can be deduced from estimation of critical components that contribute to its value. Hence the equation:-

$$\Psi = \Psi_s + \Psi_p + \Psi_m + \Psi_g$$

where s, p, m, and g, are the components of solute (osmotic), pressure

(turgor), matric and gravitational potential. Ψ_s, $_m$ & $_g$ are -ve and Ψ_p may be +ve. or -ve., though it is common for xylem Ψ_p to be -ve. (ie. exhibit a sap tension). Matric and gravitational potentials are usually small enough to be insignificant.

The nature of the project was such that -ve. xylem Ψ_p often prevailed during summer months, while Ψ_p fluctuated from +ve. to -ve. during winter. Devices for recording these pressures were therefore chosen appropriately.

a) Scholander - Hammel pressure-bomb.

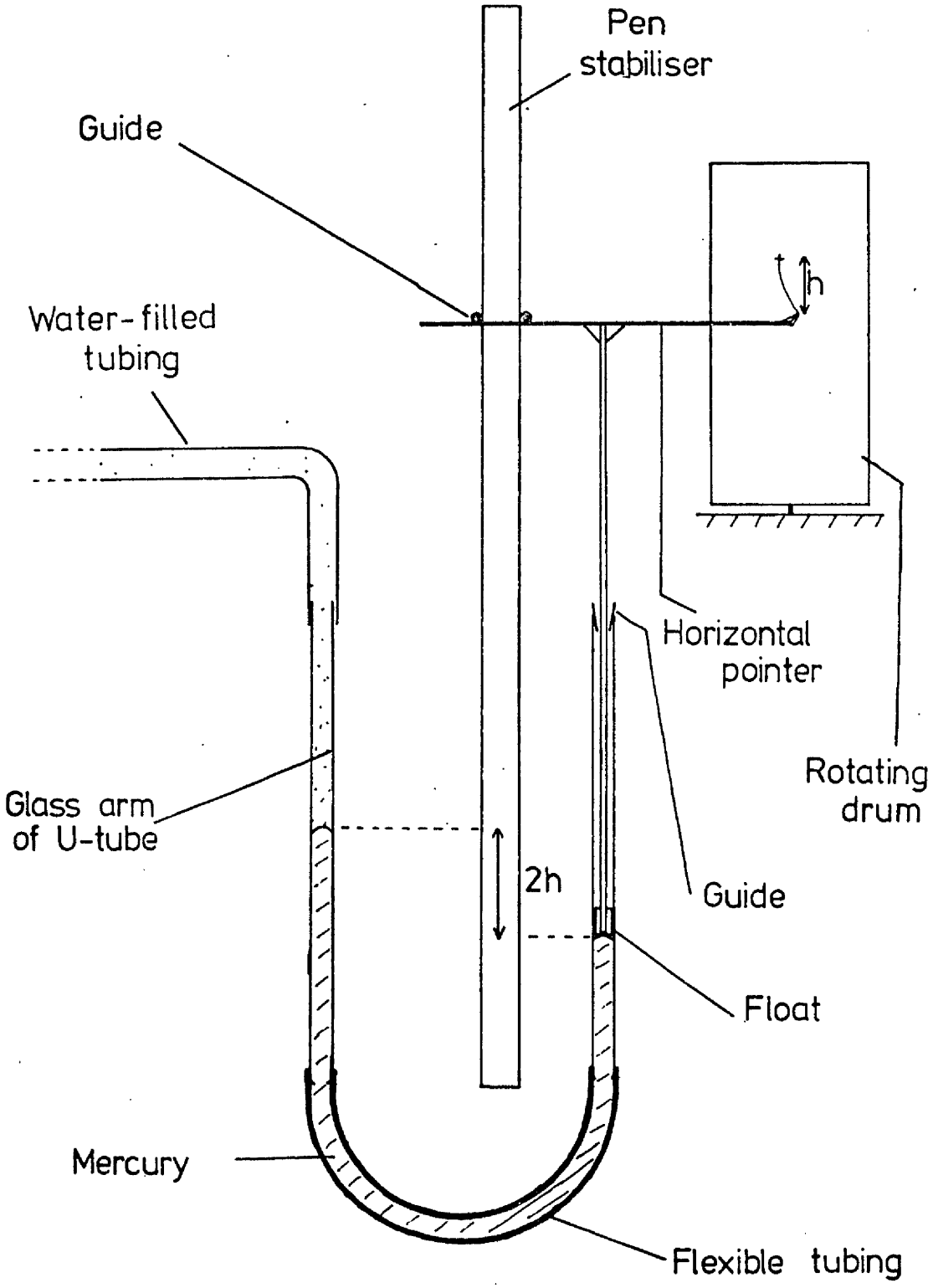
During the summer, a pressure-bomb was used to estimate sap pressure potential (Ψ_p) on mature trees and seedlings. The theoretical principles of its use are outlined by Scholander et al (1964, 1965), and many other authors have considered its use in depth, (eg. Boyer 1969, Ike et al 1978, Kaufmann 1968, Waring & Cleary 1967, Millar & Hanson 1975, Ritchie & Hinckley 1975.) A standard diving cylinder of compressed air functioned as a pressure supply, enabling use of the apparatus in the field - trees 'A', 'B' & 'C'. Determination of Ψ_p for the seedlings was accomplished by transporting samples, with great haste, into the laboratory. For each estimation samples of similar twiglets, subtending 2 or 3 leaves, were enclosed in plastic bags, sealed with wire whilst attached to the tree/seedling. All samples were left so, for $\geqslant$ 1 hour, to allow equilibration of leaf cells with xylem sap water potential so that bomb measurements were true reflections of xylem sap tension. The internal dimensions of the bomb made it necessary, when bagging, to crumple the laminas to a size small enough to fit within. After equilibration, samples were excised with a clean, sharp razor-blade, leaving 3cm. of stem protruding from the bag. The latter was inserted through a rubber bung containing a central hole. To reduce tissue damage here, a cork borer of appropriately larger size was used to thread it. The bung was then inserted into the bomb coverplate and with the bagged leaves inside the body of the bomb, the coverplate screwed down securely. Pressure inside the chamber was slowly increased, (eg. 0.4 MPa min^{-1}) to minimise temperature changes associated with pressure changes, (see Tyree et al. 1974). When xylem sap was first visible on the cut surface of the protruding stem, the corresponding positive pressure value was recorded and taken as equal but of opposite sign to the xylem sap tension (Ψ_p). To ensure the expressed sap was true xylem sap and

not that displace form embolised conduits, the chamber pressure was then slightly increased before final venting. To reduce errors, the time between excision of the bagged sample and determination of Ψ_p was kept to a minimum. Hence Ψ_p was determined for each sample before excision of the next one.

b) Manometric pressure recorder.

The principle tool of investigation was that of a simple mercury manometer designed to afford continuous monitoring of xylem sap pressures (see diag. 2.3). This system was developed because it was decided that a constant monitoring device would provide information greatly superior to any discontinuous system (eg. pressure gauges). Unfortunately, because of the isolation of the site, no mains electrically operated equipment could be used (eg. pressure transducers & chart recorder). Although fundamentally similar in design, recorders for field and laboratory work differed in detail.

Field recorder - A water-filled, plastic tube, 5m. long. of constant bore 0.65cm. (O.D. 1.3cm.) connected the tree tapping point (eg. borehole adaptor, see p.18) to a glass armed U-tube containing mercury. The latter was positioned inside a wooden hut for protection. All joints were tight fitting, but more durable seals were achieved by application of glue (impact adhesive - 'Evostik', (Evode Ltd., Stafford, U.K.), and epoxy resin 'Araldite' (Ciba-Geigy, Duxford, Cambridge, U.K.). The glass arms of the manometer were of constant bore 0.7cm. (O.D. 1.0cm.) and each 60cm. long. The U-tube junction was a flexible plastic tube of appropriate dimensions. Enough mercury was used, (approx. $35cm^3$), to give a 30cm. column in each arm. A float rested on the surface, fitting snugly inside the open arm, yet free to move. This was constructed from the end 1cm. portion of a $1cm^3$ polypropylene syringe, (Plastipak, Republic of Ireland.). Teflon tape (Chromatography Services, Wirral, Mersyside, U.K.), was wrapped tightly around it to ensure an accurate but lubricated fit. A fine glass capillary extension rod (length 85cm., diam. 0.2cm.) connected the float to a horizontal extension arm (length 25cm., diam. 0.2cm.) and ink marker. Hence, any fluctuation in mercury level was transmitted via the ink marker and recorded on a clockwork rotating

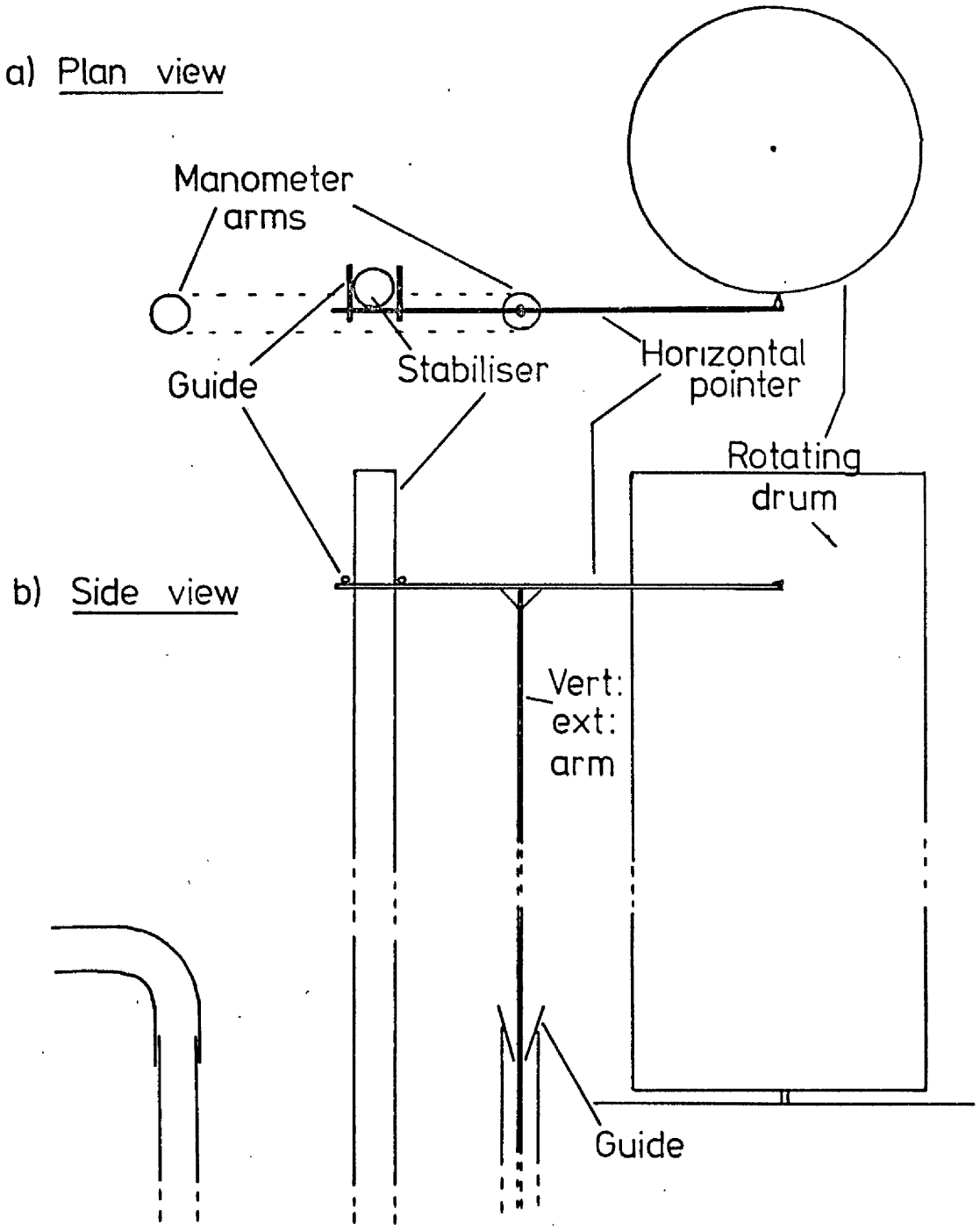


Diag. 2.3

Illustration of basic manometric recording device designed for continuous monitoring of sap pressure fluctuations.

drum, (C.F. Casella & Co. Ltd., Britannia Walk, London, U.K.). Drum rate was 1 rev./week or 1 rev./day, circumference was 28cm. The resultant trace reflected fluctuations in level of only one of the two columns of mercury. Hence, any change in marker height, 'h' cm., represented a difference in height between columns of 2 'h' cm., ie. pressure of 2 'h' cm. Hg. (see diag. 2.3). To prevent snagging of the float and to increase accuracy, a system of guides was devised which effectively restricted movement of the ink marker to a vertical plane. (see diag. 2.4). A plastic cone (ie. cut from a Finnpipette disposable tip - Jensons, Hemel Hempstead, U.K.) with a central hole (diam. 0.25cm) at the tapered end was located into the top of the open manometer arm. This prevented lateral movement of the vertical extension rod within the glass manometer arm. A stabiliser (ie. straight glass tube, O.D. 1.5cm.) was firmly held in a vertical position with the manometer. Two 3cm. long pieces of fine glass capillary were then glued, in a horizontal position, onto the end of the horizontal pointer so as to fit either side of the stabiliser. Hence, when the ink marker was in contact with the rotating drum, accuracy of recording was maintained for all values of pressure experienced.

Exposure of the water-filled tube to the environment produced problems of freezing during cold periods. This was overcome by using a water jacket system. A flexible vinyl tube (bore 1.0cm.) was coiled along the length of the water-filled recorder tube, and through this was pumped water from a reservoir situated in the hut. A 12v. D.C. immersible pump was used (Heller Marine & Caravan Equipment Ltd., Germany.) operated from a power source of 2 x 12v. car batteries, connected in parallel. The temperatures of both the reservoir and hut interior were constantly maintained above $0^{\circ}C$ by a paraffin heater. Hence, by careful lagging of all tubing exterior to the hut with expanded neoprene insulation tubing, the water in the recording system could be prevented from freezing at air temperatures as low as $-10^{\circ}C$. To monitor the temperature gradient between the water in the recorder system and the xylem sap in the tree, steel encased thermistor probes (dimensions - 5cm. x 0.32cm.), were sealed into the trunk adjacent to the borehole and also at the bleed point of the trunk adaptor. (see p. 18). Results indicated the temperature of the sap was about $5^{\circ}C$ below that of the recorder water, a value that was considered acceptable.

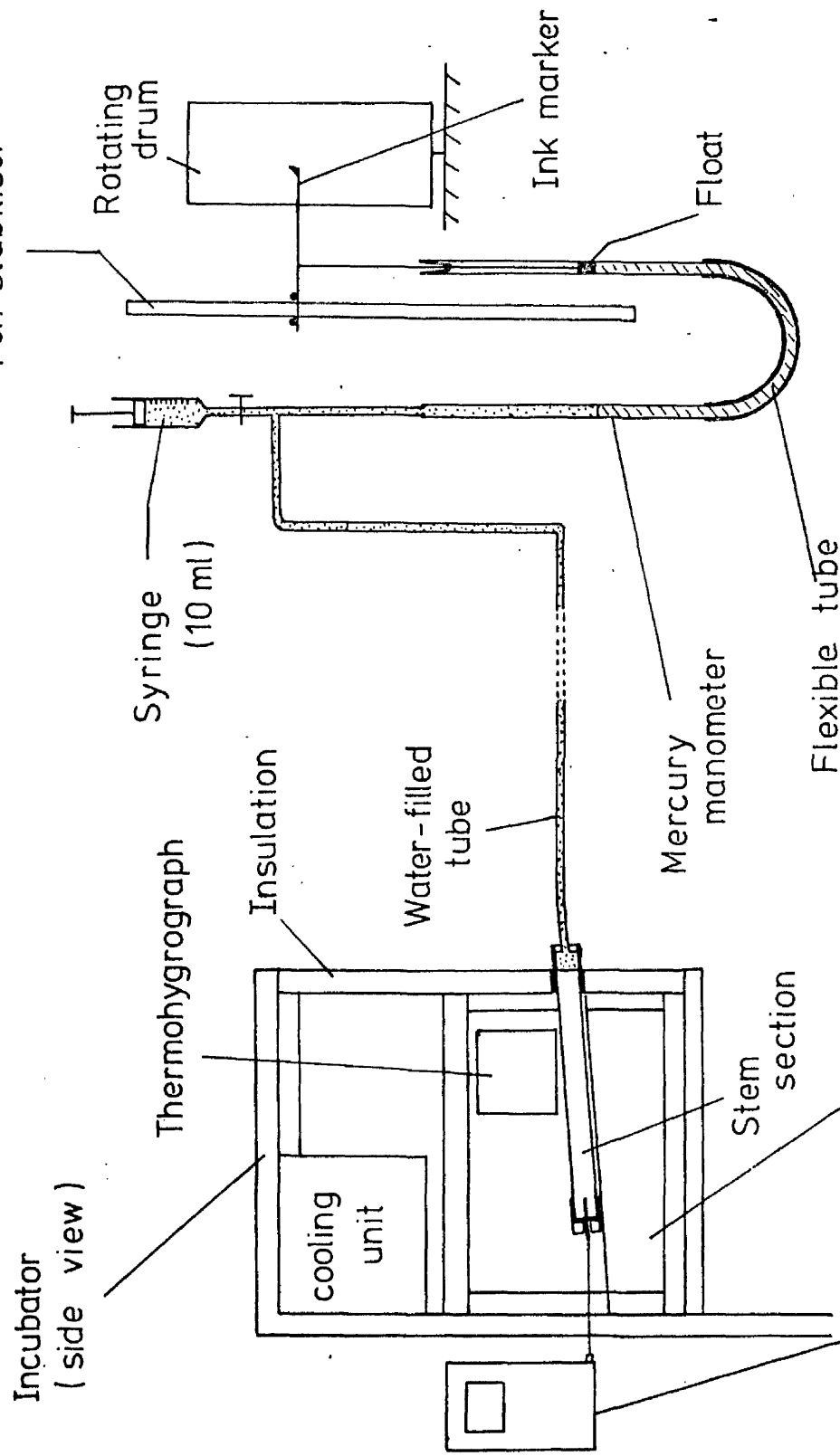


Diag. 2.4: Arrangement of guides employed to increase accuracy of manometric recorder.
a) plan view. b) side view.

Knowing the internal dimensions of the manometer arms facilitated the calculation of volume displacement required to produce a given pressure, ie. bore = 0.7cm., ∴ cross sectional area = $\pi.(\frac{0.7}{2})^2$. Hence, to record a pressure of 0.1 MPa (75cm. mercury) necessitated a volume change of $14.4cm^3$. This value was considered small enough in relation to the capacity of the tree to interpret the values recorded as true pressures, (eg. it is not unreasonable for the sugar maple tree to exude $5000cm^3$ sap in a single day - see Clark 1895, Wood 1895, Jones 1903 and Johnson 1945. To support this assumption, pressures were simultaneously recorded on the same tree ('A') using Bourdon pressure gauges, (see later). No significant differences were detected.

In total, 4 field manometric recorders were constructed. 2 were used for recording sap pressures within the tree, and 2 were used as controls to monitor fluctuations within the recording systems alone. All pressure records presented are those relative to atmospheric. This for each point of attachment to the tree was that recorded on the rotating drum prior to sealing of the system. This avoided calculations of artefactual pressure contributions from a) the weight of the float and pointer system, and b) hydrostatic pressure resulting from differences in heights of the tapping point and manometric recorder. (Both a) and b) were small anyway). Atmospheric pressure was monitored using a Fortin barometer, but fluctuations were small enough to be considered insignificant.

Laboratory recorder A water-filled tube 3m. long of constant bore 0.45cm. (O.D. 0.60cm.) connected a 30cm. length of stem (sampled from the stock of seedlings) to the mercury manometer. (see diag. 2.5). All joints in the apparatus were tight and sealed with glue (see 'field recorder'). Both glass arms of the manometer were 30cm. long, of constant bore 0.35cm. (O.D. 0.50cm.). As before, a float rested on the surface of the mercury. This was constructed from teflon tape wrapped tightly around the basal end of the vertical extension rod (length 30cm., diam. 0.2cm.) until it just fitted within the open manometer arm. A horizontal rod and ink marker facilitated recording of mercury level fluctuations on a rotating drum (as in the field). Again, the same system of guides was employed. Zeroing of mercury levels was made easy by the use of a syringe permanently incorporated at the bleed point. In total 4 such apparatuses were constructed.



Diag. 2.5 Illustration of laboratory manometric recorder and controlled temperature chamber as used during experimentation.

When used the basal ends of stem segments were positioned relative to the manometers to give zero difference in mercury levels. Hence, at the commencement of each experiment, negligible +ve. or -ve. hydrostatic pressure was imposed on each segment.

The selection of smaller manometer arms effectively increased the sensitivity of the apparatus compared to that employed in the field. The volume displaced required to record a pressure of 0.1 MPa was $3.6cm^3$ - 25% of that for an equivalent response in the field. However, this value was still considered large compared to the capacity of the stem segments used for experimentation. It is therefore incorrect to interpret resultant traces as true pressure records. Conversion of the trace fluctuations to changes in volume is possible, knowing 'h' cm. change in trace level - a difference in mercury levels of 2 'h' - is equivalent to π.(radius manometer arm)^2 'h' cm^3, = 0.096. 'h' cm^3. This approach is also inadequate however, as larger volume changes are associated with larger differences in levels of mercury, and here such pressure effects would become sources of error. Results produced from such a system are therefore a combination of simultaneous pressure and volume changes. A decision was made to adopt the arbitary term "pressure units". 1 such unit represents a difference in manometric mercury levels of 1cm., (0.0013 MPa pressure), and simultaneously an absorption (or exudation) by the stem segment under investigation of $0.048cm^3$.

c) Bourdon pressure gauges.

Manometric investigations of sap pressures in the field were supplemented by the use of the Bourdon pressure gauges, (C & A Stewart Ltd., Kilsyth, Scotland, U.K.). Responses of the gauges to pressure changes were adequately accurate, the scale showing a maximum +ve. pressure of +30lb in^{-2} and -ve. pressure of -30cm. Hg. Conversion to MPa was easy (14.5lb in^{-2} = 0.1 MPa). General attachment has been described (p.18), and specifics are to be found in appropriate chapters. Care was always taken during use to exclude air from the gauge and borehole adaptor. The system was sealed by insertion of a rubber bung at the bleed point. Readings were noted when required, and values converted to MPa.

d) Pressure transducer.

Sap pressures were monitored for seedling stem segments in the laboratory using a P.G pressure transducer, type TP78 (Penny and Giles Transducers Ltd., Christchurch, Hants, U.K.). Here, the volume displacement to produce a given pressure was smaller than that of the manometric recorder, (ie. a pressure of 0.1 MPa required a displacement of 2.5cm^3). This value was more acceptable, and considered to reflect truer values of sap pressure. For experimentation, a steady D.C. power supply of 12V. (from standard transformer) was required and variations in output were recorded on a Servoscribe potentiometric chart recorder (Belmont Instruments Ltd., Glasgow, U.K.). The pressure scale was bidirectional, (+0.1 to -0.1MPa). Atmospheric pressure (ie. 'zero' relative pressure) gave an output of 6V.

iii) Measurement of environmental factors.

a) Air temperature and humidity.

These were monitored using Casella drum thermohygrographs (C.F Casella & Co. Ltd., Britannia Walk, London, U.K.), with drum rate of 1 rev./week or 1 rev./day (drum circumference = 28cm.). One was positioned in a protective grill, 1m. above soil level, near the base of tree 'A' on the East side. This recorded true air temperature and humidity. Another was exposed $\frac{1}{2}$m. above ground level alongside the solarimeter (see p. 33). This recorded temperature as affected by direct sunlight, and served as an indicator of shade effects. A thermistor probe from the Grant temperature recorder (see below) was also positioned inside the protective grill for more accurate recording of temperature ($T \pm 0.1^{\circ}C$)

b) Tree, soil and manometric recorder temperatures.

A Grant 9-channel portable temperature recorder was available (Grant Instruments (Cambridge) Ltd., Barrington, U.K.) with thermistor probes (steel encased, 5 x 0.32cm.). Using a 0.3cm. diameter drill a hole 3.5cm. deep was made 5cm. to the right of both trunk boreholes (used for manometric pressure recording) on tree 'A'. A temperature

Table 2.1 Details of twigs on Tree A embedded with thermistor probes for recording of wood temperature.

Twig No.	1	2	3
Age (years)	11	7	10
Diameter +bark (cm.)	1.00	0.80	0.95
	x	x	x
	0.85	0.75	0.85
-bark (cm.)	0.85	0.65	0.85
	x	x	x
	0.70	0.60	0.75
Vertical height above ground (m.)	2.50	2.50	2.50
Horizontal distance from trunk (m.)	1.50	1.25	2.00

probe was embedded in each, and well sealed in position using impact adhesive, (Evostik: Manufact. see p. 18) and silicone rubber sealant and adhesive, (Dow Corning International: Manufact. see p. 18).

Three twigs were also chosen (see Table 2.1) south-west facing, at a vertical height above the ground of about 2.5m. This site was chosen because it was of approximately the same compass orientation as the trunk boreholes. After excision with a hacksaw, the twig stumps, still attached to the tree, were drilled to make a hole down the centre of each, 3.5cm. deep and 0.3cm. diameter. Probes were inserted, and sealed in position with glue as above. Great care was taken during the drilling to avoid splitting of the wood or bark. A further probe was positioned with its tip buried to a depth of 5cm. in the soil, 1m. from the base of the tree. Finally, 2 probes recorded water temperature inside the recorder tubing (see diag. 2.1) so the temperature gradient between this and sap within the tree could be monitored. They were arranged to function as stoppers at the bleed points and so could be removed and resealed in position with ease.

c) Windspeed.

A sensitive cup anemometer was positioned on the roof of the

research laboratory, at a height of approx. 3m. above ground level and some 300m. West of tree 'A'. It was impractical to record wind speeds in the vicinity of tree 'A', because of the lack of mains power supply. Results were recorded automatically on a Servoscribe potentiometric chart recorder, as integrations of each 5 minute time period. Translation of deflection to wind speed was accomplished using the standard calibration curve supplied with the instrument.

d) Incident radiation.

A Casella radiometer (model 293) was positioned $\frac{1}{2}$m. above ground level approx. 75m. from tree A. This site was chosen as it was unshaded at all times of the day. The instrument had been calibrated recently against a Kipp Solarimeter (Kipp & Zonen, Holland). 3.5 units were equivalent to 100 Wm^{-2}. The recording drum rate was the same as that of the thermohygrographs for ease of correlation of data.

e) Rainfall.

Two simple rain collectors were constructed adjacent to the solarimeter. They consisted of plastic filter funnels (diam. 17cm.) with collecting flasks (250ml. measuring cylinders). Knowing the area of collection ($A = \pi.(\frac{17}{2})^2 cm^2$) and the volume collected (V. cm^3), results could be expressed as 'mm. rainfall', ($\frac{V}{A}$ x 10).

iv) Sap collection procedures.

a) Collection of xylem sap samples in the laboratory.

Typically, a seedling was selected from those in the plot, and excised at a point 5cm, above ground level, using secateurs or a hacksaw. In the laboratory, the basal 1cm. of bark (ie. tissue exterior to the xylem core) was carefully removed and the xylem cut surface trimmed using a clean, sharp razor-blade. A clean, dry rubber sleeve of appropriate dimensions was fitted over the end of the stem. This procedure reduced contamination of xylem sap from infiltration of phloem sap, and also prevented undue evaporation (and so concentration) of sap during collection. When dormant, seedlings usually exuded after transportation to the laboratory, due to thermal effects. However,

during the summer state considerable xylem sap tensions prevailed and collection of sap required elution of the stem with distilled water. (eg. Bennett et al 1927) This was considered to displace the sap, and because of the small volume collected, not dilute it in the process. Here having prepared the basal end for sap collection, the stem was excised under distilled water some 70cm. from the basal cut surface. A tight fitting rubber tube was attached, leading to a reservoir of distilled water. Throughout, care was taken to exclude air from the apical cut surface. By raising the reservoir above the stem, the latter maintained horizontally, xylem sap was displaced by distilled water and readily collected at the basal end. A head of $\leqslant$ 2m. was usually sufficient for this.

Sap in both summer and winter was collected in 50μl 'microcap' glass capillaries (Drummond Scientific Co., U.S.A.). It was usual to disgard the first 50μl., as this may have been contaminated with sap from injured cells. The next 2 x 50μl samples were then retained. The first was used for determination of solute potential (Ψ_s) by osmometry, and % sucrose by refractometry (see later). The remaining 50μl. sample was placed in a clean plastic vial, sealed, and stored in a freezer (Temp. -15^{o}C). Carbohydrate content (by Nelson/Somogyi assay) and mineral content (Atomic Absorption spectrophotometry) were estimated for this sample at a later date (see later). Deep freeze storage did not exceed 7 days. Frequently a third 50μl. sample was taken and analysed by osmometry & refractometry. When compared with the first sample analysed from the same stem, no significant differences were recorded.

b) Collection of xylem sap samples in the field.

Sap collection in the field was a simple operation. When trees were dormant and pressurised, exudation would occur from any appropriate wound. Usually, trunk boreholes (ht. 1m.) prepared for the attachment of Bourdon pressure gauges were used (see p. 18). Having disconnected a gauge, any sap within the hole and adaptor was removed using a syringe and needle. With subsequent exudation, the hole and adaptor refilled and was allowed to drip into a clean plastic vial. When sufficient had exuded (eg. 2cm^3), the vial was sealed and transported to the laboratory for analysis. Samples were also taken from a branch on tree 'A'. Here, a tight fitting rubber sleeve had been sealed over the end of a cut limb, again as an attachment for a

Bourdon pressure gauge. Removal of the gauge and any residual sap allowed free exudation of fresh sap, which could be collected in plastic vials as before. Refractometry and osmometry were performed immediately on return to the laboratory. However, for carbohydrate analysis, (Nelson/Somogyi assay) and atomic absorption spectrophotemetry 100μl samples were stored at -15°C until needed. Storage did not exceed 7 days.

Sap collection in the field during summer was achieved using the pressure bomb (see p. 23). Having determined the xylem hydrostatic pressure (Ψ_p) on a twig, xylem sap exudate was forced from the protruding stem by fractionally increasing the chamber pressure. 25μl 'microcaps' were used to collect the sap exudate. When full, these were stored in a high humidity sealed container until analysis in the laboratory $\leqslant$ 1 hour later. On occasions, samples from single heights were pooled for analysis.

c) Collection of bark sap samples.

Extraction of bark cell sap was achieved by freeze-disruption of the tissue, (see Slatyer 1967 and Barrs 1968) and was only collected from seedling material. In each case bark was removed from a region 10cm. long, 40cm. from the base of the seedling. This was then divided into two approximately equal samples. Prior to September 1978, the samples were each forced into a new 2cm^3 plastic syringe (B. D. Plastipak, Becton Dickinson and Co. Ltd., Ireland.), the plunger replaced and the nozzle sealed. The time taken from removal of bark from the stem to sealing within the syringe was kept to a minimum to reduce evaporational losses. The samples were placed in a freezer at -15°C, for a maximum storage period of 2 days. The tissue was then thawed at room temperature and centrifuged at 2500 r.p.m.* for 10mins. (in a Gallenkamp Universal Centrifuge, Gallenkamp. London, U.K.) into a clean pyrex centrifuge tube. (see Dodoo 1978). The sap collected (usually $\geqslant$ 100μl) was then used for chemical analysis.

After November 1978, a different technique was adopted for greater speed, with no suspected loss of accuracy. Having removed the bark samples from the stem as before, each was forced into a 10cm. length of clear vinyl tube (bore 1.2cm. O.D. 1.6cm.). The two ends were sealed with rubber bungs and the tube and sample stored in a freezer as before. On removal, the samples were each immersed in liquid N_2 (temp. -196°C)

* $g \simeq 1000$

for 5 minutes. This ensured freeze disruption of the tissue, which was now being sampled from dormant seedlings and so may be hardy enough to withstand the freezer temperature of -15°C (eg. Scarth 1944, Mazur 1969, Levitt 1972, Burke et al 1976). Samples were thawed at room temperature, and then sap expressed from them with a vice. The protective vinyl tube acted as a catchment area, and the sap was collected in 100µl 'microcaps'. Usually ⩾ 100µl were obtained per sample.

v) Analytical procedures.

a) Osmometry.

Solute potential (Ψ_s) of xylem sap and bark-cell sap were determined cryoscopically, after collection, using an osmometer (Knauer Electronic Temperature Measuring Instrument with a Thermoelectric Cooling Instrument: KG.Dr-Ing. Herbert Knauer & Co. GmbH, Berlin, F.R.G.) The instrument was calibrated against standard solutions of NaCl before measurements, and careful zeroing against distilled water was performed before and during each series of samples. A standard sample size of 100µl was required. Some sap samples (ie. those of 50µl) needed dilution to this volume with distilled water. Errors resulting from this were considered insignificant. The operating procedures and relevant precautions have been described by Dodoo (1978).

b) Refractometry.

After determination of Ψ_s by osmometry, each sample was thawed, well mixed and then used for determination of its refractive index (RI). An Abbe refractometer No. 302 (Atago Optical Works Co. Ltd., Japan) was employed, with the prism thermostatically maintained at 30°C. R.I. is proportional to solute concentration, and such an instrument has a double scale relating R.I. to % sucrose - pure solution (see appendix I). Results were presented as the latter - considered to be more useful in this investigation - although sap samples, of course, were not pure sucrose solutions. Hence, recorded values are accurate relatively, although in absolute terms they contain a small degree of error due to solutes present other than sucrose.

Conversion to refractive index is possible using the graph (appendix I p.133). Values noted were corrected for any sap dilution incurred for use previously in osmometry.

c) Carbohydrate determination.

Reducing and non-reducing sugar levels were measured by the colorimetric method of Nelson (1944). The sap samples were diluted appropriately, (x 100 by volume with freshly distilled water usually sufficed) and 2 x $1cm^3$ samples taken, One sample was used for determination of reducing sugars, and the other for non-reducing sugars. Both estimations were replicated. For reducing sugars, the $1cm^3$ diluted sap sample was mixed in a pyrex test tube with an equal volume of copper reagent (made according to Somogyi, 1952). The mixture was boiled for 0.5 hours, (evaporation prevented by aluminium foil tube covers), cooled, and to it added $1cm^3$ of arsenomolybdate reagent - the colour forming agent. Distilled water was then added, diluting the solution to $10cm^3$. Care was taken to dissolve all the Cu_2O. The colour was measured spectophotometrically using a Pye Unicam SP 8000 Ultraviolet Recording Spectrophotometer, (Pye Unicam Ltd. Cambridge, U.K.) with silica-glass cuvettes. Absorbance was monitored at 600 nm usually, though no difference in linearity of response was detected at different wavelengths across the spectrum.

The assay for non-reducing sugars involved initial acid hydrolysis. $1cm^3$ of diluted sap was mixed with $1cm^3$ 10 $mM.dm^{-3}$ HCl in a pyrex test tube and incubated at 90^oC for 0.5 hours. The tubes were covered with foil to reduce loss by evaporation. After cooling, $1cm^3$ 10 $mM.dm^{-3}$ NaOH was added to neutralise the acid. $1cm^3$ of this mixture was then taken, and the procedure followed as reported for reducing sugars. For each series of samples, a standard curve was constructed to convert spectrophotometric readings to sugar concentrations. Sucrose solutions of 0, 0.25, 0.5 and 1.0 $mM.dm^{-3}$ were used, 1.0 giving full scale deflection at 600 nm. Sap samples generally gave half f.s.d. when the assay was positive.

d) Mineral content determination.

K^+ and Na^+ content was estimated for xylem sap by atomic-absorption spectrophotometry. Sap samples used were diluted, usually x 100 by

volume, with fresh distilled water. An EEL Atomic Absorption Spectrophotometer Mark 2 (Evans Electroselenium Ltd., Halstead, Essex, U.K.), was available with the appropriate hollow cathode lamp (Activion Special Products Division, Halstead Essex, U.K.). The fuel was acetylene (B.O.C Ltd., U.K.) with air as support gas (EEL Model 349 Compressor). All procedures and precautions were followed as described in the handbook. Standard curves were constructed for each series of samples - for K^+, f.s.d. was 100μM dm^{-3} and for Na^+, f.s.d. was 50μM dm^{-3}. Sap samples generally gave half f.s.d Distilled water "blanks" usually gave insignificant values, though care was taken where appropriate to use well washed glassware because of residual Na^+ from detergent.

vi) Methods employed for killing plant tissue.

Seedlings were appropriately chosen and after sap sampling cut with a hacksaw or secateurs to leave the basal 45cm. The bark was then removed, using a blunt penknife to scrape the wood if necessary. Treatment was one of the following:-

a) Heat.

This was imposed in one of 2 ways,

i) Hot water bath - stems were plunged into a water bath of distilled water at a given temperature (ie. 80°C or 90°C) and held submerged for 24 hours.

ii) Steam treatment - stems were supported in steam (ie. approx. 5cm. above boiling water) in an enclosed container (though not pressurised) for 24 hours. Temperature at the surface of the segments was approx. 90°C.

After treatment, stems were removed, the surfaces blotted dry and each segment wrapped in polythene and P.V.C. tape (see p. 22). 1-2 cm. of stem was removed from the basal end for vital staining (ie. T.T.C. test - see below). Stems were then prepared for attachment to manometric devices (see p. 22).

b) Potassium cyanide (KCN) elution.

The de-barked stem was wrapped in polythene and plastic tape. The basal end was then cut with a hacksaw or secateurs under distilled water, removing approx. 3cm. The surface revealed was

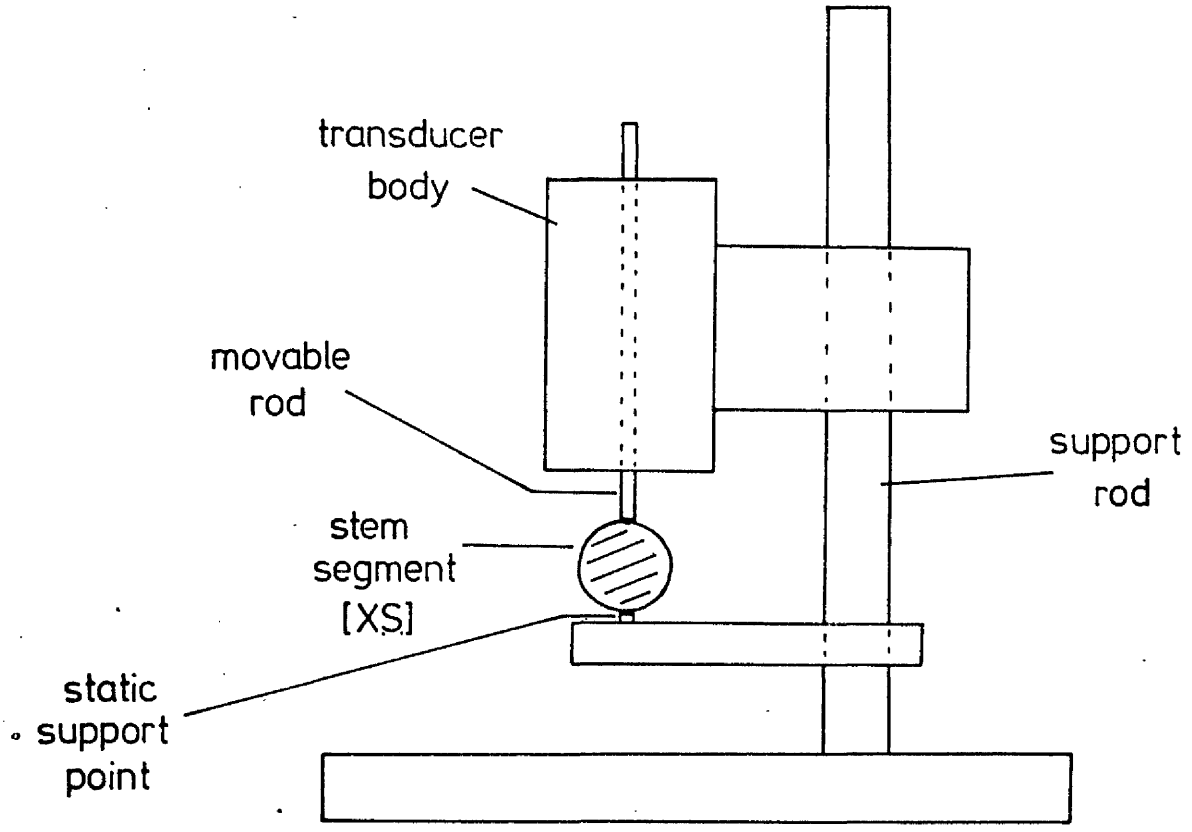
trimmed with a clean sharp razor blade and a tight fitting rubber sleeve with tapering plastic adaptor attached before removal from the water. Each segment was then linked to vinyl tubes leading from a reservoir of KCN solution, the latter 1m. above the level of the segments. Elution of the segments (from base to apex) was then undertaken with collection of the eluate to estimate volume of solution passed. This was performed in a fume cupboard for safety, usually lasting 3 days. Recutting of the basal end under distilled water (removing approx. 3cm.) was performed after 2 days to maintain a good elution rate. Solutions of 10, 20 & 50 $mM.dm^{-3}$ KCN were used, in tris buffer pH 8. After elution, 1-2cm. of stem was removed from both the basal and apical ends for use in T.T.C. test (see below). Segments were then prepared for attachment to manometer devices, (see p. 22)

T.T.C test - vital staining

Tissue was tested for signs of life using 2, 3, 5 triphenyltetra--zolium chloride dye (T.T.C.). This is a colourless compound in solution that can be reduced to a red coloured insoluble formazan in the presence of cellular activity. (see Towill & Mazur 1975). The test involved using 1-2cm. sections of stem, as sampled above, each placed in a plastic petri dish with 0.1% aqueous solution of T.T.C. Samples were then incubated for 24-48 hours in the dark at 15°C, and studied visually for signs of colouration. This was not always easy, as heat treatment caused general browning of the wood due to tannins.

vii) Recording of stem diameter fluctuations.

Stem diameters could be monitored during temperature treatment in the laboratory using a linear displacement transducer, type D2/0.1 (Sangamo Western Controls Ltd., Bognor Regis, U.K.). This was supported in the controlled temperature chamber by a bracket system, as shown in diag. 2.6. A steady 12V. D.C. was supplied from a transformer, and output monitored on a Servoscribe potentiometric chart recorder (Belmont Instruments Ltd., Glasgow, U.K.). During investigation stems were laid horizontally and diameters usually measured 4cm. from the basal end. This region rested on the static support point (diam. 0.20cm.)

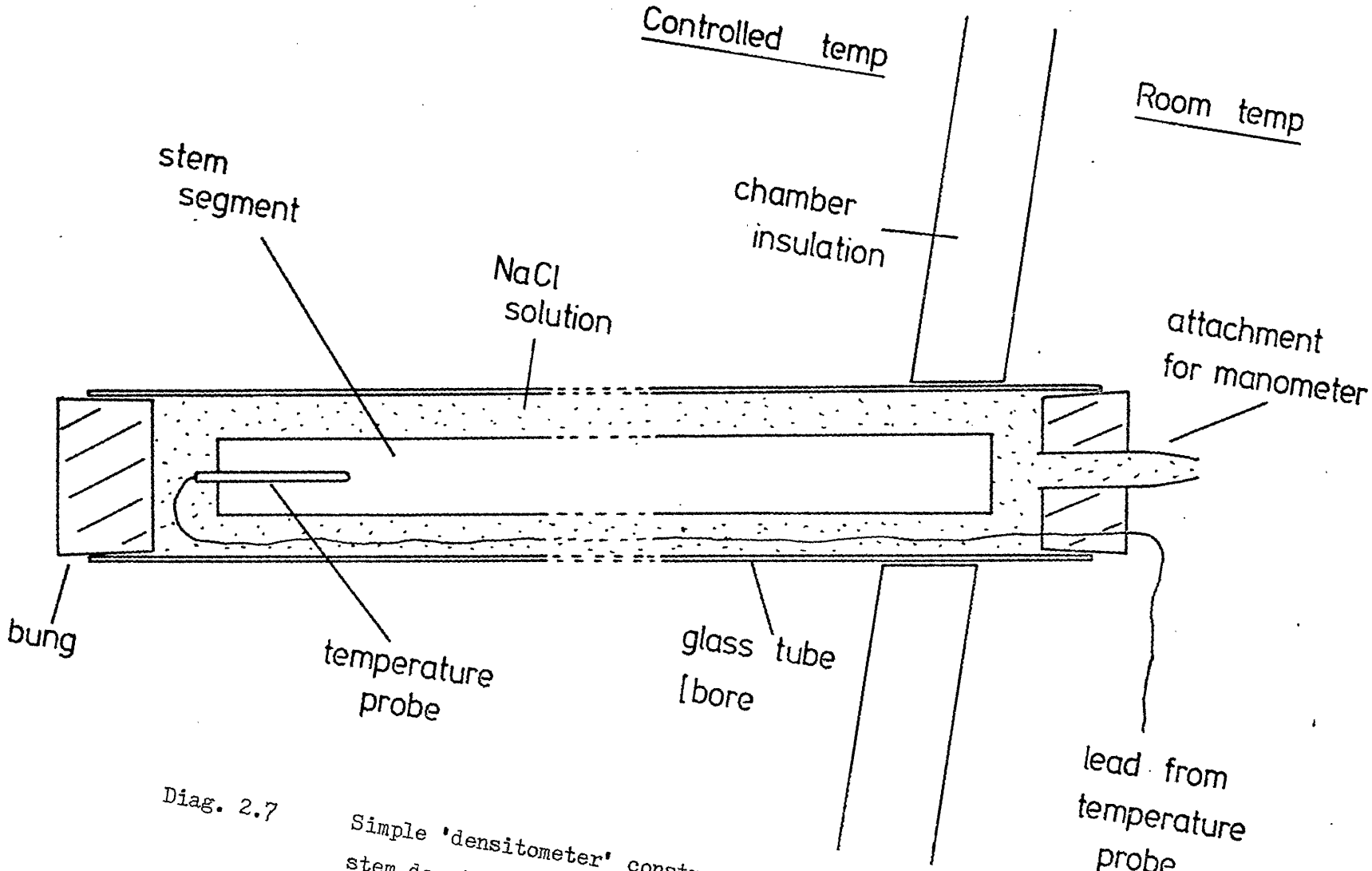


Diag. 2.6 Arrangement of linear displacement transducer (LDTr) used for measurement of stem diameter changes in the laboratory.

directly beneath the movable rod to the transducer (diam 0.12cm.) Whether the region was internodal or nodal was not noted. Measurements monitoring overall diameter changes (ie. bark plus xylem) were frequently followed by measurements of xylem core changes alone for the same region. This was achieved by carefully removing discs of bark (diameter 0.4cm.) from opposite sides (radially) of the stem, using a No.1 cork borer. The stem was then repositioned with the exposed xylem between the transducer rod and support point. Stem diameters (both bark and xylem and just xylem) were measured after experimentation using a venier micrometer (accurate to 0.001cm.). Bark thickness was deduced by subtraction. Transducer output was directly related to diameter change, and calibrated against the above mentioned micrometer, 0.5V. f.s.d was equivalent to 0.06cm. Accuracy was $\pm$2%. Effects on the system of thermal changes were estimated and corrected for by experimentation on material of known diameter and content (eg. copper block.).

viii) Sodium chloride 'densitometer'.

Experimentation was performed to monitor overall volume change of stem segments during temperature treatment. For this a simple 'densitometer' was constructed (see diag. 2.7). A wide glass tube (bore 2.3cm. O.D. 2.5cm.) 35cm. long was sealed at one end with a rubber bung. It was then filled with aqueous sodium chloride solution, (3M dm^{-3}), having a depression of freezing point ($\Delta T^{o}f$) of 11.8°C. A bung to fit the open end was then bored appropriately, and a tapering plastic adaptor and lead from a thermistor probe were located and sealed in place. When used for experimentation a stem was first selected, and after sap sampled, bark removed (see p.20). The stem was then wrapped tightly in polythene and P.V.C. tape. A hole was drilled, centrally, in the basal end and the temperature probe (see above) inserted. The stem was then cut under distilled water with secateurs to leave the lower 30cm., and the apical end trimmed with a clean sharp razor blade. The segment was quickly transferred to the densitometer and immersed in the salt solution. Care was taken during transfer to retain a layer of water on the apical cut surface. The bung and adaptor were fitted, and salt solution added to exclude all air within. The whole was then positioned in



Diag. 2.7 Simple 'densitometer' constructed to monitor stem density during temperature treatment.

the controlled temperature chamber with the adaptor protruding and the apical end of the stem segment flush with the polystyrene insulation of the chamber. A manometric recorder was attached, and temperature treatment undertaken. Recorded fluctuations reflected that of overall volume/pressure change, as measurement is made not directly on the stem segment, but on the liquid bathing medium. A control was run for each experiment, constructed to identical specifications but containing a stem segment of comparible size that had been oven dried (90°C) previously.

ix) Determination of fresh and dry weight, and % water content.

Determination was by standard gravimetric procedure. Stems were weighed (usually after experimentation), on a Mettler top loading balance of appropriate sensitivity, (Gallenkamp Ltd., London, U.K.). Tissue was then dried in an oven (Model OV100, Gallenkamp Ltd., London, U.K.) at 90°C until no further change in weight (usually left for 7 days). For estimation of bark fresh weight alone, stems were weighed with bark intact and then reweighed after its removal. Subtraction gave fresh weight of bark. Dry weight was determined by taking 2 samples weighing them on a top loading balance, and drying as above. % water content is the ratio of water content to fresh weight x 100.

x) Statistics.

Where experimental results were clear, and conclusions easily drawn, no statistical tests were employed. However, where desirable, the following tests were undertaken.

a) Standard Error (S.E.)

Standard error of the mean value of a series of observations was calculated as follows:

$$S.E. = \frac{S.D}{\sqrt{n.}}$$

Where n = number of observations.

S.D.= standard deviation.

S.D was calculated as:-

$$\sqrt{\frac{\Sigma x^2 - \frac{(\Sigma x)^2}{n}}{n-1}}$$

b) Correlation and regression analyses.

Correlation and regression analyses were used to investigate the interdependance of 2 quantitative variables, x & y. The correlation co-efficient, r, is first calculated to estimate the degree of association between x and y :-

$$r = \frac{\Sigma(x - \bar{x})(y - \bar{y})}{\sqrt{\Sigma(x - \bar{x})^2 \quad (y - \bar{y})^2}}$$

The r value was compared with the values in the r distribution table by Pearson & Hartly (1958), using n-2 degrees of freedom. If r was significant at the 5% level or less, the 2 variables were considered associated. Assuming this relationship to be linear, a regression analysis is undertaken. The best fitting regression line is estimated by the expression:-

$$y = mx + c$$

where m, the regression coefficient, is estimated by:-

$$m = \frac{\Sigma(x - \bar{x})(y - \bar{y})}{\Sigma(x - \bar{x})^2}$$

c, the constant term in the regression is estimated as:-

$$c = \bar{y} - m\bar{x}$$

To fit the regression line, 2 values of x were selected and the corresponding values of y calculated from the regression equation. A straight line was drawn through the points.

EXPERIMENTS, RESULTS AND DISCUSSIONS.

3. Preliminary Investigations And Characterisation Of The Maple Exudation Phenomenon.

As explained in the general introduction, investigations into the maple sap pressurisation phenomenon have used Acer saccharum (the sugar maple). It seemed necessary therefore, studying Acer pseudoplatanus, to obtain information of a qualitative nature initially. Later more in depth studies could be undertaken with greater surety, (presented in chapter 4). This approach also allowed development of appropriate techniques, (eg. reliable and efficient manometric devices), for use in both the field and laboratory.

Investigations concentrated on measurement of sap pressure potential in response to natural or induced environmental fluctuations Particular attention was paid to temperature, as its intimate association with sap flow/pressurisation has been noted, though not fully clarified, (eg Jones et al 1905, Wiegand 1906, Stevens and Eggert 1945, Marvin 1958). Some investigation of sap solute content was also undertaken. Exudation - the result of wounding a tree exhibiting a positive sap pressure - is observed naturally only in dormant plants. Likewise, the presence of sugar in maple sap is detected only during this period (eg. Jones et al, 1903, 1933, Marvin 1958, Sauter et al 1973). Not surprisingly then, these two factors have been associated. A distinct correlation has been illustrated between high sucrose content of trees and high sap volume yield, (general introduction p 10). However, although undoubtedly associated in the field, a direct causal relationship is as yet unproven. It was hoped that investigation of sap solute content and pressure responses might clarify this issue.

3.1 Xylem Sap Pressures In Mature Sycamore Trees During Dormancy.

The principle tool used for investigation of sap pressure potential (Ψ_p) during dormancy was the manometric recorder (see p 24). This monitored sap pressure fluctuations - continuously and automatically - providing liquid continuity was maintained between the tree and the recorder. Many records were obtained using this device, but quality varied due to technical difficulties, (eg. problems of sealing the joint between the borehole and recorder adaptor).

Figures presented are, therefore, those results best illustrating significant observations, yet lacking technical errors. Fig. 3.1 shows some early results obtained. Pressures are those recorded from one trunk borehole, representative of 2 used, (each ht. 1m., seperated by 30cm.), on the south side of tree A. Boring of the holes was originally undertaken on 21st March 1978, to a depth of 3cm., diameter 1.25cm. Redrilling of the same holes was performed in the late afternoon of April 10th, making the depth then 4.5cm. (ie. pentrating approx. 3.5cm. into the xylem). The tree was pressurised at this time and 2 samples of xylem exudate were collected from each hole. These were used for estimation of solute potential (Ψ_s) and % sucrose, by osmometry and refractometry respectively. With this data and that estimated for sap pressure potential, xylem water potential (Ψ_x) could be calculated, using the approximate equation $\Psi_x = \Psi_p + \Psi_s$. During the period shown (fig. 3.1) the manometric devices were kept sealed except for occasional bleeding to remove gas. Simultaneous recordings of air temperature and incident radiation were made.

Repetition of the whole procedure was undertaken the following year, and fig. 3.2 shows results for 17th - 21st January 1979. New holes were drilled in the trunk of tree A, each 15cm. to the west of the previous holes, and hence south-west facing. They were originally bored to a depth of 3cm. on 6th January, but with redrilling - most recently on 17th - the depth was increased to 4.0cm. (ie. penetrating approx. 3.0cm. into the xylem). Again the tree exhibited a positive sap pressure when redrilled, and sap samples were taken for analysis. Manometric recorders were attached, sealed and pressures monitored. Air temperature and incident radiation were also recorded.

It is evident from figs. 3.1 and 3.2 that positive xylem sap pressure potentials were manifested in tree A at various times during investigation. These pressures were maintained for different durations with gradual decline in value. This is particularly well illustrated in fig. 3.2, 17th - 21st January. More interesting, however, are the pressure fluctuations (indicated by arrows). Such changes from positive to negative sap pressure (relative to atmospheric) are dramatic and easily discerned. They correlate well with periods when air temperature drops below $0^{o}C$, but do not seem to be related quantitatively to temperature change. In fig. 3.2 for example, a drop

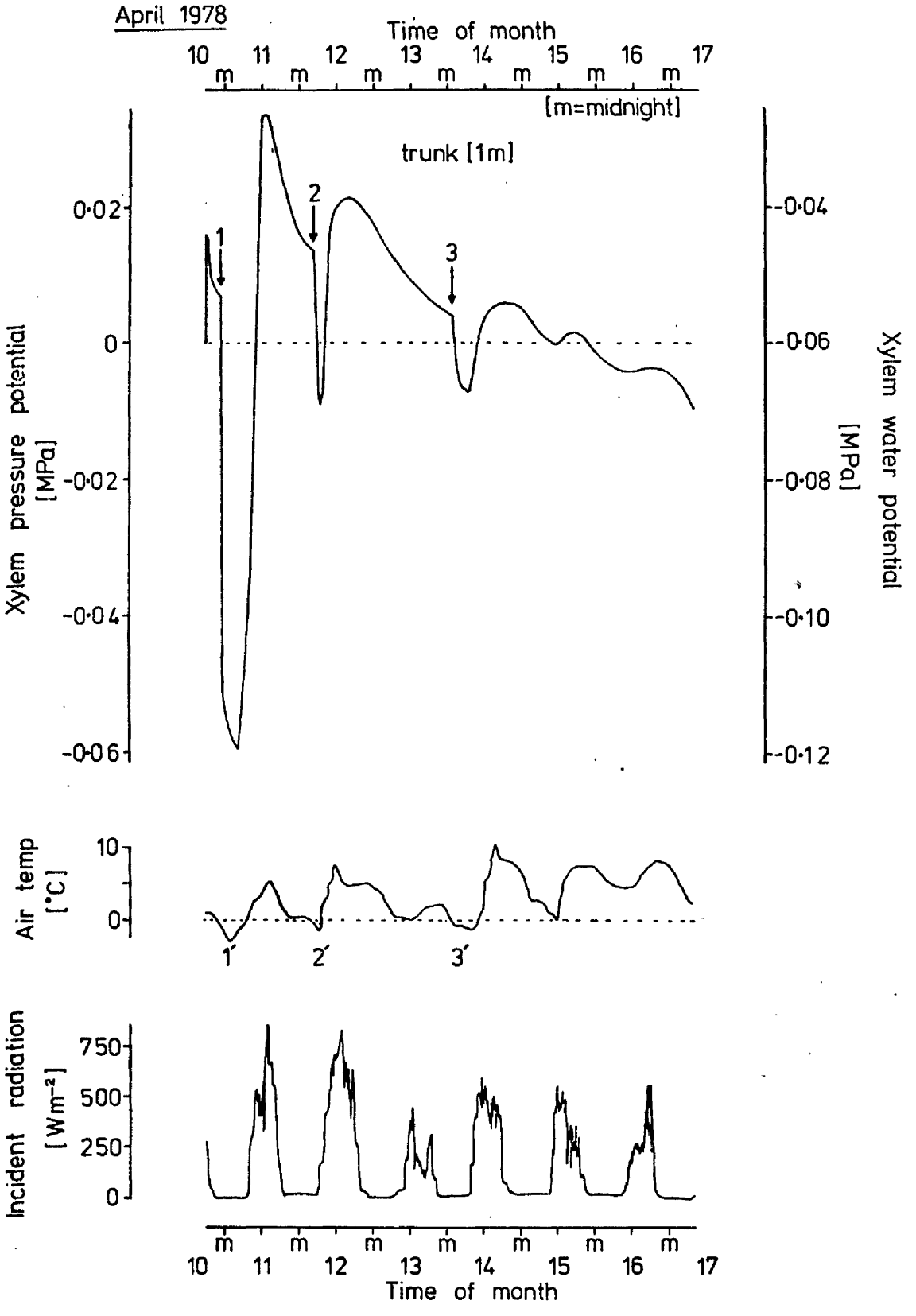


Fig. 3.1 Xylem pressure potential (Ψ_p) monitored for tree A (trunk, ht. 1m) during April 1978 using the manometric recorder. Water potential was deduced from addition of pressure and solute potentials ($\Psi_p + \Psi_s$).

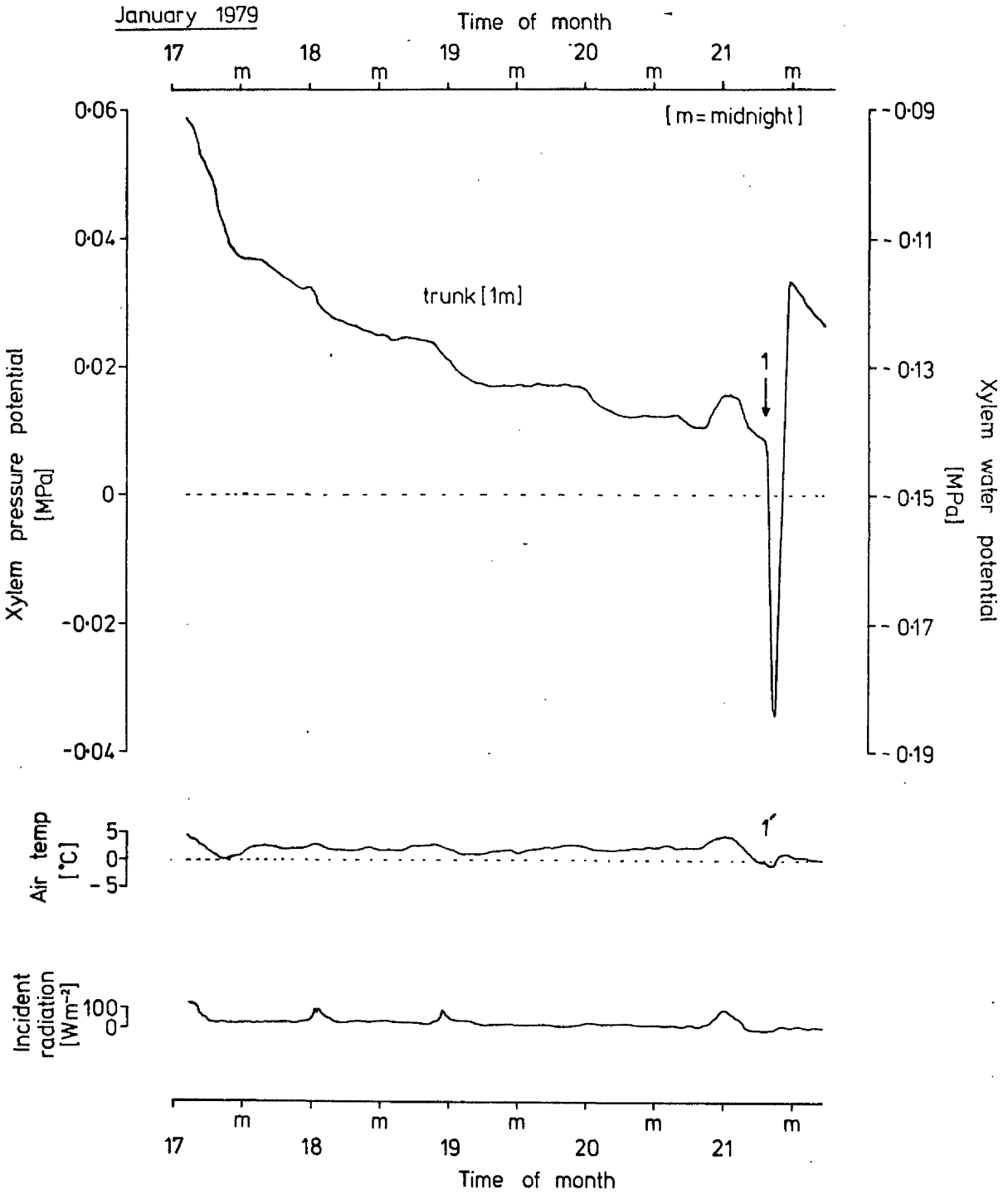


Fig. 3.2 Xylem pressure potential (Ψ_p) monitored for tree A (trunk, ht. 1m.) during January 1979 using the manometric recorder. Water potential was deduced from addition of pressure and solute potential ($\Psi_p + \Psi_s$).

in air temperature of some 5°C, (ie. 5 → 0°C) starting at approx. midday 21st January, produced a small drop in Ψ_p (ie. 0.015→ 0.009MPa). However, a continued drop of approx. 1°C (ie. 0 → -1°C) resulted in a large reduction in Ψ_p (ie. +0.009 → -0.035MPa) - arrowed. It would therefore appear that sub-zero air temperatures have a greater effect upon the induction of sap tension than changes in air temperature above 0°C.

Sap tension induced by sub-zero air temperatures were characteristically reversible. Subsequent rise in air temperature produces sudden and rapid manifestation of a positive sap pressure. It is interesting to note, however, that although positive values of pressure potential are often detected, at no time during the record is there production of a positive xylem water potential (Ψ_x). The presence of solutes in the xylem sap reduces the value of solute potential (Ψ_s) such that it more than compensates for the positive value of Ψ_p.

3.2 Xylem Sap Pressures In Mature Sycamore Trees When In Leaf.

Sap pressures were estimated for one or all of trees A, B & C on three occasions in the summer months of 1977 and 1978. For this the pressure bomb was employed, and results from two occasions are illustrated in figs. 3.3 & 3.4. The third investigation produced similar results and so has not been included. The earlier study (fig. 3.3) focused on tree A, and sap pressures were estimated every 2 hours for a 24 hour period. Shoot samples were chosen at 2 heights (10 & 3m.), vertically aligned, on the south-west side of the tree. To reduce error in alignment they were taken as near to the main stem as possible, (approx. 2m. horizontally distant). For each determination 2 samples were bagged and after equilibration, Ψ_p measured, with the pressure bomb, (see p 23). Throughout the investigation, recordings were made of incident radiation, air temperature, relative humidity, wind speed and rainfall.

The study on trees A, B & C (fig. 3.4) involved a similar procedure. Ψ_p was estimated at 3 hour intervals for a duration of 12 hours. Sample heights were those illustrated. Xylem sap samples were also obtained from each tree for estimation of solute potential (Ψ_s). This facilitated calculation of xylem water potential (Ψ_x). The sap was forcibly expressed from twiglets immediately after their use for determination of Ψ_p. This was accomplished by increasing the

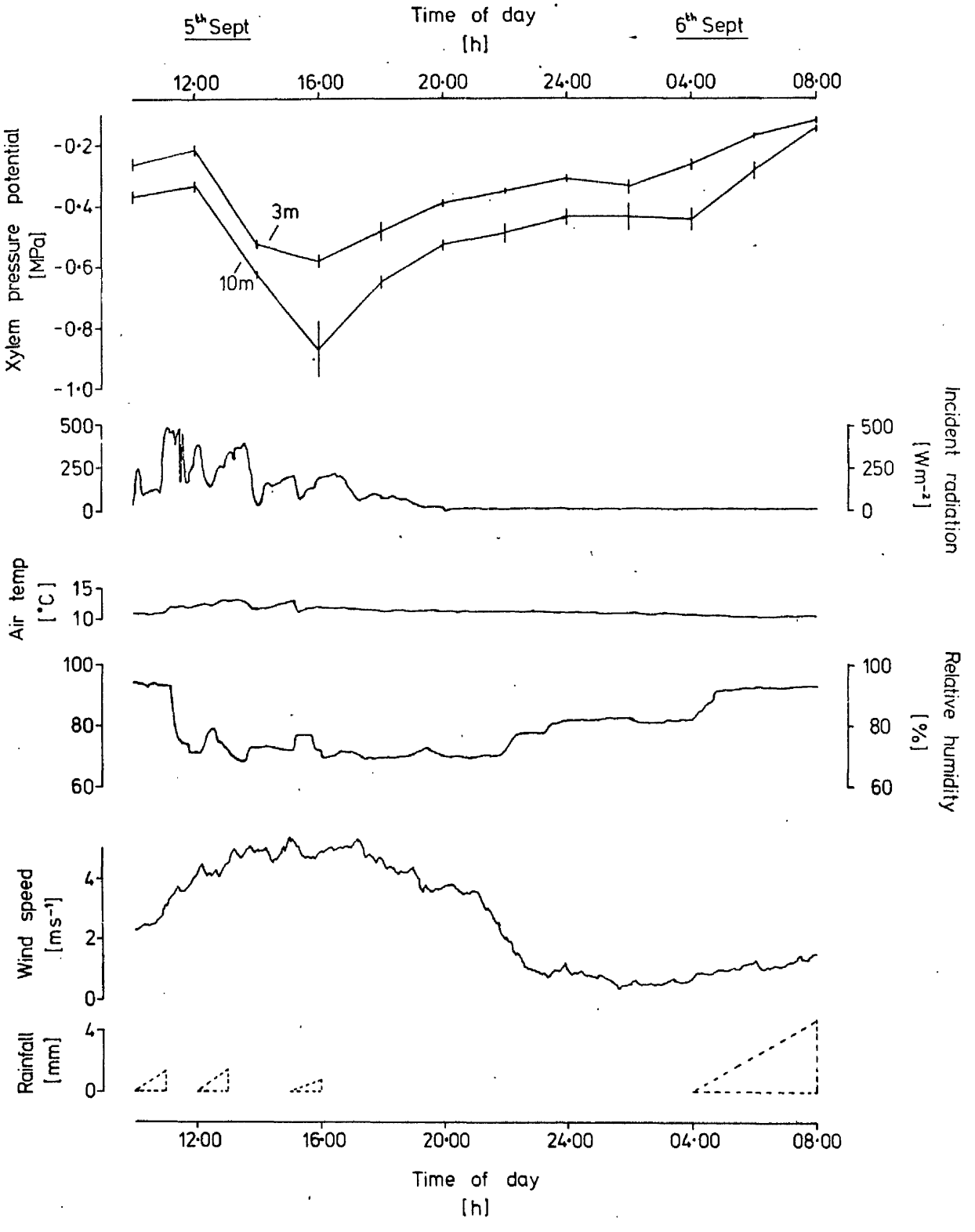


Fig. 3.3 Xylem pressure potential (Ψ_p) estimated using the pressure bomb, for tree A at heights 3 & 10m. during September 1977. Environmental factors were recorded simultaneously.

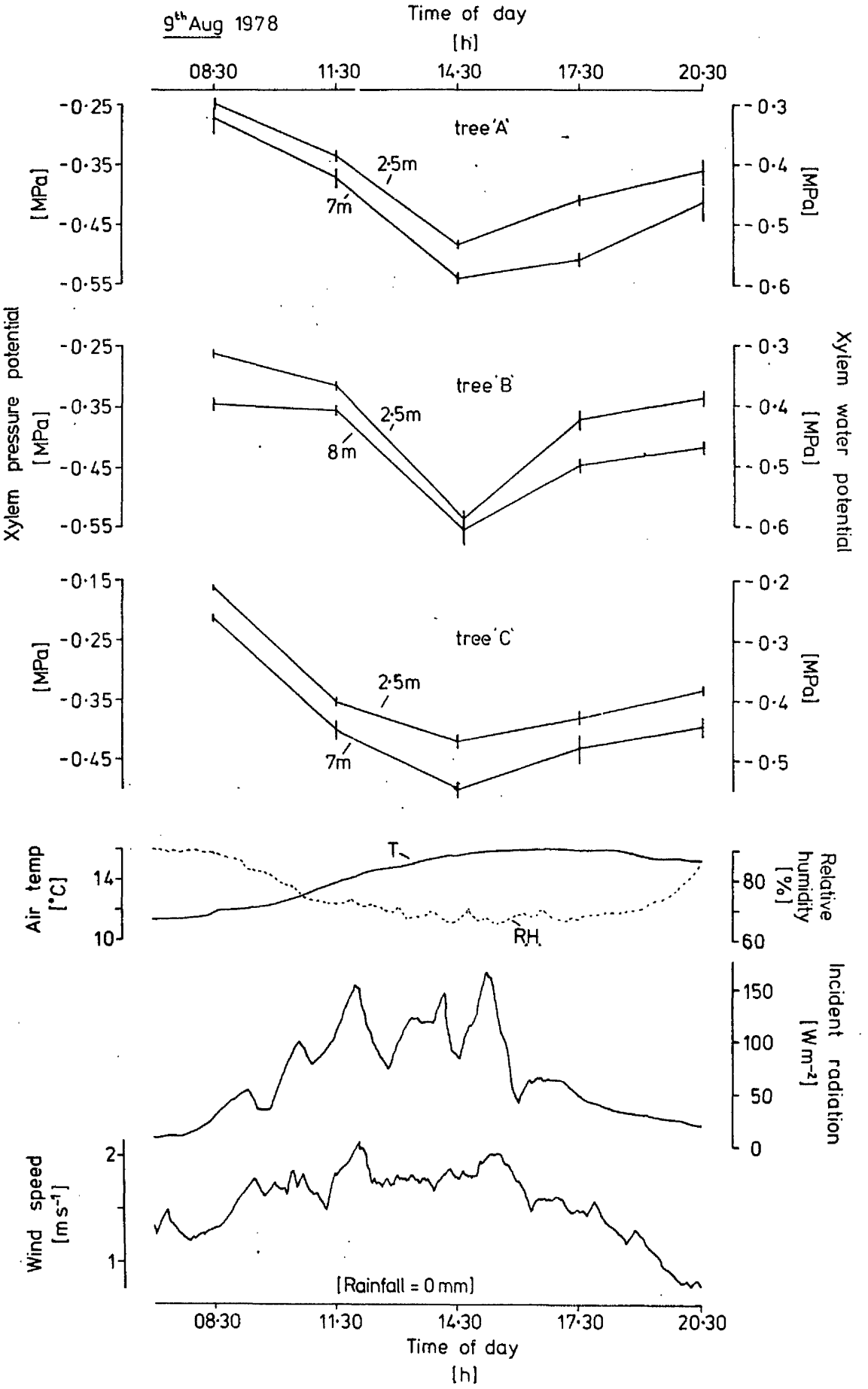


Fig.3.4 Xylem pressure potential (Ψ_p) estimated using the pressure bomb on 9th August 1978 for trees A, B & C at heights indicated. Environmental factors were recorded simultaneously.

chamber pressure slightly above the sap pressure balance point. Approx. 20 μl were collected for each height per sample time, and that for each tree pooled. This gave a large enough volume for determination of Ψ_s using the osmometer (see p 36).

Both figs 3.3 and 3.4 indicate that considerable sap tensions - ie -ve values of Ψ_p - were present within the trees when investigated. Classic diurnal fluctuations of pressure potential are evident, and these correlate well in general with the evaporative demands of the environment. Minimum Ψ_p values (ie. most -ve.) occured in mid to late afternoon when relative humidity was low and incident radiation and wind speed high. Indeed, quantitative comparison of figs. 3.3 & 3.4 shows considerably lower values of pressure potential for the former than for the latter, and inspection of environmental factors reveals that values of incident radiation and wind speed for fig 3.3 are practically double those for fig. 3.4. Examination of fig. 3.4 alone shows that although values of Ψ_p differed slightly between trees A, B & C on the day of investigation, general trends were similar. There is no evidence to suggest that tree A - used for intensive investigation - was unusually different from trees B & C.

Analysis of sap samples produced similar values of Ψ_s for each tree (approx. -0.05MPa). This magnitude of solute potential indicates the presence of very few solutes, as expected during summer when large fluxes of sap occur due to high rates of transpiration.

3.3 Xylem Sap Solute Content Of Mature Sycamore Trees During Dormancy, And Its Relationship With Pressure.

Studies of the presence of solutes - known to be principally sucrose - in the xylem sap of dormant maples have used A. saccharum as plant material. Some basic studies of sap content of A. pseudo-platanus (trees A, B & C) were therefore undertaken for comparison. Sap samples were taken from trunk boreholes prepared for attachment of Bourdon pressure gauges. For trees B & C, 2 holes were made per tree, at height 1m., 30cm. apart. Sap was collected when the trees were pressurised and exuding freely. The holes were approx. south-west facing, drilled initially (6th January 1979) to a depth of 3cm., diameter 1.25cm. Redrilling of the holes was undertaken on the dates

indicated by arrows, fig 3.5. The depth attained by 30th January was 5cm. (ie. penetrating approx. 4cm. into the xylem. It must be noted here that tree A had only one Bourdon pressure gauge, attached to a borehole situated between the two used for manometric recorders (see part 3.1). The specifications and treatment of this was the same as for holes in trees B & C. When not collecting sap, pressure gauges were attached . Alternatively at night, the borehole adaptors were sealed with rubber bungs.

Values of sap pressure were determined by direct reading of the gauges. In all cases, this was performed just prior to their removal to allow sap collection. After sap sampling, gauges were re-attached and considerable time (ie. approx. 1.5 hours) elapsed before further readings were taken. This allowed equilibration to occur. Fig. 3.5 shows data thus obtained. Values are the maximum sap pressures recorded for each day when the trees were thawed. Estimations for early January are averages of 2 boreholes for trees B & C. Differences between boreholes in the same tree were insignificant however, and only the eastern hole was used after January 15th. Pressures for tree A were supplemented by readings from manometric records. The 2 boreholes used here had identical specifications to those used for attachment of Bourdon pressure gauges. Where readings were taken from gauges and manometric recorders, no significant differences were evident. Air temperatures illustrated are maximum and minimum values for each day - the minimum value being that attained between 21.00 hours of the previous day and 00.60 hours. This information was supplied by Glasgow Weather Centre (118 Waterloo St., Glasgow, U.K.) from their station at Glasgow Airport, (Abbotsinch). No significant differences were noticed between this data and that recorded using Casella thermohygrographs at Garscube Estate.

% sucrose estimated by refractometry of sap samples collected from trees A, B & C throughout January is shown in table 3.1. Each value is the average of 1 or 2 samples taken from at least 2 sample times per day, Analysis was then performed immediately on returning to the laboratory. The results of more in depth studies are shown in tables 3.2 & 3.3. Here, samples (each~2ml) were taken at 2 hour intervals throughout 7th & 30th January. After transportation to the laboratory, solute potential (Ψ_s) and % sucrose were estimated by osmometry and refractometry respectively. Samples of 100 μl size were then placed in clean, plastic vials, sealed and stored in the

freezer (T = -15°C). Analysis for carbohydrates (Nelson/Somogyi assay) and mineral content (atomic absorption spectrophotometry) were performed when convenient. On 30th January, (table 3.3), sap was also collected from a branch (diam. 1.7cm.) situated 2m. above the trunk boreholes in tree A. This also had an attachment for a Bourdon pressure gauge.

Inspection of results for trees A, B & C, fig 3.5, reveals that the general trends for each tree were similar during the investigation. Actual values of pressure however, differed considerably between trees and a distinct categorisation is evident. Trees A & B consistantly exhibited higher values of pressure potential (Ψ_p) than tree C. Moreover, although maximum values fluctuated considerably throughout the study, this relative status was maintained. Similar ordering was sought for sap solute content (table 3.1). % sucrose content by refractometry does show that of tree C to be consistantly lower than that of trees A & B. However, highest % sucrose content is shown for tree B, which always showed pressure potentials slightly below those of tree A. If errors are small then the distinct ordering evident from maximum pressure records does not seem to correspond rigidly with % sucrose content of the sap.

Consideration of recorded pressure maxima in relation to maximum and minimum air temperatures (fig. 3.5) reveals a general increase in value of Ψ_p during periods with air temperature minima repeatedly below 0°C. Conversely a general reduction in Ψ_p is evident with minima repeatedly above 0°C. By repetition of sub-zero air temperatures, it may be that some value of Ψ_p can be attained, above which pressures cannot be increased. This possibly explains the stablising of sap pressure within tree A, evident between the 26th and 30th January. Although this influence of sub-zero air temperatures is relatively clear, the importance of the <u>absolute</u> value of minimum air temperature is not obvious.

Detailed analyses of sap samples (tables 3.2 and 3.3) support the general observations of table 3.1. It can be seen that trees A & B exhibit higher values of pressure potential (Ψ_p) than tree C, and accordingly lower (more negative) values of solute potential (Ψ_s). This correlation - as noted before - is not necessarily adhered to when values of Ψ_s and Ψ_p for different trees are similar (<u>ie</u>. trees A & B). Presumably errors in determination of these parameters will contribute here, but other factors (noted in the general introduction)

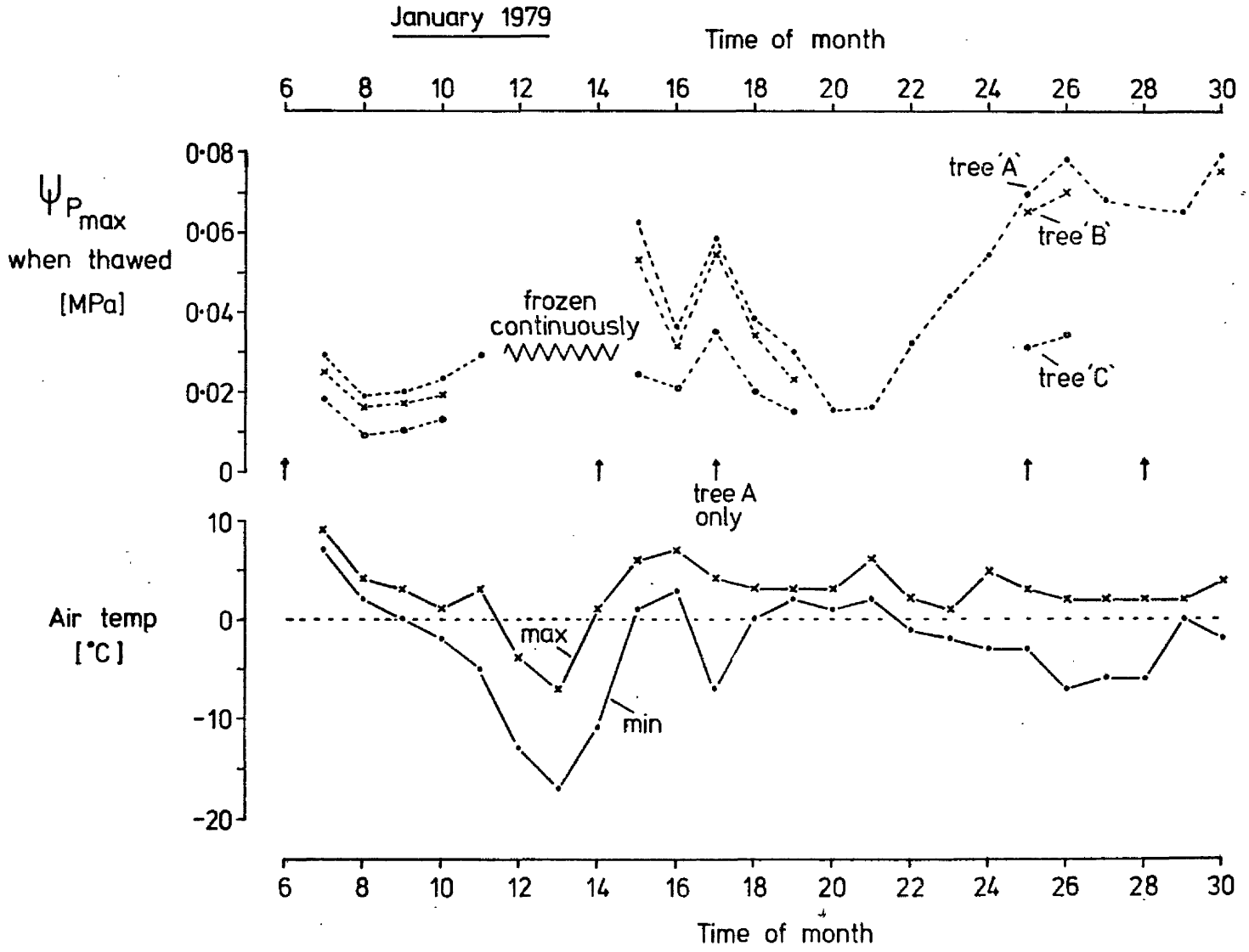


Fig. 3.5 Max. pressure potentials (Ψ_p max.) recorded for trees A, B & C (trunk, ht. 1m.) during January 1979. Max. and min. air temperatures are also plotted. Arrows indicate re-boring of trunk holes.

Table 3.1 % sucrose content of sap samples taken from trunks of trees A, B and C during January 1979, estimated by refractometry.

January 1979		Tree A	Tree B	Tree C
6th	bored	2.0	2.1	1.7
7th		1.8	2.0	1.2
8th		1.5	2.0	1.0
15th	rebored	1.8	2.3	1.1
17th	rebored	1.6	2.4	1.1
25th	rebored	1.5	1.7	0.9
26th		1.6	2.4	1.0
30th	rebored	1.9	2.2	1.6
Averages		1.7	2.1	1.2

Table 3.2 Analyses of xylem sap samples taken from trees A, B & C during a single day (7th January 1979).

	Time of day	Minerals K^+ (mM.dm^{-3})	Minerals Na^+ (mM.dm^{-3})	% sucrose (refract.) (%)	Non-reducing sugars (mM.dm^{-3})	Ψ_s solute potential (MPa)	Ψ_p pressure potential (MPa)
Tree A Trunk	10.30	3.2	0.6	1.8	48	-0.15	0.029
	12.30	3.1	0.6	1.8	50	-0.17	0.024
	14.30	3.2	0.6	1.8	50	-0.17	0.024
	16.30	3.1	-	1.8	48	-0.16	0.022
Averages		3.2	0.6	1.8	48	-0.16	0.025
Tree B Trunk	10.30	3.0	0.8	1.9	60	-0.16	0.025
	12.30	2.7	0.8	2.0	56	-0.17	0.020
	14.30	2.8	0.8	2.0	55	-0.18	0.021
	16.30	2.7	0.9	2.0	52	-0.17	0.020
Averages		2.9	0.8	2.0	56	-0.17	0.022
Tree C Trunk	10.30	1.2	0.8	1.2	32	-0.09	0.018
	12.30	1.2	0.8	1.2	33	-0.11	0.014
	14.30	1.2	0.8	1.2	32	-0.10	0.015
	16.30	1.2	0.9	1.2	31	-0.11	0.014
Averages		1.2	0.8	1.2	32	-0.10	0.015

Table 3.3 Analyses of xylem sap samples taken from trees A, B & C during a single day (30th January 1979)

	Time of day	Minerals K^+ (mM. dm^{-3})	Minerals Na^+ (mM. dm^{-3})	% sucrose (refract.) (%)	Non-reducing sugars (mM. dm^{-3})	Ψ_s solute potential (MPa)	Ψ_p pressure potential (MPa)
Tree A Branch (ht. 3m)	10.00	2.0	0.7	2.2	61	-0.23	0.034
	12.00	2.1	0.7	2.1	58	-0.21	0.050
	14.00	2.2	0.8	2.0	58	-0.19	0.040
	16.00	1.9	1.2	1.9	56	-0.17	0.034
Averages		2.1	0.8	2.1	58	-0.20	0.040
Tree A Trunk (ht. 1m)	10.00	3.7	0.7	1.5	43	-0.17	0.062
	12.00	4.1	0.8	2.0	52	-0.21	0.071
	14.00	4.5	0.8	2.1	52	-0.21	0.060
	16.00	4.4	1.0	2.0	54	-0.19	0.053
Averages		4.2	0.8	1.9	50	-0.20	0.062
Tree B Trunk (ht. 1m)	10.00	5.0	0.8	2.1	51	-0.21	0.065
	12.00	4.9	0.7	2.1	54	-0.25	0.061
	14.00	5.0	1.3	2.3	52	-0.24	0.059
	16.00	4.7	1.0	2.2	53	-0.22	0.054
Averages		4.9	1.0	2.2	53	-0.22	0.059
Tree C Trunk (ht. 1m)	10.00	2.2	0.7	1.5	37	-0.14	0.024
	12.00	2.2	0.7	1.5	44	-0.15	0.028
	14.00	2.2	0.7	1.6	41	-0.17	0.027
	16.00	2.6	0.9	1.6	36	-0.17	0.021
Averages		2.3	0.8	1.6	40	-0.16	0.024

may also be of consequence (eg. heredity, tree age, disease). The value of solute potential is determined principally by the levels of non-reducing sugars and minerals. The relative contributions of these may be seen in the tables. If it is assumed that sucrose is the only sugar present in any quantity (Jones et al 1903, Taylor 1956) then by calculation, this usually accounts for 70-80% of Ψ_s measured. Potassium is also of significance and like sucrose content, levels were observed to vary considerably both between trees and between different days for the same tree. Levels of sodium however, were relatively stable.

Sap collected from the branch in tree A (table 3.3) was found to contain generally higher levels of non-reducing sugars (sucrose) than that from the trunk. Although only one branch was investigated, and hence the significance of the data is questionable, this observation is in accordance with those previously for sugar maples (eg. Jones et al 1903). It is also of interest to note that sap sucrose levels from the branch were highest when initially sampled just after thawing, (ie. 10.00 hours). This value gradually fell during the day. The converse was so for the sap collected from the trunk. Comparison of sap pressures detected in the branch and trunk of tree A indicates a classic hydrostatic gradient (ie. 2m. height difference between branch and trunk tapping points producing a pressure difference of 0.02MPa).

3.4 Sap Solute Content Of Sycamore Seedlings Throughout The Year.

To substantiate sap analyses performed on mature trees (previous part), sap analyses were undertaken for seedlings. Samples of xylem sap were taken either as natural exudations or by elution (see p 34). Estimations of solute potential (Ψ_s) and % sucrose (by refractometry) were performed immediately. Samples of sap were stored in a freezer (sealed in plastic vials) for determination later of carbohydrate content (ie. non-reducing and reducing sugar - Nelson/Somogyi assay) and mineral content (atomic absorption spectrophotometry). Bark samples were occasionally taken and after extraction of sap (see p 35) Ψ_s and % sucrose estimated. When seedlings were in leaf, estimations of pressure potential (Ψ_p) were performed using the pressure bomb, (see p 23). Values for each seedling were an average of 2 samples

taken at one height. Sample heights between seedlings ranged from 60-150cm. Using this data and that obtained for Ψ_s, xylem water potential could be plotted (Ψ_x - see fig. 3.6). During dormancy, pressure potential was not determined for seedlings under natural conditions. An estimated range was therefore plotted (see fig. 3.6, dotted lines Ψ_p). This represents maximum temperature-induced fluctuations of sap pressure (approx. -0.06 → +0.03MPa) predicted from laboratory studies.

Results in fig. 3.6 are presented as monthly averages, vertical bars indicate ranges of values. The sample size for each month totalled between 4 and 20 seedlings, (usually approx. 15) sampled on 1 to 4 dates, in batches of 3-8 seedlings. A full tabulation of data is presented in appendix I.

Particularly obvious in fig 3.6 is the detection of non-reducing sugars (presumed to be mainly sucrose) in sap collected from dormant seedlings (Nov-April). Both non-reducing and reducing sugars were found to be completely absent from sap collected from seedlings in leaf, (June-Sept.). This rise in sap sugar content also appeared to be selective. Reducing sugars remained undetected, (by the method employed) throughout the year. Levels of non-reducing sugars varied quite extensively (eg. 30-100mM.dm^{-3} for December and February), and this may be expected due to inbuilt variation and environmental influences (see later). As for mature trees, sucrose is the principle component of solute potential during dormancy, and hence seasonal changes referred to are paralleled by changes in sap refractive index (ie. % sucrose by refractometry) and mirrored by xylem solute potential changes (Ψ_s). Minerals also contribute to Ψ_s although presumably are of more significance during summer months. Again, as noted for mature trees, values of K^+ vary considerably between seedlings and throughout the year, whereas Na^+ levels appear relatively stable. It is also interesting to note that changes in bark Ψ_s, where recorded, parallel those of xylem Ψ_s. Values during winter are lower than those during summer, implying increased bark cell solute content. These changes are substantiated by similar fluctuations in bark sap refractive index (see appendix I.)

Calculation of xylem water potential (Ψ_x) by addition of Ψ_s and Ψ_p produced interestingly similar values for both summer and winter periods. The principle component of Ψ_x during summer is Ψ_p. Values of Ψ_s are usually high (Kramer 1949) whereas xylem sap tensions may

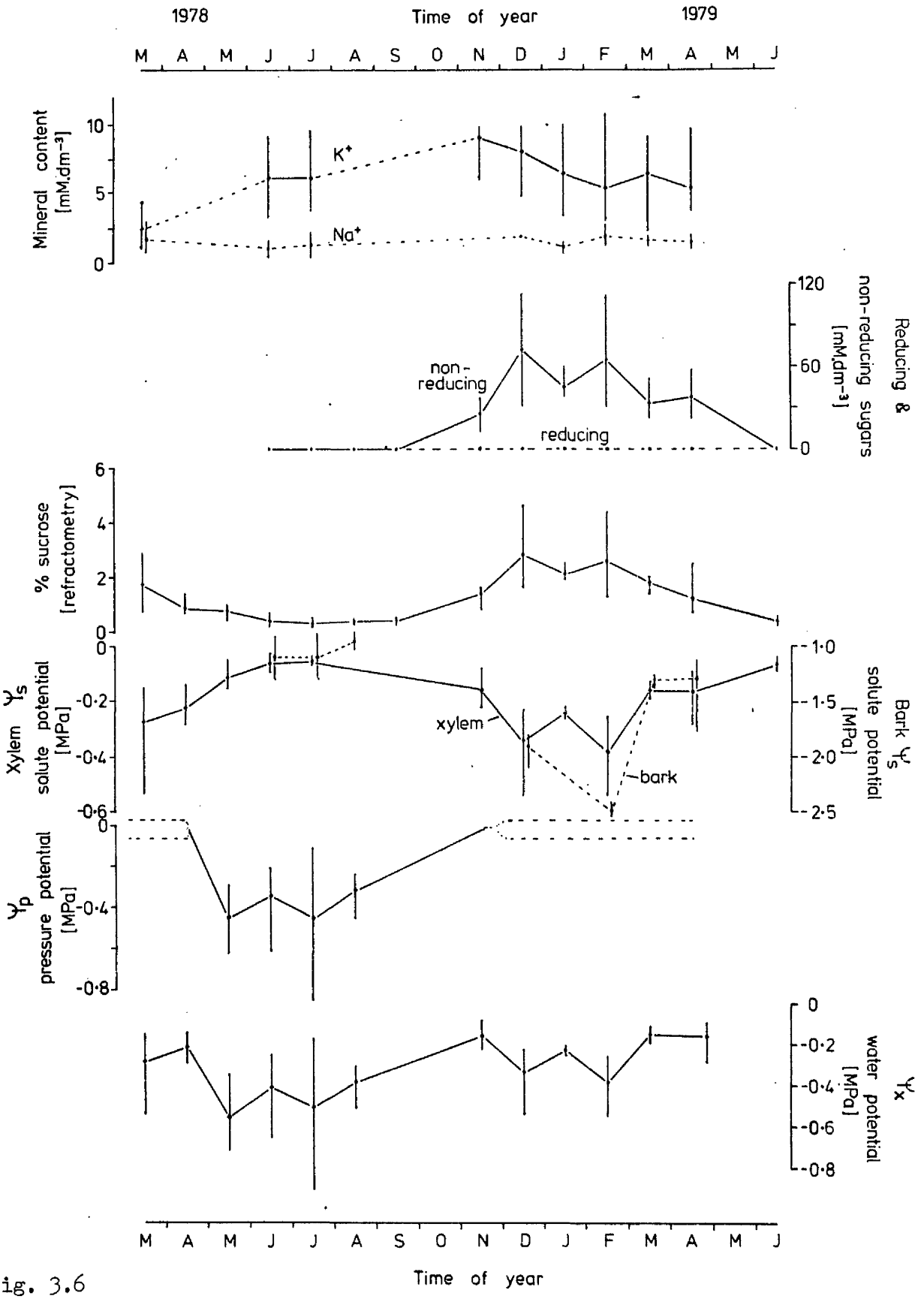


Fig. 3.6

Analyses of xylem sap samples taken from seedlings throughout the period March 1978 to June 1979. Xylem pressure potential and bark sap solute potential were also estimated. For further details see text.

be considerable (ie. Ψ_p may be very low). Transpiration rates and soil water potential are principal factors of influence here. In contrast during dormancy transpiration is much reduced and Ψ_p values are relatively high (ie. less negative). Xylem solute potential (Ψ_s) however, is characteristically low due to increased solute content, and this correspondingly lowers the value of Ψ_x.

3.5 Manometric Investigations Of Sycamore Seedling Stem Sap 'Pressures' In The Laboratory.

Field investigations reported in part 3.1 revealed unusual pressure fluctuations associated with periods of air temperature below 0°C. Experiments were therefore undertaken in the laboratory under more controlled conditions to investigate this further. Principally, xylem sap 'pressure' fluctuations were monitored in response to induced temperature changes. N.B. Sap 'pressures' monitored in the laboratory using manometric recorders are not true estimates of pressure. Development of a sap tension in these devices mecessitates absorption of relatively large volumes of water by the stem from the recorder. (see p 30). Accordingly, the term 'pressure units' was adopted, and results shown may ge taken as indications of xylem sap pressure fluctuations, but are not absolute measurements. In discussion, these pressures appear in inverted commas.

Seedlings were chosen for similarity from the stock available, and excised 5cm. above ground level with secateurs or a hacksaw. Usually samples of xylem sap were taken for analysis (see p 33 & p 36). Stem segments, (ie. the basal 30cm. of each stem), were then prepared for attachment to manometric recorders (see p 20). Segments were positioned in the controlled temperature chamber and 'pre-treated' for 12hours, (ie. supplied with distilled water at +15°C - see p 22). They were then attached to manometric devices and pressures monitored as the chamber temperature was lowered from +15 → -5°C, and raised to +15°C. Each phase was of 12 hours duration. Mercury levels were then re-zeroed, and the temperature cycles repeated. Stem temperatures were simultaneously recorded on a Grant temperature recorder (G.T.R.) from a probe embedded in the basal end of each segment. Chamber air temperature was recorded using a Casella thermohygrograph and/or a G.T.R and probe. After experimentation, fresh and dry weights of

wood (and bark if appropriate) were determined for each stem by standard gravimetric procedures.

Figs. 3.7a & b illustrate results from stems with bark intact. Barkless stems were also treated (figs. 3.8a & b) the naked xylem protected by polythene and plastic tape (see p20). Records shown are from individual dormant stems representative of those (totalling at least 8 in each case) used for experimentation at different times. These results could be obtained from one stem when treated appropriately. Long periods of treatment however, (eg $\gtrsim$ 5 days) gave reduced responses. This was presumed partially due to wound effects, initiating sealing of the apical end. Experiments were therefore performed on different batches of stems. It must also be stressed that although results are presented from dormant stem segments, similar results were obtained using tissue in the summer state.

Immediately apparent from inspection of fig. 3.7a is the sudden drop in stem sap 'pressure' (ie. production of a sap tension) - indicated by arrows (eg. points b → c). Due to the nature of the recording device, such a response must reflect absorption of water by the stem. This event is observed after the period of rapid temperature change (eg. a' → b') during which a more gradual drop in sap 'pressure' is detected. (eg. a → b). A similar biphas pressure response during cooling was observed previously for mature trees in the field (part 3.1). Laboratory investigations however, detected freezing of sap within the stem associated with the phase of rapid induction of sap tension. Release of latent heat from supercooled sap produces a sudden rise in stem temperature (eg. from approx. $-4^{\circ}C$ → $-1^{\circ}C$, b' → c', fig. 3.7a) which is easily detected. Such a phenomenon is commonly called an exotherm, and was prominent as ice formation usually occured during the period when temperature was changing slowly. If freezing failed to occur (fig. 3.7b) no such sudden sap absorption/tension phase was observed.

The 'ice-induced' tension described above was characteristically reversed by thawing. Warming of the stem (eg. d' → e') produced rapid pressurisation (eg. d → e). Full recovery of the system was not observed however. Stem pressures plateaued at a value (eg. e) below that originally (eg. a). This infers that water is retained by the stem (eg. by gas dissolution, growth) or lost from the system (eg. transpiration, evaporation). Results shown in figs. 3.8a & b are repeats of treatments in figs 3.7a & b using stem segments

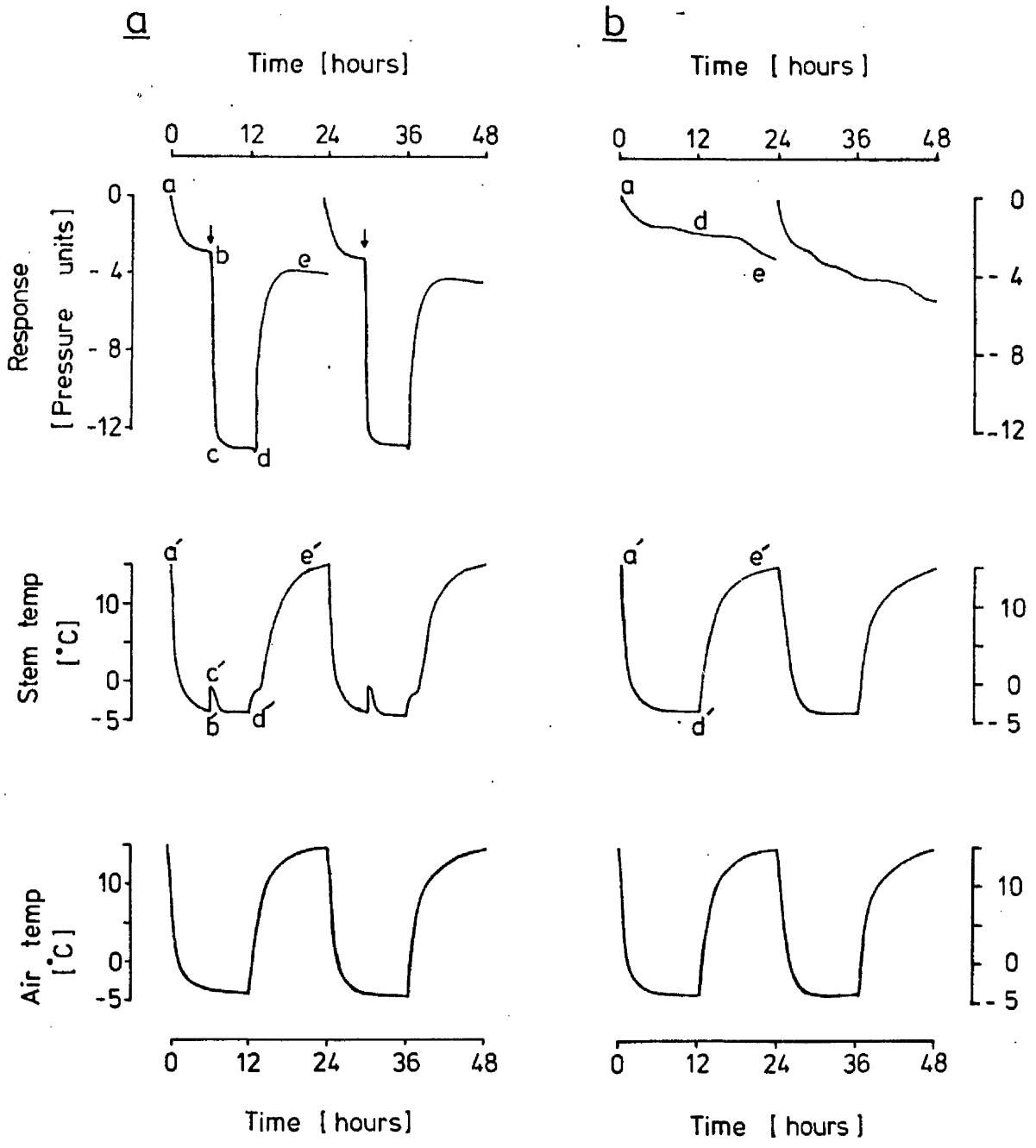


Fig. 3.7 Seedling stem sap 'pressures' monitored with manometric recorders in response to controlled temperature changes. a) with ice formation b) without ice formation. Stem segments were used with bark intact.

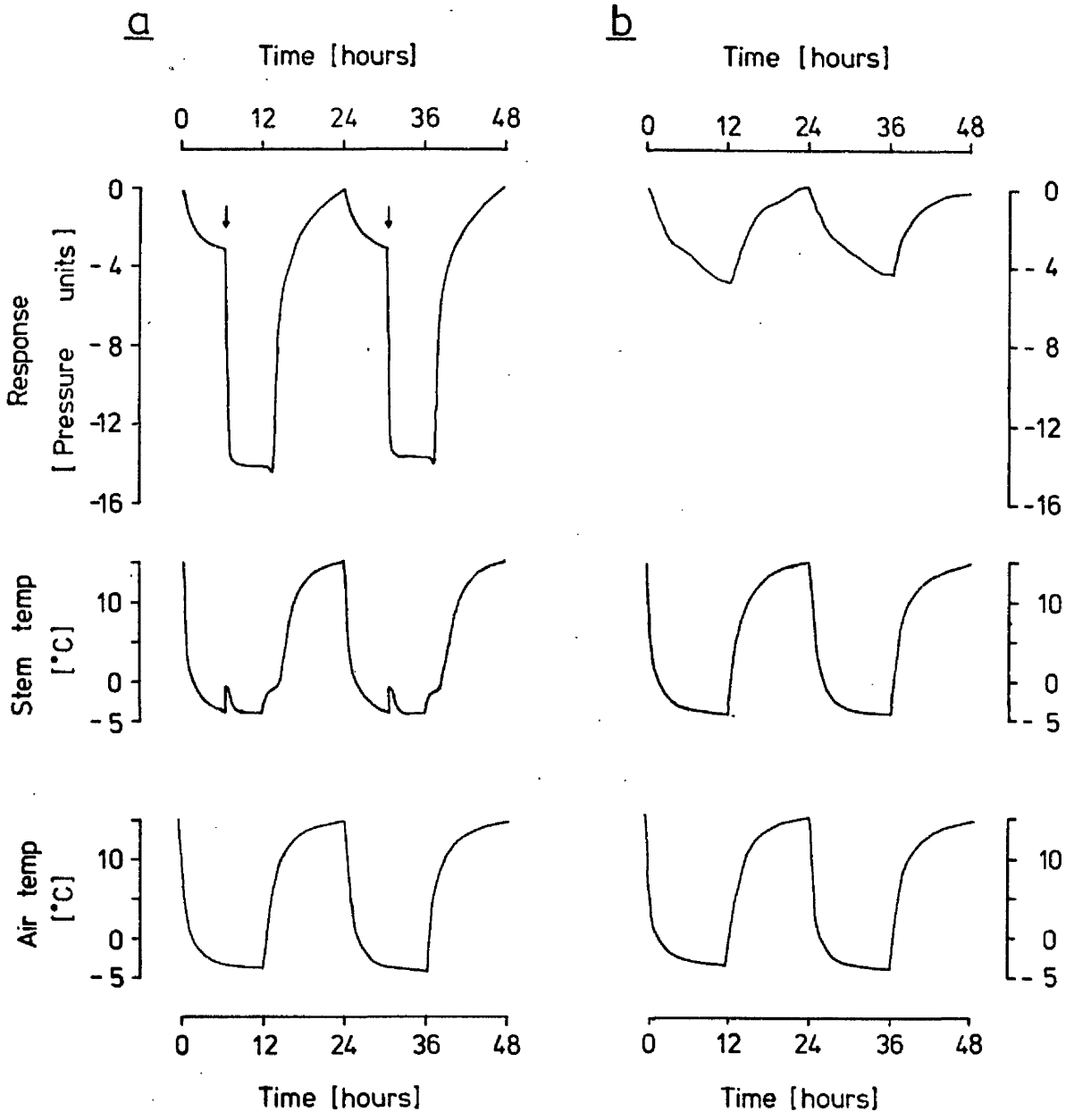


Fig. 3.8 Seedling stem sap 'pressures' monitored with manometric recorders in response to controlled temperature changes. a) with ice formation b) without ice formation. Stem segments were used with bark removed.

lacking bark but protected with polythene and tape. Clearly the ice-induced pressure response is qualitatively similar to that from stems used with bark intact. Response to thawing differed however, producing full recovery of sap pressure. This difference is also reflected in the sap pressure response for segments cooled and warmed without freezing (figs. 3.7b & 3.8b).

3.6 Measurement Of Sap Pressure Potentials (Ψ_p) And Sap Uptake During Cooling Of Seedling Stem Segments.

Seedling sap pressures monitored in the laboratory using manometric recorders are merely indications of pressure fluctuations. Further investigations were therefore conducted to estimate truer values of sap pressure using the pressure transducer (see p 31). Priority was given to study of the response during cooling, as the importance of this phase has been established. Similarly, experiments were undertaken to quantify sap uptake during cooling of segments freely supplied with water.

a) Volumetric estimations of sap uptake.

Stem segments (30cm. long) with bark intact were prepared for experimentation (see p 20). After pretreatment they were maintained attached to pipettes supplying distilled water, and subjected to standard temperature treatment in the controlled temperature chamber ie. 12 hour cooling ($+15^{o}C \rightarrow -5^{o}C$) followed by 12 hour warming ($-5^{o}C \rightarrow +15^{o}C$). Absorption and exudation during each period was monitored by taking pipette readings at 15 minute intervals. Fig. 3.9 shows the standard response, plotted from results for one segment representative of 12 used, (2 batches of 6). Later experiments monitored uptake only (ie. response during cooling.). Estimation of this alone was considered sufficient. Segments were cooled until freezing had occured and equilibration was complete. (ie. sap absorption had ceased). This was usually approx. 6 hours in total. Pipette readings were taken at frequent intervals throughout. After experimentation, bark and wood fresh and dry weights were estimated by standard gravimetric procedures (see p 43.). Results compiled from different segments collected during summer and winter are shown in tables 3.4 and 3.5 .

Uptake is presented as volume absorbed per unit dry weight of

wood, although stems investigated had bark intact. The data is considered more useful expressed this way as experiments revealed that the absorption phenomenon is based in the xylem cylinder. Absence of bark did not apparently influence the response.

b) Estimations of pressure potential (Ψ_p).

Stem segments were prepared and pretreated as above. Only one pressure transducer was available and hence experiments were repeated using single segments. Standard response was first determined by cooling and warming stems in the controlled temperature chamber whilst monitoring pressure. Results were characteristically similar in form to the free absorption response (fig. 3.9). Again, later experiments monitored response only during the cooling phase. (ie. until freezing had occured and equilibration was complete - approx. 6 hours). Results are shown in table 3.6 compiled from data for both summer and winter tissue.

Experiments were also conducted to determine values of positive sap pressure potential (Ψ_p) during warming. Stem segments were prepared and pretreated in the standard way. Each was then cooled to $-5^{\circ}C$ in the controlled temperature chamber, during which free absorption of distilled water was allowed. Once freezing and associated sap uptake was complete, the pipette was removed and the pressure transducer attached. Pressure was then monitored during thawing to $+15^{\circ}C$. Special care had to be taken to ensure the rubber seals - particularly that at the basal end of the segment - did not leak under the positive sap pressure. Table 3.7 shows some results obtained by this method.

Comparison of fig. 3.9 with seedling sap pressure response recorded using manometric recorders (part 3.5, fig. 3.7a) shows little qualitative difference. This may be expected as pressure and volume changes are intimately associated. However, it does verify that responses monitored using the manometric devices are representative of true pressure and volume changes during both freezing and thawing cycles.

Examination of sap uptake during cooling (tables 3.4 & 3.5) is of particular interest. This response is characteristically biphasic, and table 3.4 shows results obtained during cooling ($+15^{\circ}C \rightarrow -5^{\circ}C$) prior to freezing. Sap uptake is relatively similar for each segment, per unit dry weight, averaging $2.0 \times 10^{-2} \pm 0.07$ $cm^3.g^{-1}$. Calculation of predicted absorption due to thermal contraction of sap

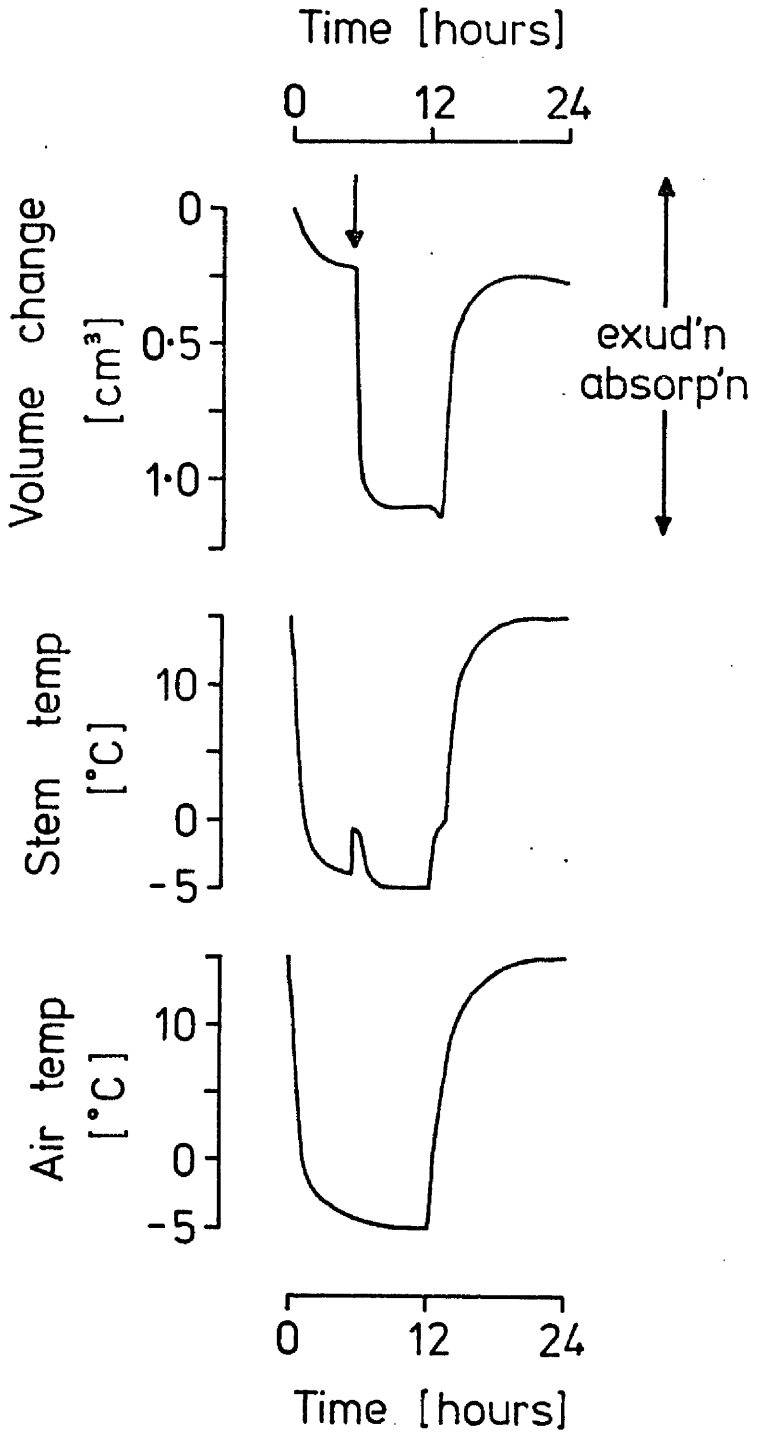


Fig. 3.9 Absorption/exudation by seedling stem segments, monitored using simple pipettes, in response to freeze/thaw cycles. Segments used with bark intact.

within the stems is clearly inadequate to account for this, (av = $1.5 \times 10^{-3} \pm 0.04$ $cm^3.g^{-1}$). In contrast, similar estimation of volume changes resulting from gas dissolution (namely CO_2) more than account for sap uptake observed. (av. = $6.9 \times 10^{-1} \pm 0.02 cm^3.g^{-1}$. Such calculations overestimate predicted uptake as it is assumed that sap within the wood is saturated with CO_2 at all temperatures. Also the value of water content employed for calculation is the total for each stem, and hence includes intracellular water of living cells. Even considering these sources of error, gas dissolution may still contribute significantly to sap uptake during cooling prior to ice formation. Its contribution to the response during ice formation (table 3.5) cannot be envisaged however - although here estimation of simple thermal effects are impractical. Paradoxically, the ice associated response appears contrary to tnat expected from freezing. Water uptake is observed during a period when liquid water is expanding within the stem, due to change of state! Absorption associated with this phase is relatively large and can be seen to vary extensively between stems (eg. $3.3 \times 10^{-2} \rightarrow 9.8 \times 10^{-2} cm^3.g^{-1}$). Explanation of the processes responsible here would be of obvious advantage, and experiments investigating this are presented later, (chapter 4).

Investigation of seedling sap pressure potential in response to freezing (table 3.6) revealed that considerable tensions may develop. Values were variable but considerably lower than those recorded using manometric recorders, (eg. average for pressure transducer $\sim$ -0.03MPa vs typical value for manometric recorder $\sim$ -0.015MPa). This in part reflects the consequence of relatively large changes in volume within the manometric recorder necessary to produce a pressure response, (see p 30). For true estimation of pressure this displacement must be minimal. The volume displacement of the pressure transducer is much reduced ($2.5 cm^3$ displacement to record 0.1MPa pressure), but still may be a source of error. Further experimentation is considered necessary using a more sensitive transducer (or alternative pressure device) to verify that the values of pressure presented are indeed true estimates of xylem sap pressure potential (Ψ_p). Similar investigations must be undertaken to substantiate observed values of positive Ψ_p (0.1MPa, table 3.7). However, although actual values may contain error, manifestation of positive pressure was real. Hence exudation on thawing, from previously 'conditioned' stems, may be considered, at least in part, to be forcibly driven from within.

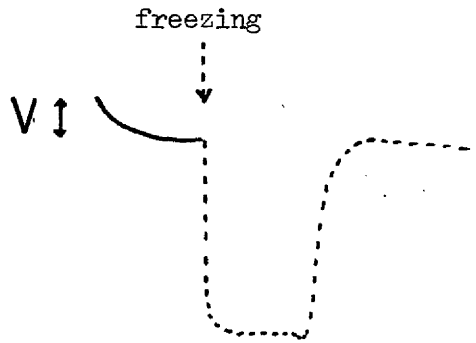
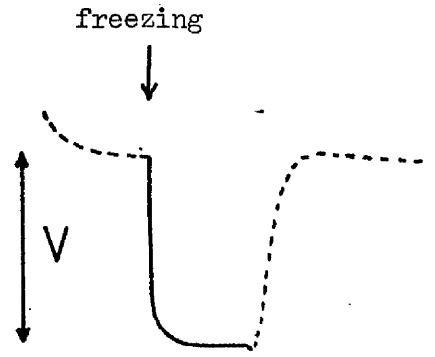


Table 3.4 Absorption of water by stem segments during cooling period <u>prior</u> to initiation of ice formation

Stem No.	Fresh weight + bark (g)	Fresh weight - bark (g)	Dry weight + bark (g)	Dry weight - bark (g)	Volume absorbed, V. (cm^3)	Vol. absorbed per g.d.w. wood ($cm^3.g^{-1}$) $x10^{-2}$	Predicted thermal contraction of water in wood per g.d.w. wood ($cm^3.g^{-1}$) $x10^{-3}$	Max. predicted vol. change from CO_2 dissolution per g.d.w. wood ($cm^3.g^{-1}$) $x10^{-1}$
1	45.1	32.5	24.8	18.7	0.43	2.3	1.3	6.2
2	39.6	29.7	23.6	17.8	0.30	1.7	1.2	5.6
3	42.2	31.3	23.0	17.0	0.29	1.7	1.6	7.0
4	41.4	30.4	22.4	16.8	0.40	2.4	1.5	6.8
5	39.5	28.6	22.8	16.8	0.43	2.6	1.3	5.8
6	39.6	28.1	23.1	16.8	0.28	1.7	1.2	5.6
7	35.0	25.7	19.8	14.7	0.28	1.9	1.4	6.3
8	34.8	25.8	19.0	14.1	0.26	1.8	1.5	6.9
9	33.7	24.5	19.3	14.0	0.24	1.7	1.4	6.3
10	34.9	25.1	18.2	13.8	0.30	2.2	2.0	9.1
11	36.2	25.6	18.9	13.7	0.27	2.0	1.6	7.3

Stem No.	Fresh weight + bark (g)	Fresh weight - bark (g)	Dry weight + bark (g)	Dry weight - bark (g)	Volume absorbed, V. (cm^3)	Vol. absorbed per g.d.w. wood ($cm^3.g^{-1}$) $x10^{-2}$	Predicted thermal contraction of water in wood per g.d.w. wood ($cm^3.g^{-1}$) $x10^{-3}$	Max. predicted vol. change from CO_2 dissolution per g.w.w. wood ($cm^3.g^{-1}$) $x10^{-1}$
12	32.4	23.4	18.2	13.6	0.21	1.5	1.3	6.0
13	35.3	25.2	18.1	13.6	0.20	1.5	1.5	7.1
14	33.8	23.9	19.0	13.6	0.34	2.5	1.4	6.3
15	34.1	24.5	18.5	13.4	0.32	2.4	2.0	9.4
16	34.0	23.7	19.0	13.4	0.20	1.5	1.4	6.4
17	37.2	25.5	19.5	13.4	0.31	2.3	1.6	7.5
18	35.7	25.7	18.1	12.9	0.28	2.2	1.8	8.3
19	32.3	23.7	17.5	12.8	0.27	2.1	1.5	7.1
20	30.9	21.6	17.9	12.7	0.23	1.8	1.3	5.8
21	30.6	20.9	16.7	11.8	0.22	1.9	1.4	6.4
22	28.9	20.6	15.9	11.7	0.21	1.8	1.4	6.3
23	28.7	19.5	14.9	10.9	0.25	2.3	1.4	6.6
24	29.9	20.4	15.3	10.8	0.20	1.9	1.6	7.4
25	26.2	17.3	15.5	10.4	0.18	1.7	1.2	5.5
26	26.7	20.0	12.8	9.7	0.20	2.1	1.9	8.9
27	23.7	16.4	12.1	9.4	0.13	1.4	1.3	6.2
28	23.9	17.9	11.2	8.9	0.23	2.6	1.8	8.4
					Averages	2.0 $\pm$0.07	1.5 $\pm$0.04	6.9 $\pm$ 0.2

Table 3.5 Absorption of water by stem segments during ice formation



Stem No.	Fresh weight + bark (g)	Fresh weight - bark (g)	Dry weight + bark (g)	Dry weight - bark (g)	Volume absorbed, V. (cm^3)	Vol. absorbed per g.d.w. wood ($cm^3.g^{-1}$) $x10^{-2}$
1	39.6	29.7	23.6	17.8	0.68	3.8
2	42.2	31.3	23.0	17.0	1.21	7.1
3	41.4	30.4	22.4	16.8	1.09	6.5
4	39.5	28.6	22.8	16.8	0.85	5.1
5	39.6	28.1	23.1	16.8	0.72	4.3
6	35.0	25.7	19.8	14.7	0.82	5.6
7	34.8	25.8	19.0	14.1	0.68	4.8
8	33.7	24.5	19.3	14.0	1.05	7.5
9	34.9	25.1	18.2	13.8	0.69	5.0
10	36.2	25.6	18.9	13.7	0.62	4.5
11	32.4	23.4	18.2	13.6	0.59	4.3
12	35.3	25.2	18.1	13.6	0.79	5.8
13	33.8	23.9	19.0	13.6	0.63	4.6
14	34.1	24.5	18.5	13.4	0.80	6.0
15	34.0	23.7	19.0	13.4	0.60	4.5
16	32.5	23.7	17.5	12.8	0.56	4.4
17	30.9	21.6	17.9	12.7	1.24	9.8
18	30.6	20.9	16.7	11.8	0.50	4.2
19	28.9	20.6	15.9	11.7	0.83	7.1
20	28.7	19.5	14.9	10.9	0.35	3.2
21	29.9	20.4	15.3	10.8	0.53	4.9
22	26.2	17.3	15.5	10.4	0.70	6.7
23	23.7	16.4	12.1	9.4	0.58	6.2
					Average	5.5 $\pm$0.3

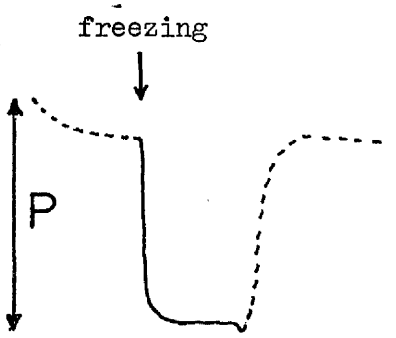
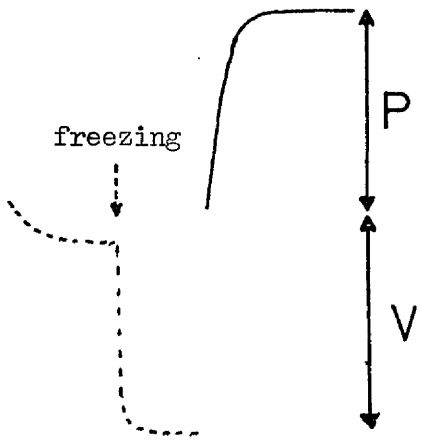


Table 3.6 Minimum pressures (ie. maximum tensions), recorded with pressure transducer, in stem segments during ice-induced absorption phase.

Stem No.	Fresh weight + bark (g)	Fresh weight - bark (g)	Dry weight + bark (g)	Dry weight - bark (g)	Pressure P (MPa)
1	84.0	-	46.2	-	-0.041
2	80.0	-	44.1	-	-0.036
3	77.6	-	34.3	-	-0.024
4	71.2	-	34.5	-	-0.061
5	65.6	-	32.0	-	-0.031
6	64.4	-	31.5	-	-0.030
7	56.4	-	23.5	-	-0.033
8	48.7	-	23.1	-	-0.035
9	48.0	37.4	24.0	18.9	-0.026
10	41.7	-	21.8	-	-0.017
11	40.6	29.1	23.0	16.8	-0.016
12	38.1	28.4	18.5	13.4	-0.015
13	33.3	24.7	17.5	12.8	-0.019
Avs.	57.7		28.8		-0.030

Table 3.7
Maximum positive pressures, P recorded with pressure transducer in stem segments on thawing. Stems were previously cooled until frozen, during which they were supplied with water, V.



Stem No.	Fresh weight + bark (g)	Dry weight + bark (g)	Vol. absorbed (ice-induced) (cm^3)	Max +ve pressure, P (MPa)
1	40.6	23.1	0.72	0.010
2	38.1	18.5	0.80	0.005
3	36.3	19.0	0.63	0.010
4	40.5	22.8	0.85	0.010
Averages	38.9	20.9	0.75	0.009

3.7 Discussion

Dramatic, reversible fluctuation of xylem sap pressure from a positive to a negative value (relative to atmospheric pressure) has been an inexplicable characteristic of dormant maple trees. Investigations of this have, until now, used systems estimating pressure only intermittently, (eg. hourly intervals throughout a day- Jones et al 1903). Such readings were then plotted, and correlated with various imposing factors. In this way the general influence of temperature has been clearly demonstrated (Jones et al loc. cit, Marvin 1958), whereas its precise involvement has remained concealed. Moreover, change in pressure associated with temperature change has been considered as a single continuous event, ie. either a decrease in sap pressure with lowering of air temperature, or an increase associated with increased temperature. Enigmatically, it has been observed that pressure fluctuations are greatest when air temperature oscillates about $0^{o}C$. Pressure changes associated with temperature changes above $0^{o}C$ are small. Further resolution of these responses has not been achieved however, and attempts to relate temperature and pressure changes quantitatively have been unsuccessful (Marvin loc. cit). It has been determined by Marvin (loc. cit) that the event of most influence on exudation is the previous period of cooling - termed the 'conditioning period'. During this, sap absorption and retention occurs. Subsequent rise in temperature is considered merely as a trigger initiating exudation (ie. increase in sap pressure).

Present investigations in both the field (part 3.1) and laboratory (part 3.5) using devices monitoring pressure continuously clearly show that maple sap pressure response during cooling is biphasic. Field investigations showed that during cooling, sub-zero air temperature changes influenced sap pressure far more than changes in air temperature above $0^{o}C$. Experiments undertaken in the laboratory imply that these differences are due to effects prior to, and associated with, freezing within the stem. Cooling without (or before) ice formation induces only gradual uptake of water by the stem. Effects of temperature change are considered of most influence here, although transpirational loss may contribute fractionally. Quantitatively, absorption during this phase can be attributed to simple contraction of liquid and gas, and more importantly dissolution of gas, (see part 3.6). This suggestion

is supported by work using sugar maple (Sauter, personal communication). Investigation of absorption/exudation from segments subjected to temperature fluctuations above 0°C. (ie. without freezing of sap), revealed that thermal expansion/contraction/dissolution of gas/liquid was adequate to account for volume changes observed. These results were fundamental in the conception of the gas powered system proposed by Sauter, (1974).

More significant than sap pressure changes prior to ice formation, however, is the response associated with ice formation - to which no specific reference has been made by previous workers.. Here sap tension is rapidly developed as water is absorbed by the stem. This response is surprising as it is contrary to that predicted. Liquid water (ie. sap) undoubtedly expands during change of state to ice, yet uptake of water was observed during this process! Absorption was characteristically reversible - exudation (ie. pressurisation) was achieved by thawing the stem.

This freeze-induced reversible uptake of water is considered to be that fundamental to 'conditioning' (ie. sap replenishment during cooling). Hence it is crucial in determining sap exudation, (Marvin 1958). The participation of freezing in this process has not been demonstrated previously, and indeed had been an issue of considerable controversy. Sole support for its involvement was presented by Stevens and Eggert (1945). They proposed an ice-induced sap absorption mechanism to explain maple sap exudation (see general introduction), although no direct, specific evidence was included. General opinion however, has excluded freezing from explanation of maple exudation, (eg. Marvin 1958, Sauter 1974).

Experiments have shown the freeze-absorption mechanism to be based in the wood, (eg. part 3.5). Ice formation is considered to be extracellular (apoplastic) and hence the bulk of ice formed will be from freezing of xylem sap. Intracellular freezing (generally resulting in death of the cells involved) is not expected at the temperatures used for experimentation, particularly when using dormant tissue. (Levitt 1972, George et al 1974, Burke et al 1976).

Although pressure response during cooling is clearly biphasic, sap pressurisation during warming does not appear so. Thawing of a stem segment in the laboratory (part 3.5) produces an initial rapid increase in sap 'pressure', which stabilises as stem temperature attains equilibrium with air temperature. On initial warming, 'pressure' response appears to lag slightly - presumably until melting of sap

occurs. That melting of ice should trigger pressurisation/exudation accords well with general observation of thawing response, (Marvin 1958). Results of further investigation of this however, are presented in chapter 4.

As with the involvement of freezing in the maple exudation mechanism the role of sucrose in the system has been a topic of similar controversy. Correlation of high sucrose content with high sap yield/pressure has been proven clearly (Morrow 1952, Marvin et al 1967). Marvin (1951) presented direct evidence for the profound influence of sucrose and other solutes on the efficacy of the mechanism, (see general introduction). In contrast, Sauter, (personal communication), has observed sap exudation from maple stem segments (when appropriately treated) the sap of which when investigated lacked any significant sucrose content. In parts 3.5 and 3.6, results are presented from experiments using sycamore stem segments from both dormant seedlings and those in leaf. The freeze/absorption response, with pressurisation/exudation on thawing, was found to be similar for both states of tissue. The observation that maple sap from trees in leaf lacks sucrose (eg. Jones 1903, Sauter et al 1973) has been verified for sycamore seedlings (part 3.4). Hence results imply that the presence of sucrose in xylem sap is not crucial to the sap exudation mechanism. The proven association of high sucrose content with high sap yield/pressure is therefore considered to be indirect. Further discussion of this relationship is presented later.

In summary therefore, preliminary experiments disclosed that freezing of sap within the wood was fundamental to the maple 'conditioning response', and hence also in determining subsequent pressurisation/exudation. Water is absorbed and retained during freezing and this excess may then be forcibly exuded on thawing. These observations accord well, in general, with previous investigations of sugar maple, although past authors have not distinguished between sap pressure response during cooling a) prior to, and b) associated with, freezing. Failure to resolve these two responses must be considered therefore when interpreting data from previous publications. Absorption of sap inducing change in sap pressure during cooling before freezing is considered most probably a result of simple thermal effects. Sap uptake and resultant pressure response associated with freezing is rapid and considered a very different, as yet unexplained, event. Although undoubtedly

exudation can be obtained by warming stems previously cooled but not frozen, (Sauter, personal communication) the 'freeze-absorption' response is considered to be that event unique (as yet) to maple exudation.

4. Detailed Laboratory Investigation Of The Freezing Process.

Information presented in the previous chapter revealed that maple sap pressure response during cooling appears biphasic. Some absorption - and hence reduction in sap pressure - undoubtedly results from simple thermal effects. More important however, is change in pressure associated with freezing within the wood. Response here is characteristically rapid, and reversible. Increase in pressure is achieved by thawing. Further, more detailed investigation of the ice-induced response was therefore considered necessary

4.1 Investigation Of The Natural Freeze/Thaw Process.

To resolve further the association of the freeze/thaw process with changes in sap pressure, experiments were undertaken using manometric recorders with a faster drum rate (1 rev./day). Barkless stem segments (30cm. long) were used, prepared as described on p 20. After pretreatment they were attached to manometric devices and subjected to standard cooling and warming cycles ($+15^{\circ}C \leftrightarrow -10^{\circ}C$). Each cycle was of 12 hours duration. Fig 4.1 shows the response for 1 segment (representative of at least 8 used at different times). Seedlings were used both when in leaf and dormant with similar results.

Experiments were also performed to highlight the thawing part of the cycle (fig. 4.2). Barkless segments were prepared as above. After pretreatment (still attached to pipettes) they were first equilibrated at $+2^{\circ}C$ for 6 hours. Manometric recorders were then attached and the stems cooled (to approx. $-8^{\circ}C$). After 6 hours, temperature within the chamber was raised to $-2^{\circ}C$, and stems equilibrated for 6 hours. Finally the temperature was raised to $+2^{\circ}C$. Results shown in fig. 4.2 are again from 1 segment representative of 8 used. (2 experiments each of 4 dormant segments).

Results in fig. 4.1 emphasise the rapidity with which sap tension is induced during freezing, and reversed on thawing. Clearly stem temperature profiles reveal exo- and endothermic regions associated with these changes. The former is more obvious as supercooling of sap is characteristically observed, and the release of latent heat by spontaneous freezing produces a dramatic rise in stem temperature.

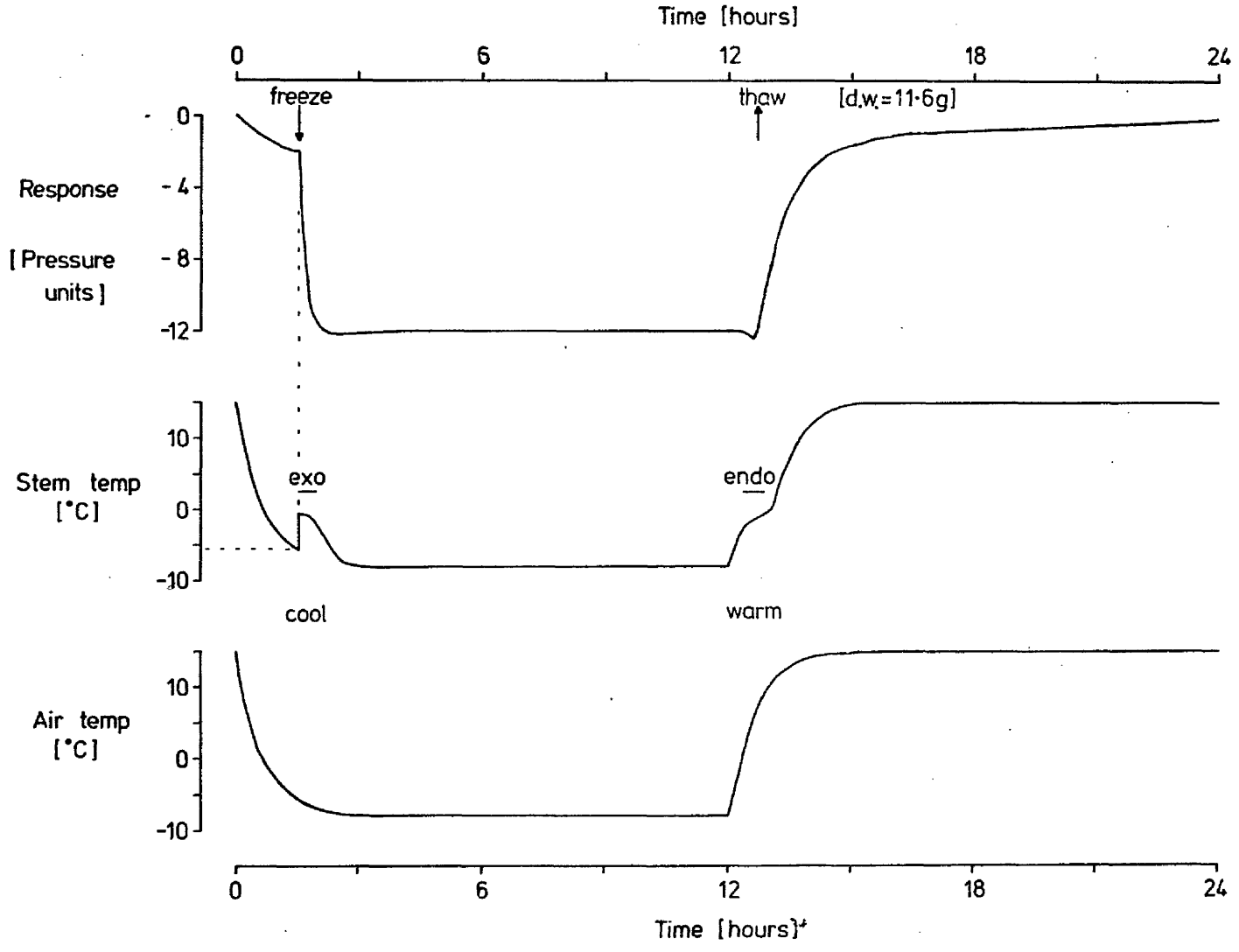


Fig. 4.1 Detailed record, using a manometric device, of seedling sap 'pressure' fluctuations in response to freeze/thaw cycles. Stem segments used had bark removed.

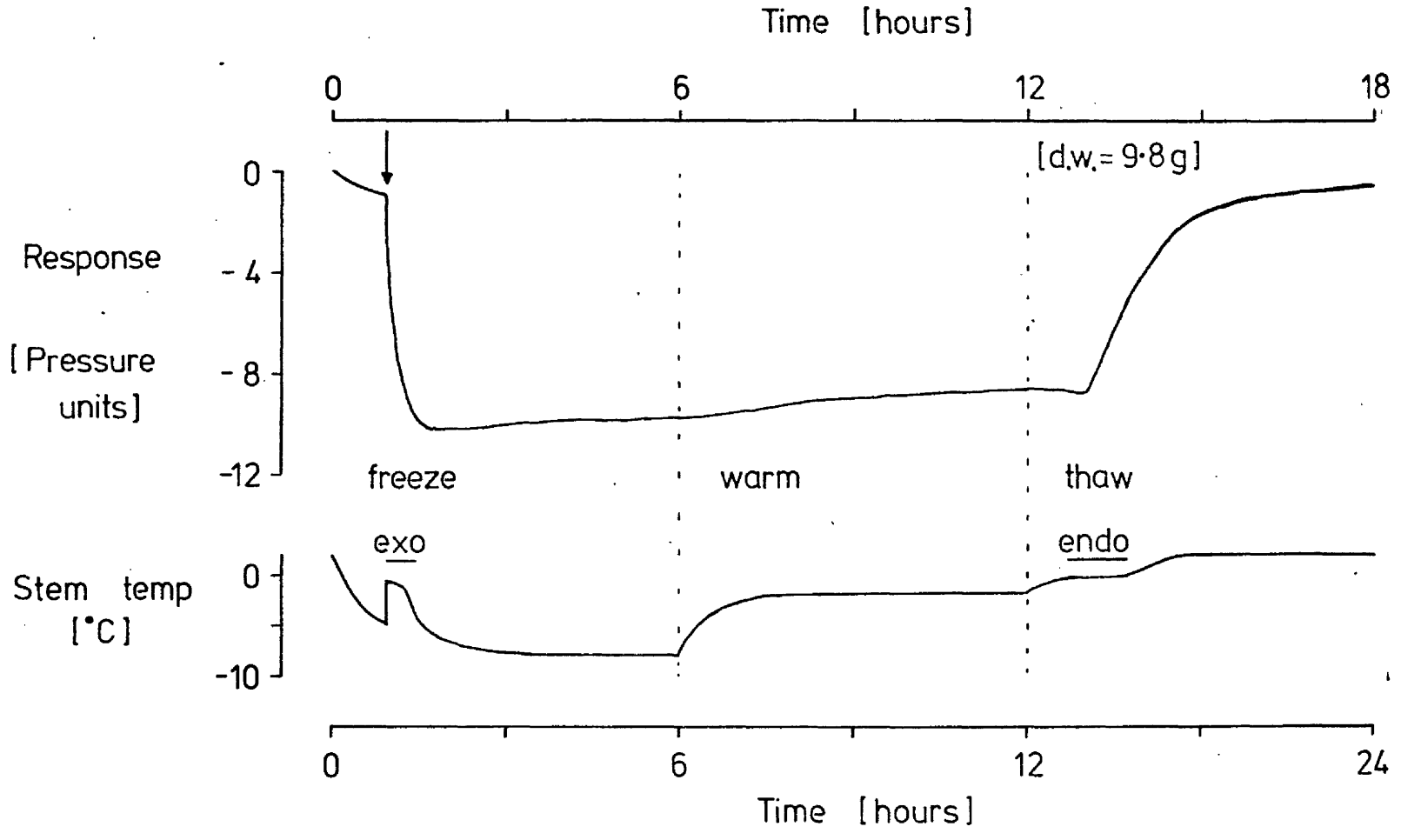


Fig. 4.2 Seedling sap 'pressure' fluctuations, recorded using a manometric device, in response to initial freezing, followed by warming to $-2^{o}C$ and final thawing at $+2^{o}C$. Stem segments used had bark removed.

The period of sap absorption - inducing sap tension - appears to last only for the duration of freezing, as determined by the exotherm.

Response on thawing appeared slightly less rapid than that during freezing. Pressurisation in fig, 4.2 is particularly slow in comparison with response to freezing. Here temperature change was small ($-2^{o}C \rightarrow +2^{o}C$). The temperature gradient between stem and environment, and the degree of temperature change, might be important factors therefore influencing this event. That the act of thawing (ie. melting of ice) is crucial for exudation (pressurisation) is also illustrated in fig. 4.2. Raising stem temperature from $-8^{o}C$ to $-2^{o}C$ produced little increase in pressure. Pressurisation was triggered however by only small increase in stem temperature, ($-2^{o}C \rightarrow +2^{o}C$) as this presumably induced full thawing of the tissue. Frequently, but not always, stem pressures were observed to decrease slightly on initiation of thawing, (see fig. 4.1). The reason for this, however remains unclear.

4.2 Effects Of Induced Ice Formation.

In experiments described so far, freezing has been allowed to occur naturally. During this, considerable supercooling of sap occurs. Experiments were therefore performed to determine the response when ice formation was induced near $0^{o}C$, and hence supercooling reduced to a minimum. Barkless stem segments (30cm.) were prepared as usual. After pretreatment they were attached to manometric recorders, cooled to $-2^{o}C$ and equilibrated for 6 hours. Ice formation was then induced in the following way. The segments were quickly removed from the temperature chamber and the basal 2cm. of stem immersed in liquid nitrogen ($T = -196^{o}C$) for 30 seconds. They were then repositioned in the chamber ($-2^{o}C$) as rapidly as possible. One of two courses of action was then adopted. Stems were further equilibrated at $-2^{o}C$ (fig. 4.3). Alternatively they were cooled to $-8^{o}C$, (fig. 4.4) following the usual air temperature pathway imposed by the controlled temperature chamber. After 6 hours equilibration, thawing was induced by raising the temperature to $+15^{o}C$. Experiments were repeated, using 4 dormant segments each time. Each result shown is that from 1 representative segment. Dotted sections of the temperature profiles are predicted courses of stem temperature. Actual temperature was off scale, due to effects of the liquid N_2.

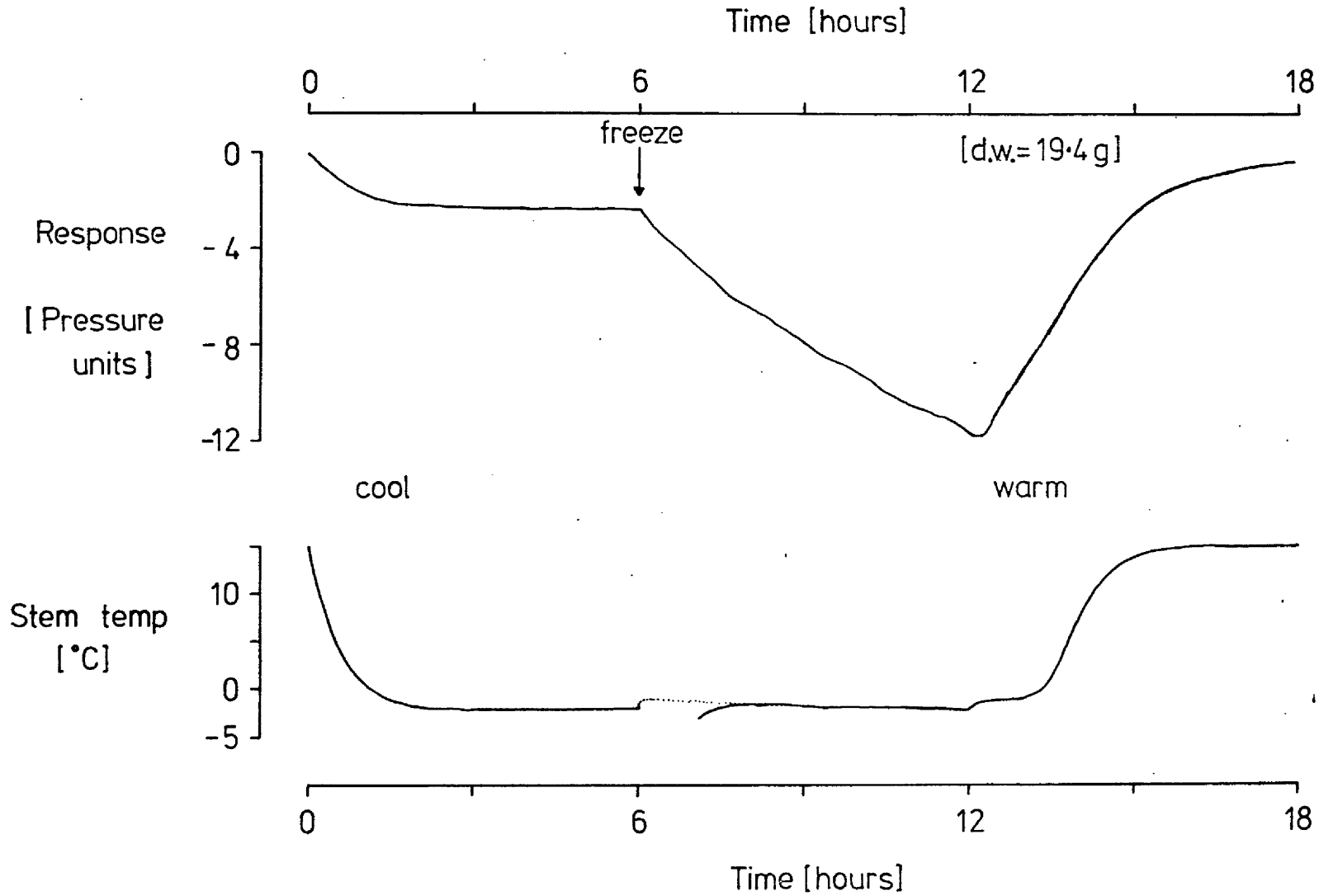


Fig.4.3 Seedling sap 'pressure' changes recorded using a manometric device in response to initial cooling, subsequent induction of ice formation, and final thawing. Stem segments used had bark removed. Dotted line on stem temp. record indicates predicted value.

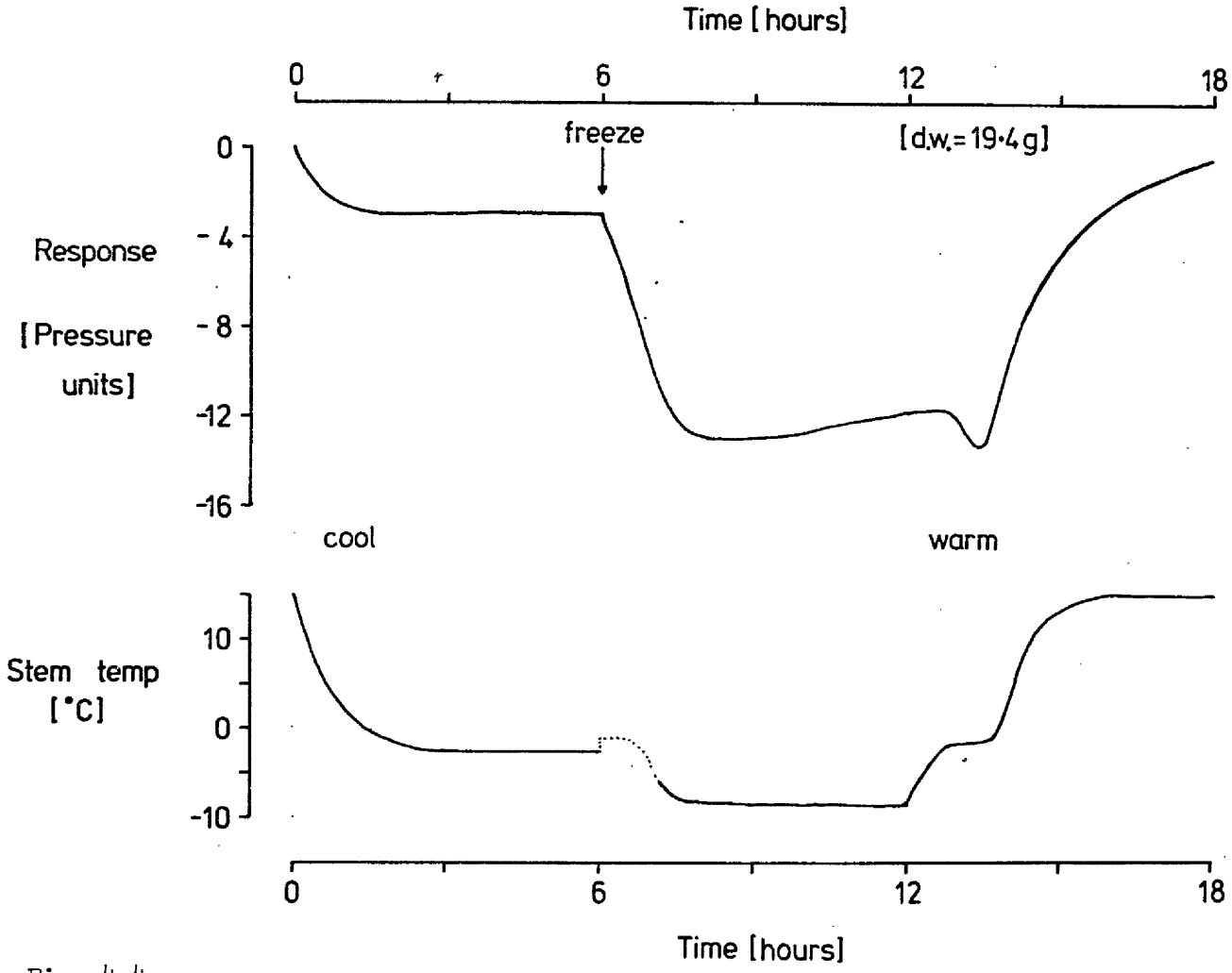


Fig. 4.4

Seedling sap 'pressure' changes recorded using a manometric device, in response to initial cooling, subsequent induction of ice formation with continued cooling, and final thawing. Stem segments used had bark removed. Dotted line on stem temp. record indicates predicted values.

Both figs. 4.3 and 4.4 show ice-induced absorption (producing sap tension - arrowed), which is reversed on thawing. Rates of change of pressure differ however. A faster drop in stem pressure was evident when stems were cooled during ice formation (fig. 4.4). This might be expected if rate of ice formation/growth is a factor of influence, as this would be determined in part by the temperature gradient between the freezing sap and environment. Minimum temperature attained in each experiment also differed, however ($-2^{o}C$, fig. 4.3 vs. $-8^{o}C$, fig. 4.4). This may be influential in its effect upon the quantity of ice formed within each stem at each temperature. Nuclear magnetic resonance (N.M.R) investigation of freezing (appendix II) suggests that relative values of liquid and frozen extracellular water differ significantly between samples of wood equilibrated at $-2^{o}C$ and $-8^{o}C$ (eg. $-2^{o}C$, 86% water unfrozen, vs. $-8^{o}C$, 73% water unfrozen). Results presented in fig. 4.2 (part 4.1) however, suggest the effect is small, as little difference in sap tension was detected for change in temperature from $-8^{o}C \rightarrow -2^{o}C$. Hence differences in response between figs. 4.3 and 4.4 are presumed mainly due to differences in thermal gradient between stem and environment during freezing.

It was mentioned in part 4.1 that a small drop in sap pressure was often detected during early thawing. This is particularly pronounced in fig. 4.4, - the response from stems cooled during ice formation - , but absent from fig. 4.3 - the response from stems equilibrated at $-2^{o}C$ after ice induction. Again, causes of this phenomenon are unclear.

4.3 Are Living Cells Fundamental To The Absorption/Exudation System?

Experiments were conducted to determine whether living cells were directly involved in the freezing (absorption) response. Barkless, dead tissue was used, killed using one of three basic treatments:- a) elution with KCN solution, b) immersion in hot water and c) steam treatment, (see pp 38 - 39). After appropriate preparation and pretreatment, (p 20) responses of the dead segments to standard cool/warm cycles were monitored using manometric recorders. For each experiment 4 segments were used, and experiments were repeated at least once. Results shown are responses of individual segments. Fig. 4.5 a & b are from segments eluted with $10mM.dm^{-3}$ KCN solution.

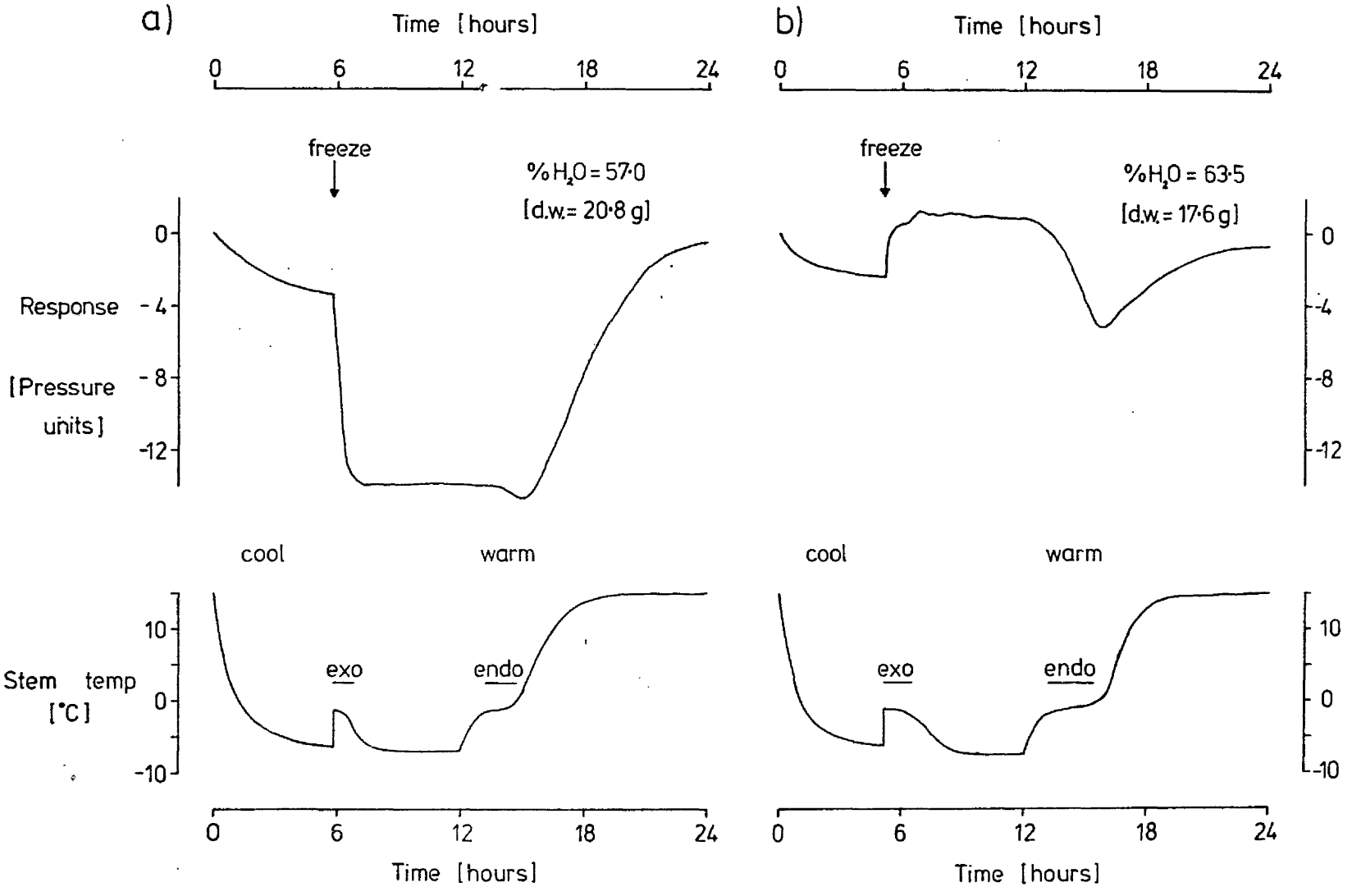


Fig. 4.5 Sap pressure fluctuations recorded using manometric devices from KCN-killed tissue in response to freeze/thaw cycles. a) low stem water content. b) high stem water content. Stem segments used had bark removed.

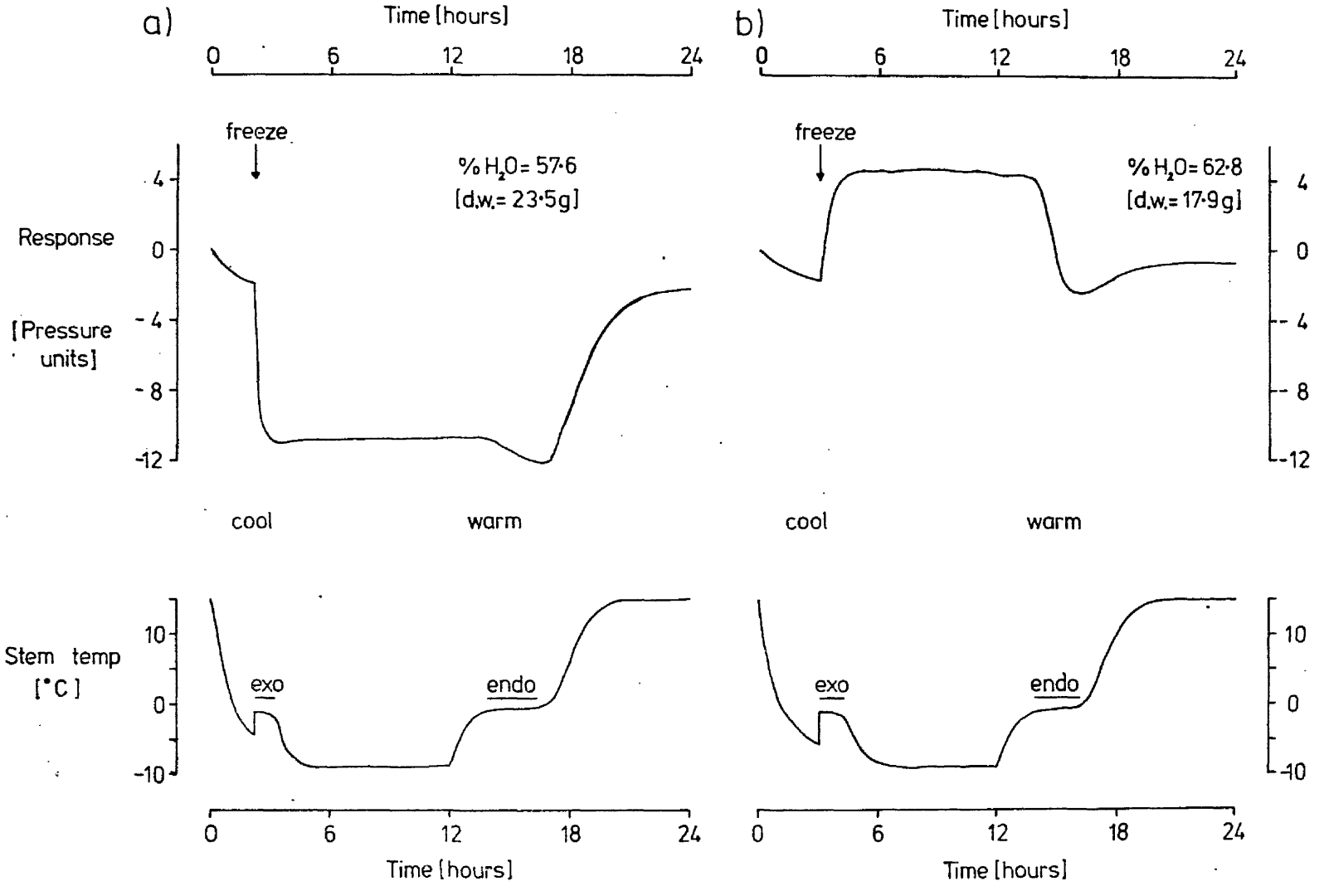


Fig. 4.6 Sap pressure fluctuations recorded using manometric devices, from heat (water) killed tissue in response to freeze/thaw cycles. a) low stem water content. b) high stem water content. Stem segments used had bark removed.

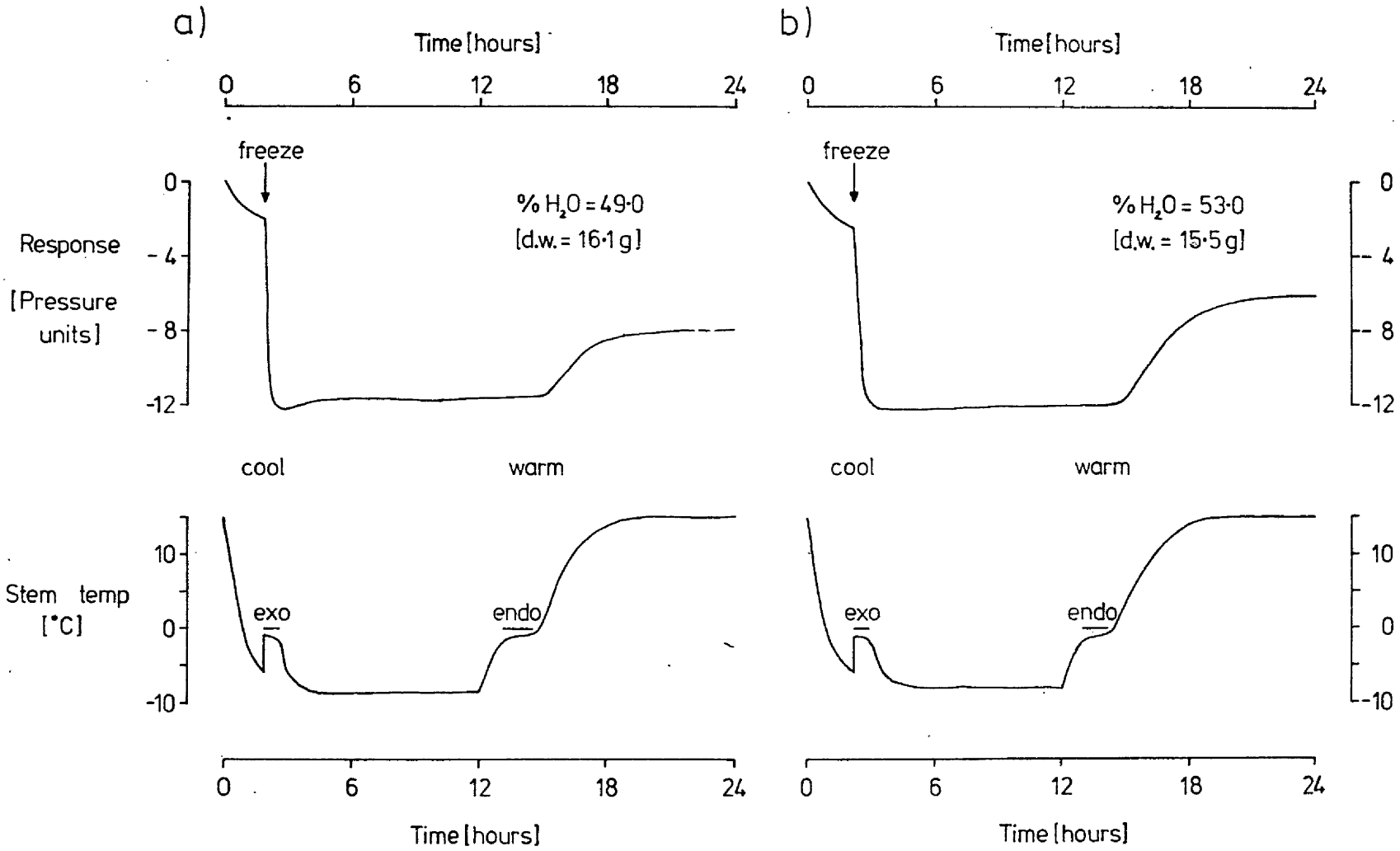


Fig 4.7 Sap pressure fluctuations recorded using manometric devices, from heat (steam) killed tissue in response to freeze/thaw cycles. a) low stem water content. b) high stem water content. Stem segments used had bark removed.

Fig. 4.6 a & b are responses from segments after hot water treatment (80°C). Fig. 4.7 a & b are responses from steam treated tissue (90°C).

Upon initial inspection, results appeared inconclusive. As well as could be determined from vital staining (TTC test, p 39) all segments used were dead, yet experiments gave a mixture of positive and negative responses to freezing. Some segments developed a sap tension during freezing (ie. exhibited normal response of living tissue) others showed the reverse response. In all cases, steam killed tissue exhibited rapid uptake during ice formation, producing a sap tension - although exudation on thawing seemed impaired (see fig. 4.7). These stems were considered dead, and confirmed so by TTC test. Hence it may be deduced that the absorption response shown to be associated with freezing of sap (ie. the process considered here as fundamental to sap replenishment during 'conditioning') does not require the direct involvement of living tissue. Explanation is necessary however of the reduced response of steam killed tissue to thawing, and this is discussed later.

Subsequent inspection of % water content of dead wood used in experimentation (see figs. 4.5, 4.6 and 4.7) revealed a general correlation between water content and the nature of the response. Those segments exhibiting absorption (and hence sap tension) on freezing were of lower water content, whereas those exhibiting exudation (increased pressure) were of higher water content. Further investigation of this relationship was undertaken, (part 4.4)

4.4 Relationship Between Water Content Of Tissue And Response During Ice Formation.

A general relationship between water content of stem wood and response during freezing was inferred for dead tissue in part 4.3. On this basis, analysis of data aquired through general experimentation was undertaken. This included both living and dead tissue. Response to freezing was categorised into resultant decrease in pressure (ie. absorption) or increase in pressure (ie. exudation). The nature of of the response was correlated with % water content of the wood, measured after experimentation. Segments sampled were totalled (figures in brackets, fig. 4.8a) for each 5% increment in water content and corresponding response expressed as a percentage of that sample

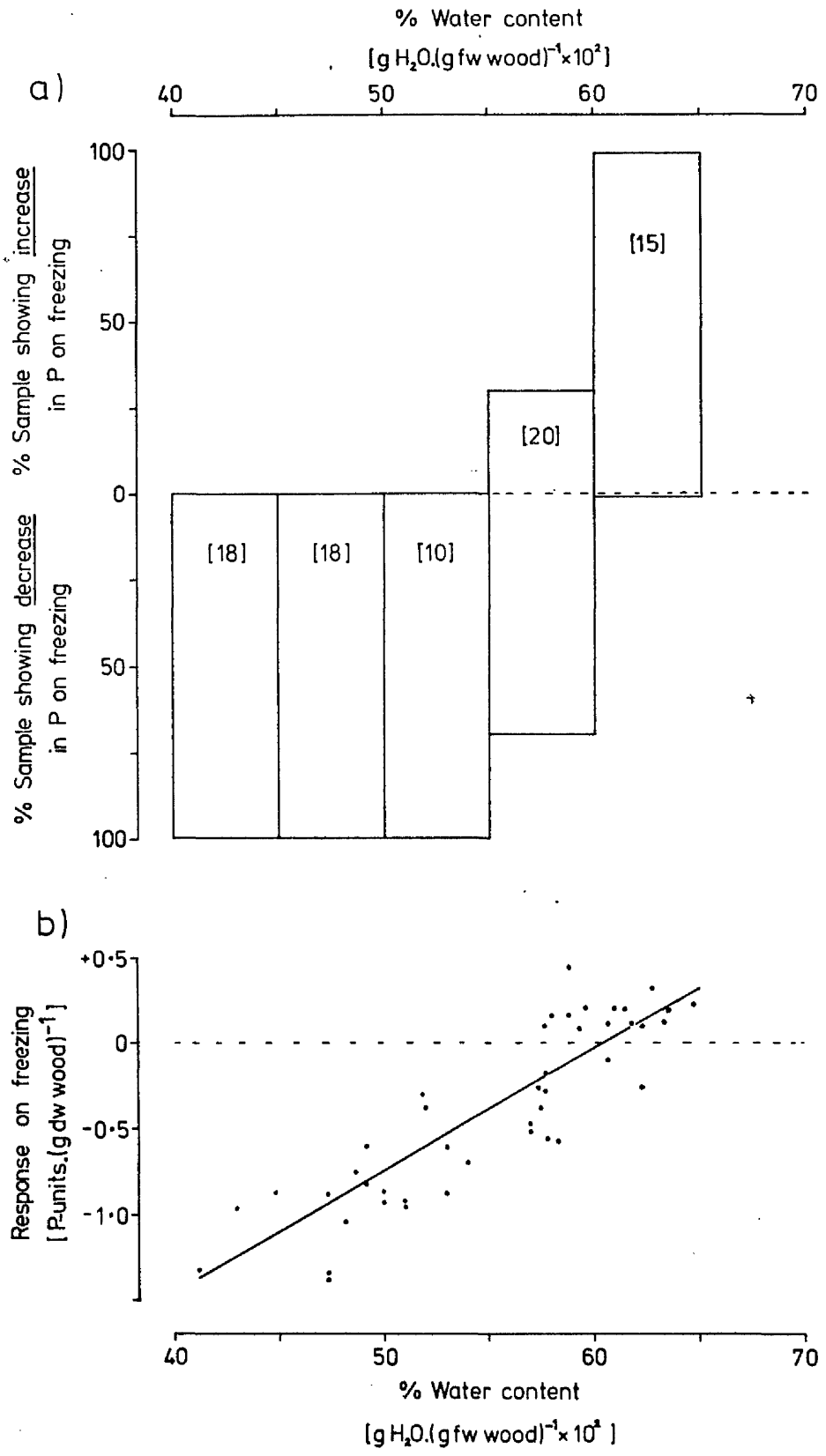


Fig. 4.8 Response of stem segment on freezing related to water content (of wood), a) expressed qualitatively b) expressed quantitatively. The line is fitted by least square analysis to y = 0.0355x - 2.15 (r = 0.83). For further details see text.

number (see fig. 4.8a). Further analysis of a quantitative form was also undertaken (fig. 4.8b). Response during freezing was measured (pressure units) for barkless segments monitored using manometric recorders during standard temperature cycles. This was related to the dry weight of stem (wood) and again correlated with % water content measured after experimentation. Results include living, dead, summer (in leaf) and dormant tissue.

In fig. 4.8a, a distinct change in nature of response is apparent in the region of 55-60% wood water content. Segments exhibiting values of water content below approx. 55% tend to exhibit sap tension (ie. absorption) during freezing. Freezing of sap in stems with wood content above approx. 60% tends to produce increase in pressure (ie. exudation). This transition is reflected in fig. 4.8b. Here quantitative analysis shows a reasonable correlation between wood water content and response to freezing, as monitored using manometric recorders, (from regression analysis, regression coefficient, $r = 0.83$, $y = 0.0355x - 2.15$). Scatter is considerable however, and may be attributed in part to the wide variation of treated material used for experimentation (noted above).

4.5 Measurement Of Stem Diameter Changes During Temperature Treatment.

Experiments previously described have revealed that during the process of freezing - during which liquid water undoubtedly expands to form ice - absorption of water occurs. (ie. induction of stem sap tension). This appears to be a physical phenomenon. Dead tissue is capable of the same response during cooling, although its water content appears of critical consequence. It seemed profitable therefore to monitor stem diameter during freezing, in an attempt to detect any change in dimensions. A linear displacement transducer (L.D.Tr - see p 39) was employed to this effect. Living stem segments were prepared for temperature treatment in the standard way, and positioned in the temperature chamber. The L.D.Tr was located 4cm. from the basal end. Stem diameter was monitored during standard cooling and warming phases ($+15^{\circ}C \leftrightarrow -10^{\circ}C$), and stem pressures and temperatures recorded simultaneously, (using a manometric device and temperature probe respectively). Overall diameter changes (bark & xylem) were recorded,

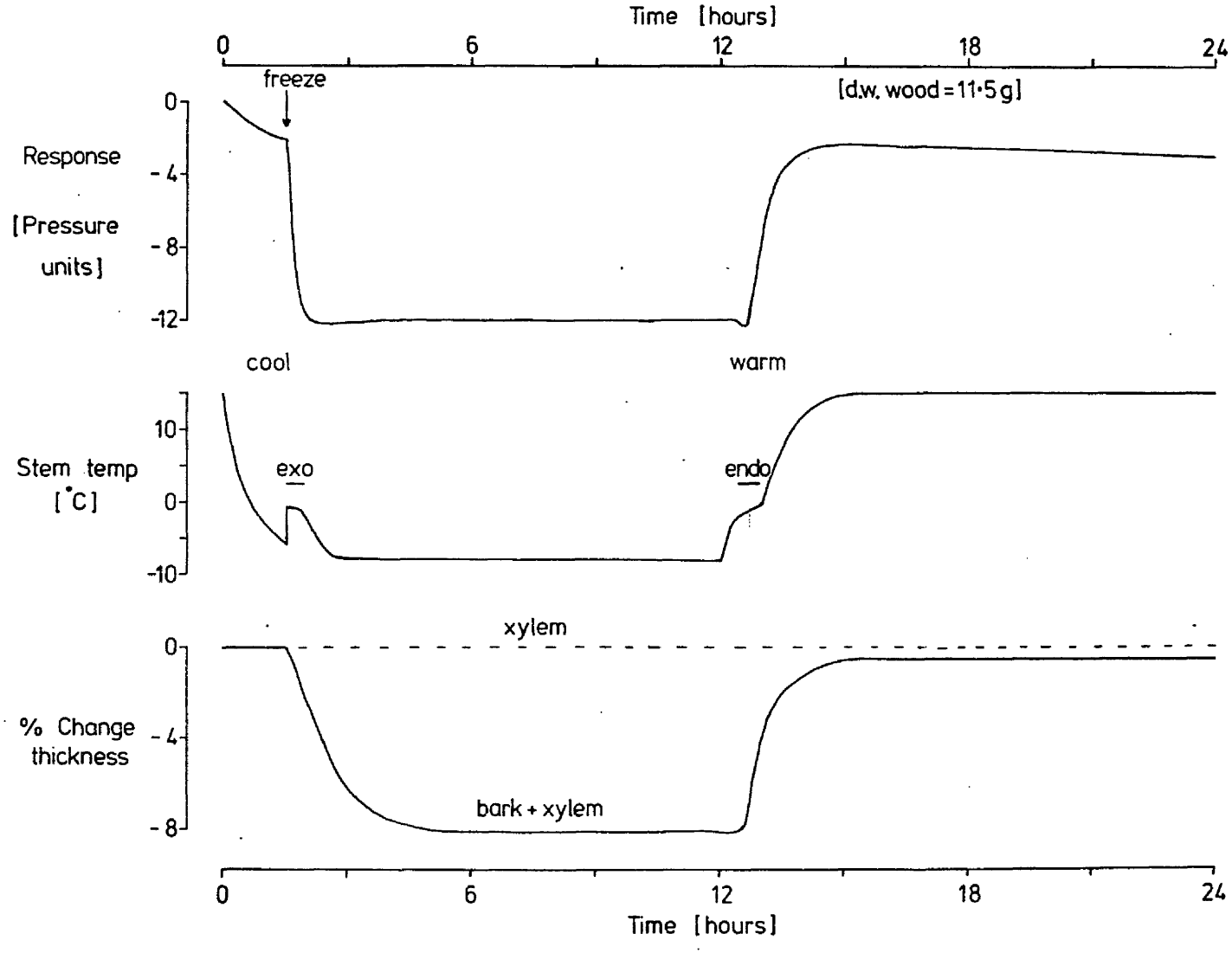


Fig. 4.9 Simultaneous measurement of stem sap 'pressure' fluctuations and changes in diameter of stem in response to freeze/thaw cycles.

Table 4.1 Recorded changes in thickness of bark during freezing of stem segments

Stem No.	Bark thickness (cm)	Change in bark thickness (cm)	% change (%)
1	0.120	-0.012	10.0
2	0.150	-0.016	10.6
3	0.150	-0.015	10.0
4	0.150	-0.015	10.0
5	0.175	-0.014	8.2
6	0.161	-0.019	11.5
7	0.190	-0.017	9.2
8	0.150	-0.016	10.7
9	0.160	-0.019	11.6
10	0.160	-0.016	9.8
		Average	10.1

and frequently just xylem diameter changes for the same stem. The latter was also monitored (with the same results) using barkless tissue. Results shown (fig. 4.9) are from 1 dormant segment representative of those used (see table 4.1). Bark thickness and xylem core diameters were estimated after each experiment using a vernier micrometer.

It is evident from fig. 4.5 that a very sudden contraction of overall (wood & bark) stem diameter was recorded simultaneous with freezing of sap associated with induction of sap tension (absorption). Stem diameter was regained, though not completely, on warming. No such changes were observed if freezing did not occur. Measurement of xylem core (just wood) diameter showed insignificant change during freezing (sensitivity of equipment determines that changes during freezing were $\leqslant$ 0.1%). Diameter fluctuations appear to be associated with the bark. Results from experiments using bark physically detached from the xylem support this deduction. Here decrease in bark thickness is detected during freezing, although % change was reduced. It seems likely therefore that although easy to detect (averaging 10% of bark thickness) and well correlated with freezing (and reversed on thawing) these changes in diameter are unlikely to be fundamental to the wood-based pressure fluctuations under investigation.

4.6 Measurement Of Changes In Stem Density During Temperature Treatment.

It seemed crucial to explain how absorption of water could occur - simultaneously with freezing of stem sap - yet with no detection of increase in diameter of the xylem core. A simple 'densitometer' was therefore constructed (see p 41) to monitor density (ie. overall volume) of stem segments during temperature treatments. Stem segments without bark (though covered with polythene and PVC tape) were used, each duly prepared and positioned in the 'densitometer'. (ie. submerged in $3M.dm^{-3}$ NaCl.) Once arranged in the temperature chamber with the corresponding control apparatus, response was monitored using manometric recorders. Standard cooling and warming cycles were induced, ($+15^{o}C \leftrightarrow -10^{o}C$), and stem temperature was recorded simultaneously. Fig. 4.10 shows results from 1 dormant segment, representative of 6 used at different times.

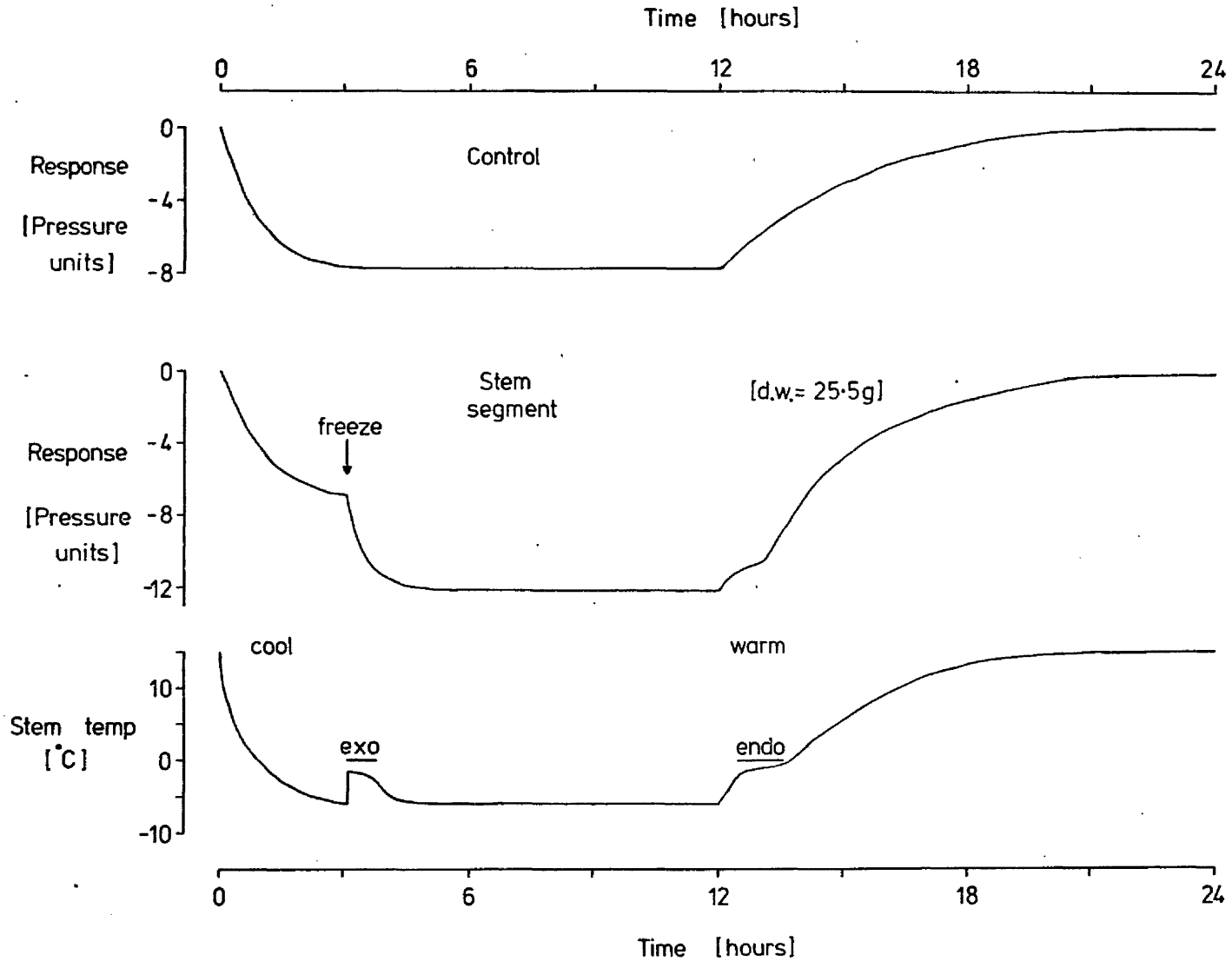


Fig. 4.10 Results obtained using the 'densitometer' (control and with segment) in response to freeze/thaw cycles.

It must be emphasised that results shown are from manometric recorders and are presented accordingly in 'pressure units' (see p 30). However, a decrease in recorded pressure is equivalent to a decrease in volume (ie. increase in density) of the system under investigation. Hence it is clear (fig. 4.10) that a sudden increase in stem density was detected during freezing (arrowed). It is unlikely that contraction of the wood is suddenly taking place. Moreover, sap within the stem is freezing - hence expanding and decreasing its density during this phase. Change in gaseous volume seems the most plausible explanation. That this may be the result of sudden dissolution of gas is highly improbable. Liquid water liberates dissolved gas on freezing. It is therefore suggested that gas is being compressed during freezing. However, this compression must not only compensate for the increase in sap volume as a result of change of state, but also account for further uptake of water during freezing, (ie. growth of ice). Possible explanation of such phenomena will be discussed in the following chapter.

4.7 Discussion

It was established in chapter 3 that sap absorption during freezing was fundamental to maple exudation. Results presented in this chapter support this view. In addition, experiments presented in part 4.2 imply that the freezing process directly influences the kinetics of water uptake during this phase. The temperature gradient imposed between the stem and its environment during freezing - which undoubtedly influences the rate of ice formation within the stem - appears to affect the rate of sap absorption. In this way supercooling of xylem sap which was invariably detected in laboratory experiments, may be of considerable consequence. When cooled and allowed to freeze naturally, sap of stem segments supercooled considerably (eg. $-5^{\circ}C$ below melting point of sap). Upon freezing, stem temperature was observed to rise rapidly (as latent heat was released) to plateau at the sap melting point near $0^{\circ}C$ (ie. the exotherm response). Hence a considerable temperature gradient was suddenly imposed between the stem and surrounding air, driving the process of freezing. As noted above, this is expected to have influence upon the rate at which water was absorbed during freezing.

Freezing appears to occur simultaneously in both wood and bark.

Freeze-absorption (a wood based phenomenon) and contraction of bark (associated with freezing), occur in unison (part 4.6). Possibly freezing advances rapidly after initial seeding, from one or more sites. Again, supercooling will influence this considerably. The progression of ice and its distribution within a stem during freezing has not been determined. A temperature gradient (magnitude unknown) must exist within the stem during cooling - the centre being more insulated and hence warmer than the periphery. Presumably ice forms initially in the latter, propogating inwards at a rate dependent upon the temperature gradient and the thermal conductivity of the moist wood.

Contraction of bark during freezing is interesting but considered unimportant to the wood based 'freeze-absorption' process. Experiments show that contraction is intrinsic to the bark, an observation supported by previous investigation of snow gum (Pook and Hall 1976). Bark shrinkage on freezing has been noted previously for sugar maple (Marvin 1949) and snow gum (Pook and Hall loc cit.) although values presented for sycamore seedlings (part 4.6) are considerably greater (ie. 10% c.f. 0.1% sugar maple and 5% snow gum). The previous studies however used mature trees, and hence the bark may have been more mechanically rigid, possessing a higher proportion of dead cells. Consequently less contraction would be exhibited during freezing than for younger tissue. Shrinkage during freezing is attributed to contraction of living cells caused by efflux of water to ice loci. As such this is a well documented process (Mazur 1963, 1970, Olien 1967, 1971 Johansson 1970, Dennis 1972, Levitt 1972, Stout et al 1977). Efflux occurs because the vapour pressure (ie. free energy) of the ice is lower than that of the unfrozen intracellular water. However, for the overall dimensions of the tissue mass to decrease during such a process, (water expands on change of state to ice) growth of ice is assumed to be in suitable intercellular air spaces (Olien 1967, Idle and Hudson 1968). Movement of water laterally from the bark to the xylem during freezing is thought unlikely (Turner and DeRoo 1974). However, observations by Marvin (1958) showed the water content of maple bark when frozen to be lower than when thawed. The reverse was true for the xylem core. Hence, sap may indeed move laterally during freezing. Alternatively, excess xylem sap replenished by absorption, longitudinally, during freezing may have moved laterally from the wood to the bark on thawing. Further investigations are required to clarify this issue.

Experiments in part 4.3 revealed that living cells are not directly involved in the absorption (conditioning) process. The mechanism is therefore purely physical. This view is supported by previous studies using sugar maple (Marvin 1958). However, it appears that water content of the stems has profound consequence regarding the ability of a stem to absorb water during freezing. A distinct reversal in nature of the freeze response was detected in the critical region of 55-60% wood water content (fig. 4.8). For reference, the water content of wood from typical living seedlings investigated was 40-45% of the fresh weight. Increase in water content of individual stem segments necessitates displacement or dissolution of gas. The importance of the gas content of the wood was inferred in part 4.6, where it was suggested that the formation and growth of ice during freezing (the latter responsible for inducing further uptake of water) resulted in compression of trapped gas. The abundance of 'gaseous pockets' would therefore have a direct affect upon this process. Increase in wood water content - reducing the gaseous content - would correspondingly reduce the capacity for absorption. This is indeed that observed. Moreover, a critical value of water content can be envisaged when gas available for compression is balanced by the expansion of sap from change of state. No nett absorption or exudation would occur during freezing. Above this critical value, exudation (ie. increase in sap pressure) is predicted on freezing. Here the wood is effectively waterlogged and too little gas is present for its compression to accomodate expansion of sap and growth of ice. The proposed mechanism by which gas is compressed during freezing is discussed in the following chapter.

Factors other than water content of the tissue are expected to influence the ability of a stem segment to absorb water during freezing. Indeed some of the scatter in fig 4.8 (p 91) may be from these sources. Variations in anatomy may affect the availability of gaseous sites. The ratio of non-conducting to conducting tissue may also influence the absorptive capacity. It must be stated here that results presented in fig 4.8 for high values of water content are from dead tissue only. Waterlogging of living tissue was difficult, presumedly due to gas production within the wood by living cells. Hence small errors may also arise from changes in water content of living tissue due to gas production during experimentation. No such changes are expected for dead tissue.

Although the conditioning phase is of fundamental importance, some consideration must also be given to the thawing, pressurisation phase. Increase in temperature is thought to act merely as a trigger for exudation(Marvin 1958). Results in part 4.1 support this view. Melting of frozen sap (detected by an endothermic response) appears to be the release mechanism for pressurisation. Care must be taken however, when interpreting thawing responses monitored using the manometric recorders. Increased pressure is actually recorded as a decrease in tension. No positive pressures are recorded due to the nature of the investigative procedure, (see p 28). Results in chapter 3 (table 3.7) however suggest stem segments posess some ability to produce positive sap pressures on thawing - when previously conditioned. Sap is forcibly exuded, though pressures are small. Considering this, it is interesting to note that little pressurisation occured on thawing of steam killed tissue (fig. 4.7). This cannot be attributed alone to the low water status (ie. % water content) of the segments (cf values of 49% & 53%). Full pressurisation is detected using living tissue (eg. fig.4.1) which has usually 40-45% water content. It may be, therefore, that living cells play a role in this part of the cycle. This was inferred by Marvin (1958) from studies with sugar maple, in which he showed dead tissue exhibited sap absorption during cooling, but exudation was much reduced or absent on warming. It can be envisaged that if compression of gas occurs during freezing, then on thawing an initial positive gas pressure may exist in the presence of liquid sap. This, aswell as driving exudation, may favour dissolution of gas that previously was not so. Further production of gas may therefore be necessary for full recovery and pressurisation of the system, (see later).

5. Natural Occurence Of Ice-induced Sap Tension (With Subsequent Pressurisation), And Proposed Mechanism.

Laboratory investigation has clarified certain aspects of the maple pressurisation system - particularly regarding the critical conditioning (cooling) period. It seemed necessary however, to relate such observations to the situation in field grown trees. Further investigations of a more detailed nature were therefore undertaken using tree A. Consequently, a possible mechanism was formulated, at both the cellular and whole plant level, and this is proposed.

5.1 Detailed Investigation Of Sap Pressure Fluctuations In The Field.

Using manometric recorders with fast drum rate (1rev/day), pressure potential (Ψ_p) was monitored for tree A during January 1979. 2 bore holes were made in the trunk (ht. 1m.), south-west facing, drilled initially to a depth of 3cm. (diameter 1.25cm.) on 6th January. Tapering plastic adaptors were sealed in position and manometric recorders attached (as described in chapter 2, part 3). Redrilling of the holes was undertaken at various times, (see fig. 3.5, p 56) such that the depth of each, on 25th January, was 4.5cm. (ie. penetrating approx. 3.5cm. into the xylem). The sap pressure record for this day is presented in fig. 5.1. This is representative of many obtained throughout January, but chosen as that best illustrating certain important features. On this date, redrilling was undertaken (mid-morning) when the tree was pressurised. A sap sample was taken for estimation of solute potential (Ψ_s) and % sucrose. This was collected from a third borehole, located midway between those attached to manometric recorders. Specifications of this hole were the same as the ones adjacent, but pressure was monitored here using a Bourdon pressure gauge. No significant differences were found between pressure values from the three tapping points.

Certain environmental factors were recorded simultaneously with sap pressure potential. Air temperature and humidity were monitored at the base of the tree using a Casella thermohygrograph. Air temperature records were supplemented by values from the Grant's recorder. Soil temperature was monitored from a probe buried at 5cm. depth, trunk temperature from probes embedded (3.5cm. depth), and sealed, adjacent to each borehole. Most importantly, temperature was monitored

for 3 exposed twigs from probes embedded in them. (see table 2.1 p 32). Temperatures of all 3 twigs were found to differ insignificantly, and hence twig temperature presented in fig. 5.1 is-that from one twig only. All probes employed were so positioned on 6th January. Further details are presented in chapter 2, part 3, iii .

Fig 5.1 illustrates the familiar oscillation of xylem sap pressure potential from a positive to a negative value (a→c) relative to atmospheric pressure, during cooling, with reversal on warming (d→e) That the cooling response is biphasic is also clearly evident. A slow drop in sap pressure in the positive range (a→b) is followed by a more rapid drop (b→c) to produce a sap tension. In the laboratory, these phases were attributed to effects prior to and a consequence of freezing (part 3.5). This is clearly also the case in the field. Change in air temperature during cooling before freezing produced only small, gradual changes in stem pressure (a→b). Laboratory experiments (part 3.6) suggest these changes are likely to be the result of simple thermal effects - eg. contraction of sap in conjunction with gas dissolution. Verification that this is so in the field will need further investigation of a quantitative nature.

More importantly however, the critical phase of cooling - ie. the rapid induction of a reversible sap tension (b→c) - appears to correlate well with freezing of xylem sap in the twigs. An exotherm (indicated fig. 5.1) indicative of freezing was detected just prior to the initiation of this absorptive phase. Detection was near simultaneous in each of the 3 twigs monitored for temperature. Only slight supercooling of the twig sap was observed (eg. approx. -1.5°C), considerably less than that noted using excised seedling stem segments in the laboratory. Reasons for this are unknown. To ensure that the method of embedding temperature probes in the twigs had not somehow influenced the freezing event, probes were strapped to the surface of undamaged twigs. Although more difficult to detect, exothermic responses were recorded at temperatures similar to those from twigs with probes embedded.

On thawing, dramatic increase in sap pressure was recorded (d→e) reaching a value exceeding that prior to freezing. Presumably this is associated with melting of ice within the tree. Endothermic temperature response of twig xylem sap is not prominent however, as air temperature stabilised near 0°C. It is again interesting to note that, as indicated in chapter 3, positive values of xylem water

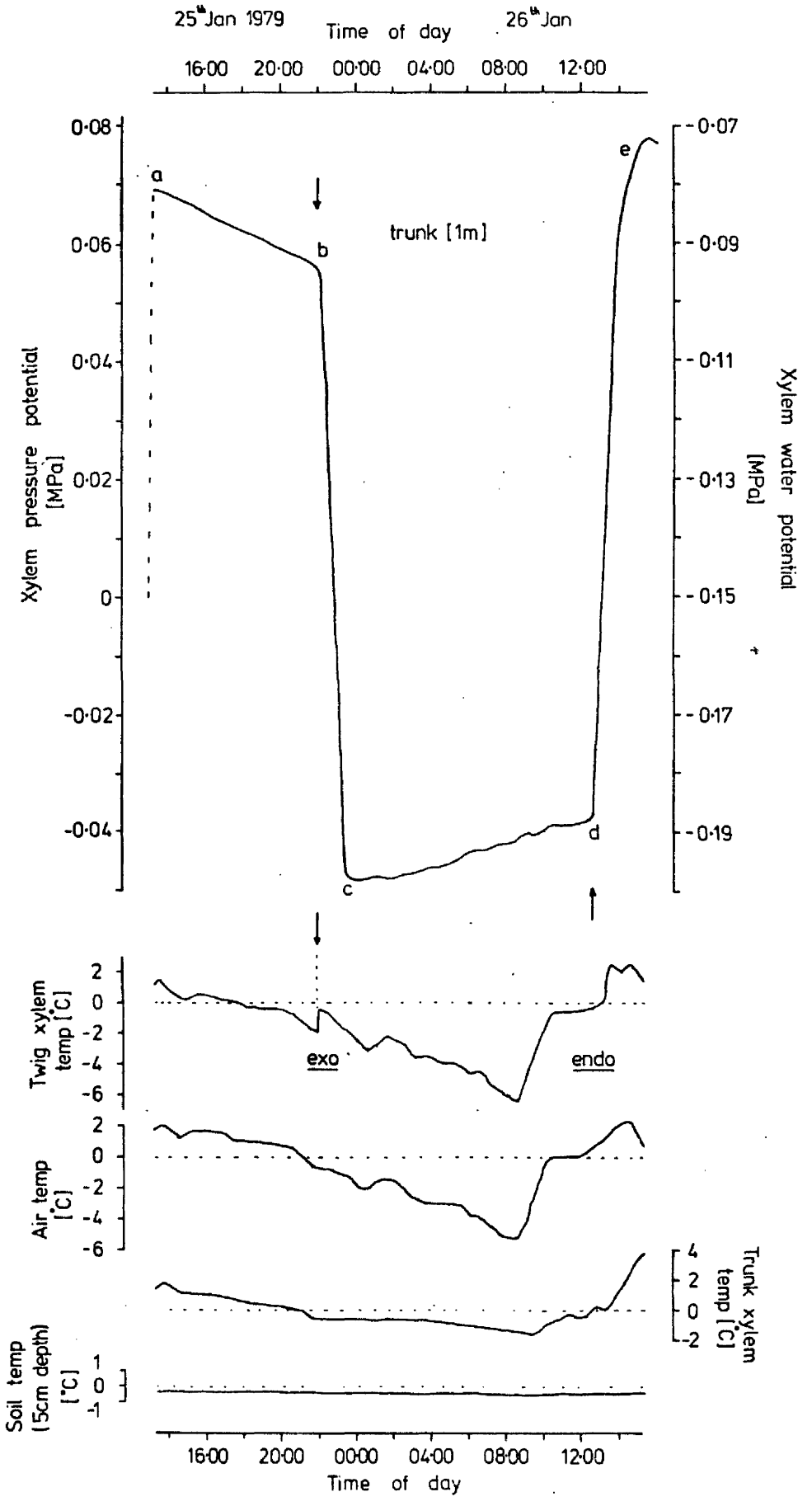


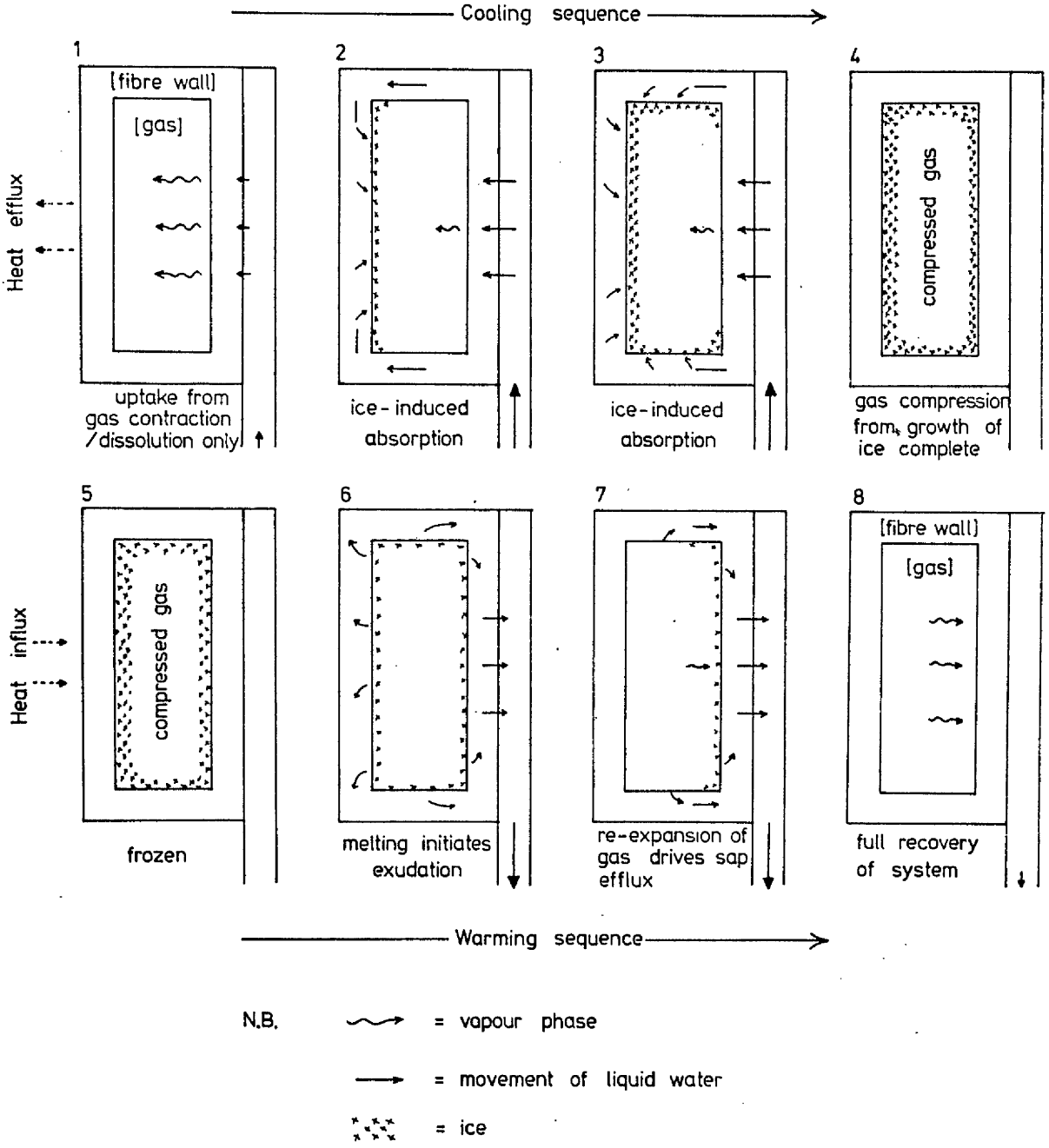
Fig. 5.1 Detailed record of sap pressure potential for tree A, correlated with temperature.

potential (Ψ_x) were not detected. Negative values of solute potential (Ψ_s) ensured a negative value of Ψ_x even when xylem pressure pontential (Ψ_p) was high.

5.2 Proposed Mechanism At The Cellular Level And Consequences For The Whole Plant.

It is known that maple sap exudation is determined by the preceding period of cooling (conditioning). Experiments indicate that this 'sap priming' is a purely physical process associated with freezing of extracellular water (sap) in the wood. The presence of sucrose in the sap is not an essential requirement. An important factor however, has been shown to be the water:gas ratio within the tissue (part 4.4). It was suggested from wood density experiments (part 4.6) that gas may be compressed during the freezing process. It was stressed that this must compensate not only for sap volume expansion due to change of state, but also further growth of ice, necessitating absorption of water. It is proposed that the latter is responsible for the enigmatic observation of uptake of water by the stem during freezing.

Intercellular air spaces may be considered as likely sites for such a system to operate. According to Jones (1903) however, intercellular spaces are absent from maple wood. Probable sites therefore are the abundant gas-filled fibres found closely associated with the xylem vessels (Wiegand 1906). These characteristically surround the diffuse vessels in maple wood (Sauter 1974). Anatomical studies of sycamore seedlings show a similar arrangement, (appendix III). It is proposed that ice forms on the inner surfaces of these fibre cell walls compressing the entrapped gas. Diagram 5.1 presents in diagrammatic form the sequence of events envisaged during cooling and warming. Initial cooling of the stem (before freezing) induces gas contraction - and its dissolution, as the fibre walls contain sap. This develops a gas tension within the fibre lumen, giving slight absorption of sap and wetting of the fibre wall inner surfaces. High relative humidity of the entrapped gas results (diag. 5.1, caption 1). Upon freezing of the stem, free water inside the fibre will crystallise (on the wall inner surface). Ice formation does not advance into the capillaries of the cell wall however. Here the sap freezing point is depressed considerably due to surface adsorption effects, and hence remains liquid. (Haynes 1968, Drost-Hansen 1969, Vignes & Dijkema 1974). Ice



Diag. 5.1

Diagrammatic representation of freezing/thawing processes within a maple fibre and the consequences for sap absorption/exudation.

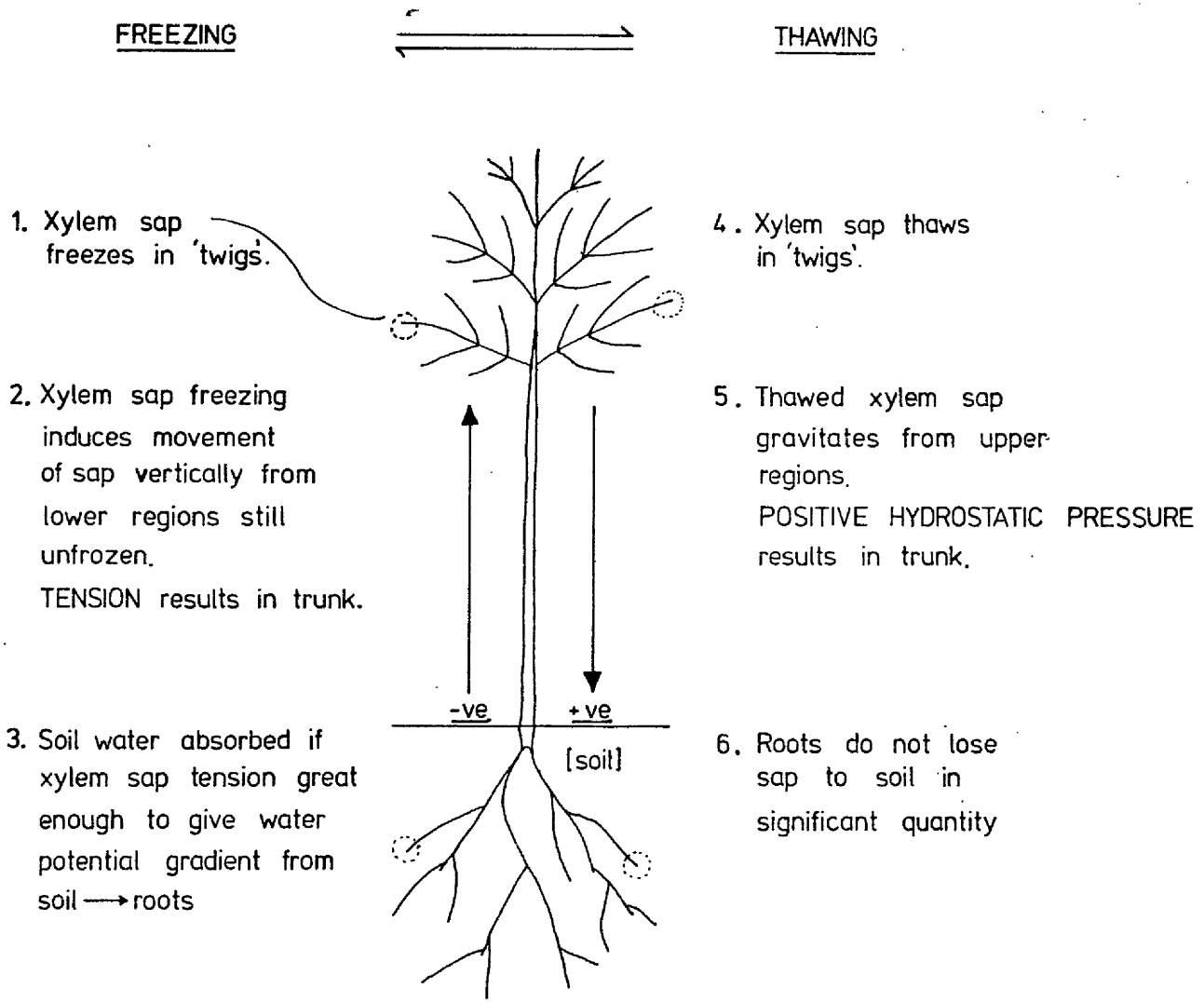
formation is also expected to be initiated assymetrically. A temperature gradient will be radially orientated within the stem (as heat is lost to the surrounding air), and so freezing is expected to occur first on the cooler outer wall of each fibre (see fig. 5.2, caption 2). Rate of propagation of ice will depend upon rate of heat removal.

That growth of ice continues after initiation of freezing is fundamental to the mechanism, as this necessitates further uptake of water. This is induced by removal of heat, and such a situation is clearly observed in laboratory experiments. Change of state of supercooled sap releases latent heat, and stem temperature rises accordingly (exotherm) to the sap melting point. Usually sap supercooled to approx. $-5^{\circ}C$. (melting point of sap $\geqslant -0.5^{\circ}C$). Hence on freezing, a temperature differential of approx. $4.5^{\circ}C$ is suddenly established between the stem and surrounding air. The rate of growth of ice is dependent upon the temperature gradient. If little/no supercooling occurs, then change in air temperature will establish the temperature gradient directly (see part 4.2).

The nature of growth of ice during this process must be considered. Contraction by vapour transfer across the fibre lumen and condensation as 'frost' is thought to be real but small. Growth of ice directly from the liquid water held by the capillaries of the cell walls is considered more significant. For reasons stated above, it is expected that propagation of ice is restricted to the surface of the fibre walls - penetration within is halted at the capillary mouths. Though no direct evidence is presented to support this assumption, it is considered highly likely (eg. Idle & Hudson 1968, Olien 1967, George & Burke 1977). Further growth of ice - energetically favoured in the process of equilibration - therefore necessitates extraction of water from the cell wall (diag. 5.1, caption 2.3). This same system has been proposed to explain frost heaving of soils and ice-lens formation. The forces responsible for soil disruption are not those resulting from simple freezing. Instead, growth of ice masses occur within the soil, extracting liquid water from neighbouring soil pores in the process, (Takagi 1970). This is a purely physical process, and has received considerable attention. It may be explained thermodynamically, and fundamentally involves consideration of the relative free energies of bulk ice and liquid capillary water, in contact, and the consequences of disequilibration due to imposed temperature gradients between the two. (Everett 1961, Haynes 1968, Vignes and Dijkema 1974, Biermans et al 1976).

In maple wood, clearly sap movement caused by extraction of liquid water from cell wall capillaries by growing ice crystals will be transmitted through the cell wall continuum to vessels as yet unfrozen (eg. those in deeper tissues or tissues that show increased thermal capacity and consequently show temperature lag during cooling). Hence water uptake is observed (diag. 5.1, captions 2 and 3). Absorption will continue until growth of ice stops and equilibration is complete ie. the temperature gradient between stem and environment has been eliminated. Thawing effectively reverses the process. Little melting of ice will occur until the temperature reaches the melting point of the sap (near $0^{o}C$). Exudation is then triggered and efflux of sap is driven by the re-expansion of the entrapped, compressed gas, (diag. 5.1, captions 5→8). Full recovery of the system will occur if the previous gas content/pressure of each fibre is attained.

Relating the system to a whole plant (tree), ice is most likely to form initially in the fine twigs, as these will cool rapidly having the largest surface area : volume ratio and smallest heat capacity. During freezing, water (ie. sap) would be pulled towards these sites from other unfrozen regions of the tree. (eg. larger branches and trunk). General movement of sap vertically would therefore result in development of a sap tension within the trunk, (which is indeed evident), if some resistance to sap movement existed. Permeability of the roots is a factor considered of importance here. A schematic diagram of events is presented in diag. 5.2. As freezing progresses, more sap is redistributed within the tree to the extremities and sap pressure potential (Ψ_p) in the trunk will continue to drop. The rate of decline here will be determined to some extent by the reservoir capacity of the trunk. However, the lower the value of Ψ_p attained, the greater the water potential gradient produced between the soil and the root (xylem) and hence the more water is absorbed. Thawing simply reverses the process and clearly the act of melting will be a trigger inducing pressurisation. As sap reverts to a liquid state, an excess of water (relative to that state prior to freezing) will exist in the upper regions of the tree. Efflux of water from here in a basal direction, will be due in part to re-expansion of gas within fibre cells, and further production of gas by living cells. In mature trees, gravitational effects are considered most important however. As a result, a surplus of water will exist in the lower regions of the tree, giving manifestation of a positive hydrostatic pressure - or exudation if allowed. The value of pressure/exudation will be determined by factors affecting the quantity of this



Diag. 5.2 The system as envisaged in the whole plant.
(N.B. Tension = negative sap pressure.)

excess sap - ie. affecting previous sap absorption (conditioning) eg. severity and duration of freezing, availability of gas sites (fibres), relative tree crown size, exposure, resistance to sap movement within the stem, permeability of the roots.

5.3 Discussion.

A mechanism has been proposed to explain the maple exudation phenomenon. Freezing of sap is fundamental to the system, as observed in various experiments presented in this thesis. In particular, detailed investigation of sap pressure potential (Ψ_p) of tree A during dormancy (part 5.1) recorded rapid freeze-induced response, attaining considerable tension. It has been proposed that this is a result of redistribution of sap to peripheral regions of the tree. Freezing was detected within the twigs near simultaneous with (though just prior to) the initiation of this phase (see fig. 5.1). Ice formation is expected to commence in the fine twigs and thinner branches of the tree as these will cool more rapidly that other parts, (see part 5.2). Moreover, a small time lag is also anticipated between detection of freezing in the twigs and detection of change in sap pressure in the trunk, as the locations are physically separated. Resistance to flow through the stem, and the reservoir capacity of the stem, will affect the rate of movement and redistribution of sap - and hence transmission of pressure change. Elasticity effects (eg. turgor displacement volume - T.D.V) for the xylem vessels are considered to be small.

Once freezing had been initiated, sap pressure monitored within the trunk was observed to fall to some minimal value (eg. $\sim$ -0.05MPa, fig 5.1). The corresponding value of xylem water potential (Ψ_x) is determined by the contributions of solute potential ($\Psi_s = \sim$ - 0.2MPa) and pressure potential (Ψ_p). Stabilisation of Ψ_p at a minimal value may indicate that freezing within the tree was complete - and hence associated sap absorption and redistribution had ceased. Alternatively it may be that absorption of water from the soil compensated for further internal redistribution of sap. Clearly the rate at which water is absorbed by the roots from the soil will be determined by the water potential gradient between the two, and the permeability of the roots.

Pressurisation of the tree (fig. 5.1) is a characteristically rapid single event. It has long been regarded as a triggered response (Marvin 1958) and clearly this appears to be the case. Little change

in pressure is recorded until twig temperatures approach 0°C. Rapid pressurisation then ensues (eg. peaking near 0.08MPa, fig. 5.1). Thawing of the upper frozen regions of the tree and gravitation of sap adequately explain such observations. It is also of interest to note that the resultant value of pressure potential is greater than that in the thawed state before the tree was frozen. This is thought due to absorption of sap by the roots during 'conditioning', as it is the volume of excess sap within the tree made available for exudation that will determine the magnitude of standing hydrostatic pressure. The value and retention of this 'head' of sap will, of course, be influenced to a lesser extent by factors other than sap replenishment. Simple thermal effects will be of consequence (eg. expansion of gases and liquids). Gas may be forced into solution by the positive hydrostatic pressure within the trunk (eg. refilling vessels previously embolised). Transpiration, although much reduced during dormancy, will also be influential. Considerable loss of sap may also occur by leakage from the roots (by 'reverse osmosis'). It is here, however, that the abundance of solutes (ie. sucrose) in sap of dormant trees is considered important. It has been stressed that positive values of water potential (Ψ_x) are not detected. Values of Ψ_s ensure that values of Ψ_x rarely exceed -0.05MPa. Hence, even when pressure potential (Ψ_p) is maximal, a water potential gradient may be maintained favouring retention of sap by the roots. Such an effect will oppose a loss of water from the roots to the surrounding soil.

FINAL DISCUSSION & CONCLUSIONS

6. FINAL DISCUSSION & CONCLUSIONS

The uniqueness of maple sap exudation has become increasingly apparent as the topic has been investigated more fully. The phenomenon has been shown to be a property of the stem (Stevens and Eggert 1945, Marvin 1951). Root pressure plays no direct role in the pressurisation system. Moreover, experiments presented in this thesis (part 3.5) demonstrate that the presence of bark is unnecessary for sap exudation. The mechanism responsible is based in the wood. In addition, the presence or absence of sucrose within the xylem sap appears to have little effect upon the functioning of the exudation mechanism, (part 3.5 & 3.6). Understandably, this has been an issue of debate, as natural maple exudation is only observed from trees during dormancy (Marvin 1958) - the only period when sucrose is detected in the xylem sap (eg. Sauter et al 1973). Sucrose and sap pressure have been associated further by distinct correlation of magnitude of pressure/yield with sucrose content (Morrow 1952, Marvin et al 1967). Marvin (1951) also claimed direct evidence for the influence of sap solute content upon sap exudation from treated stems. Experiments performed in this thesis however, suggest that the sucrose/sap pressure relationship is indirect. Further discussion of this will be presented later.

It has long been demonstrated that the external factor of most influence on maple exudation is temperature, (Jones 1903). Oscillations of stem pressure correlate well with oscillations of temperature. Sap tension (ie.negative sap pressure relative to atmospheric pressure) inducing absorption is evident during temperature fall. Pressurisation or exudation results from temperature rise. It has also been established that response during cooling is responsible - and indeed necessary - for subsequent exudation, (eg. Stevens & Eggert 1945, Marvin 1958). Sap is absorbed and retained by the stem (ie. wood) when cooled - termed 'conditioning', (Marvin loc cit.). Enigmatically, however, this does not appear directly related to temperature change (Marvin & Erickson 1956). In whole plants, sap is absorbed through the roots during cooling. Clearly, soon after leaf fall, such replenishment must initially overcome tissue water deficits before excess water is available for exudation. Later, replenishment must support both exudation and transpiration from buds, leafless twigs and bark.

Understanding of the conditioning response is crucial. Experiments throughout this thesis have clearly demonstrated that this critical

absorption response is associated with freezing within the wood. Absorption during cooling prior to freezing is quite distinct from during freezing. Hence, quantitative correlation of absorption / pressure change with change in temperature throughout cooling is not to be expected - as shown by Marvin & Erickson (loc cit). It is necessary to consider the responses seperately. Mechanisms proposed to date (excepting that of Stevens & Eggert 1945, see p 4) however, viewed sap uptake as a single continuous event (eg. dissolution of gas, Sauter, 1974, see p 6). This accounts for certain inadequacies of hypotheses proposed to date, (eg. see table 6.1, p 117).

Experiments suggest that sap absorption and exudation induced by temperature change without freezing of sap may be due to simple thermal effects (see chapter 3). Dissolution of gas is considered of most consequence here. This accords well with investigation of sugar maple by Sauter (personal communication, 1979). In contrast, the freeze-induced absorption response appears more complex. Water uptake is observed, yet sap undoubtedly expands during change of state to ice. Freeze-absorption is also more significant than sap uptake during cooling prior to freezing. The latter was observed to average approx. $2 \times 10^{-2} cm^3$ per g.d.w wood of stem segment (see table 3.4, p 71) for a change in temperature of $20^{o}C$. Freeze-absorption was commonly 3 times this value, averaging approx. $5.5 \times 10^{-2} cm^3$ per g.d.w. wood, (table 3.5, p 75). Uptake associated with freezing has not been demonstrated previously with any clarity. Stevens & Eggert (1945) suggested that such a system may operate, although no direct evidence was presented. They envisaged sap uptake to be caused by an osmotic effect resulting from 'freezing out' of sap solutes. (see General Introduction). Recently S. Essiamah,(Forestry Inst., University of Göttingen, personal communication, 1979) has observed strong correlations between sap exudation and freezing of sap. General opinion however, has excluded freezing from explanation of 'conditioning' (eg. Marvin 1958, Sauter 1974).

During freeze-induced uptake the density of the stem is observed to increase (part 4.6) with no apparent dimensional changes of the wood (part 4.5). This response appears to be purely physical (part 4.3). A mechanism has been formulated (part 5.2) which appears to account for these, and other, enigmatic observations. It was suggested that ice formed within the lumens of gas-filled fibres, compressing the entrapped gas. Such a system is analogous to the well documented frost heaving

response (eg. Everett 1961, Vignes & Dijkema 1974). The consequences envisaged for the whole plant were such that internal redistribution of sap resulted in sap movement towards the regions of ice formation - deemed principally the extremities of the tree as these are considered to freeze first. As a result, a sap tension (ie. negative pressure) is produced in the trunk of the tree (as yet unfrozen) and consequently water is absorbed by the roots from the soil environment. Uptake would continue until freezing was complete (the trunk is expected to freeze also by 'seeding' of ice from the upper regions). Thawing results in gravitation of sap from the upper region producing exudation/ pressurisation. Clearly the capacity for sap redistribution within the tree during freezing will affect the magnitude of sap absorption and hence exudation (although these processes are plainly influenced by a variety of factors - see below). Gas content of maple trees has been estimated to be 24% of the volume of the wood (Wiegand 1906). This provides adequate capacity to account for the volume of the sap absorbed/exuded (eg. in New England, U.S.A., exudation often averages c.a. 5000cm^3 of sap/tree/day).

Simple change of state within the tissue will not induce absorption of sap. It has been stressed that growth of ice is necessary after initial seeding. Temperature disequilibrium must therefore exist between the stem and environment during freezing. In the laboratory, super-cooling ensured this. Field investigations however showed supercooling of sap was less pronounced, and here changes in sub-zero air temperature may have direct influence. The extent to which freezing and growth of ice occurs will clearly determine the volume of sap absorbed (ie. replenished) during this process. This is crucial as this excess sap (effectively) is fundamental in determining the magnitude of standing hydrostatic pressure, (or volume to be exuded) on thawing. It is therefore understandable that the severity and duration of freezing influences exudation (noted by Marvin, 1958). Moreover, any factor that affects the efficacy or capacity for freeze-induced sap uptake will also influence subsequent sap pressure/exudation, (eg. tree crown size (Morrow 1955), exposure, innate abundance of fibres, trunk reservoir, vascular conductivity, root permeability - see part 5.2).

Considerable evidence has been amassed linking the sap sugar content of mature sugar maple trees with the magnitude of positive sap pressure (Morrow 1952, Marvin et al 1967). This was also evident from field studies of sycamore (part 3.3). Laboratory experiments presented in

parts 3.5 & 3.6 have indicated that this relationship is indirect. Sap exudation can be induced from stem segments when in the 'summer state', the sap of which contains no sucrose, (see part 3.4) Clearly further explanation of this association is necessary. As discussed above, dormant trees with large crowns may be expected to produce greater positive sap pressures (or volumes of exudation) as the capacity for freeze-absorption (ie. sap replenishment) is greater. However, such trees also possess a greater capacity for photosynthesis (during the preceding summer), and hence may be expected to have greater potential for solute loading (Morrow 1955). One consequence of cells loading sucrose at a faster rate - or a greater number of cells, per unit volume of wood, loading sucrose at average rate - would be to produce more gas from respiration. Tissue gaseous content will therefore be higher - particularly in the twigs and branches of the tree as potential for sucrose loading is greatest here, (Jones 1903). Freeze-absorption and hence resultant exudation is influenced accordingly. Physiologically then, high sap sucrose content could be linked indirectly with the potential for production of high sap pressure potential. Support for this concept comes from the observation by Morselli et al (1978) that the association of high sap pressure and high sucrose content is a consequence of larger and more abundant vascular rays. These have already been shown to be the locations of contact cells, responsible for the loading of sucrose, (Sauter et al 1973).

It has been observed by Jones (1903) that the greatest contribution to exudation is by sap flow from above, rather than below, a borehole. The capacity for internal redistribution of sap will influence sap replenishment and hence exudation. The gaseous content of the upper regions of a tree is clearly important in the proposed mechanism. The process of gas production may indeed aid exudation directly - indicated in parts 4.3 and 5.2. The importance of living cells in the exudation response of excised stems has been noted previously by Marvin (1958), though their contribution towards pressurisation in a mature tree is debateable. Here exudation (or pressurisation) is considered more a result of gravitation of excess sap (redistributed during freezing) from the upper regions of the tree (see part 5.2). Clearly, gas subjected to positive sap pressure will tend to dissolve. It is thought unlikely that positive pressures of any magnitude or duration exist in the upper portions of a tree, and this would presumably also help maintain the gas content of these regions, so necessary to the proposed

pressurisation mechanism. Dissolution of gas within the basal regions however, (eg. tree trunk) would be particularly advantageous to a diffuse porous tree. Conduits previously embolised may be refilled and made available for use. The duration of positive sap pressure would plainly affect the efficacy of this process. Retention of sap (and hence pressure) by the tree is therefore of importance. Some losses are expected due to transpiration. In addition, considerable losses could occur through the roots by reverse osmosis. Here however, the xylem sap solute content is considered critical. It has been noted that positive values of xylem water potential (Ψ_x) are not detected. (see parts 3.1 & 5.1). The low sap solute potential (Ψ_s) ensured by the presence of sucrose appears to compensate for the manifestation of positive values of sap pressure potential (Ψ_p). It is considered therefore that xylem water potential may be consequently maintained at a low enough value to favour retention of sap by the roots (see chapter 5.). Clearly then, from ecological considerations, sap pressure and sap sucrose content may be expected to be physiologically interdependent, as inferred above.

Any mechanism proposed to explain maple exudation must also account for the uniqueness of the phenomenon. Clearly, freezing of xylem sap is not restricted to the genus Acer, (eg. Stevens & Eggert 1945, Levitt 1972, Burke et al 1976). Some degree of specificity however, may result from anatomical requirements. The scarcity, or even absence, of gas-filled fibres of suitable location would plainly be of influence. Similarly wood anatomy may affect the nature of the freezing process, eg. rate of propagation of ice during freezing. If ice were to form too rapidly throughout the tree then associated sap redistribution could be impaired or made impossible. Alternatively, the freeze-redistribution mechanism may indeed function in trees of other genera but without subsequent manifestation of positive sap pressure. Sap must be absorbed through the roots, and retained, for exudation to be possible. Clearly root permeability, hydraulic resistance, and the ability to retain positive sap pressure (eg. by lowering of sap Ψ_s) are but a few factors to be considered here. Further investigation is necessary however, to clarify this issue.

The mechanism, based on freezing of sap, which has been proposed to explain maple sap exudation can be usefully compared and contrasted with previous theories (Table 6.1). Further experimentation must be undertaken to confirm its validity, and clearly quantitative knowledge

Table 6.1

Synopsis comparing and contrasting different theories presented to explain Acer sap pressurisation. Certain licence is necessary to simplify sufficiently, but most points included have been discussed more fully in previous chapters.

Environmental effect or experimental result	Osmotic Stevens & Eggert	Gas expansion & dissolution Sauter*	Internal freezing O'Malley & Milburn
Tree exposure	✓	✓	✓
Tappers recommendation (ie. freezing nights & warm days)	✓	0	✓
Temperature rise	✓	✓	✓
Temperature fall	✓	✓	✓
Freezing necessity	✓	0	✓
Freeze-absorption	✓	X	✓
Triggered pressurisation	✓	X	✓
Solutes, absence of	X	0	0
Absence of CO_2 (specifically)	0	X	0
Anatomical considerations	X	✓	✓
Functional in dead tissue	?	0	0

Correlation key - positive ✓
neutral or indirect 0
negative X
not known ?

No theory can be regarded as valid if identified by 'X'

*Sauters original hypothesis suggests wood is compressed directly by CO_2 pressure which is most unlikely. We assume that his theory implies displacement of sap by gas expansion.

of the freezing processes within maple tissue would be invaluable. However, it is considered that the freeze-absorption system described in this thesis accords well with the observations presented here and those already in the literature.

APPENDICES.

APPENDIX I

Table Ia) Xylem sap analyses for samples taken from sycamore seedlings at various times throughout 1978 & 1979.

Date 1978	Minerals K^+ (mM. dm^{-3})	Minerals Na^+ (mM. dm^{-3})	%sucrose (refractometry) (%)	Non-reducing sugars (mM. dm^{-3})	Ψ_s (solute potential) (MPa)	Ψ_p (pressure potential) (MPa)	Ψ_{xylem} ($\Psi_p + \Psi_s$) (MPa)
Mar 14th	-	-	1.7	-	-0.15	-	-0.15
(Dormant)	-	-	1.9	-	-0.21	-	-0.21
	-	-	2.0	-	-0.42	-	-0.42
	-	-	2.9	-	-0.54	-	-0.54
Mar 20th	1.9	4.6	2.4	-	-0.26	-	-0.26
	1.8	2.0	2.5	-	-0.32	-	-0.32
	4.2	5.6	2.8	-	-0.34	-	-0.34
	3.6	-	2.2	-	-0.26	-	-0.26
	1.6	2.6	2.3	-	-0.39	-	-0.39
	4.4	2.6	2.6	-	-0.39	-	-0.39
	2.8	4.5	2.6	-	-0.26	-	-0.26
	3.4	3.2	1.8	-	-0.31	-	-0.31
Mar 28th	1.2	1.4	1.0	-	-0.20	-	-0.20

Date	Minerals K^+ (mM. dm^{-3})	Minerals Na^+ (mM. dm^{-3})	% sucrose (refractometry) (%)	Non-reducing sugars (mM. dm^{-3})	Ψ_s (solute potential) (MPa)	Ψ_p (pressure potential) (MPa)	Ψ_{xylem} ($\Psi_p + \Psi_s$) (MPa)
Mar 28th cont.	2.1	1.2	1.0	-	-0.19	-	-0.19
	3.8	1.1	1.5	-	-0.21	-	-0.21
	1.6	-	0.8	-	-0.18	-	-0.18
	2.6	1.4	0.9	-	-0.18	-	-0.18
	1.3	1.7	0.8	-	-0.18	-	-0.18
	1.2	1.4	1.0	-	-0.22	-	-0.22
	3.2	1.8	1.0	-	-0.28	-	-0.28
Averages	2.5	2.5	1.8	-	-0.27	-	-0.27
Apr 1st	-	-	0.8	-	-0.20	-	-0.20
	-	-	0.6	-	-0.20	-	-0.20
	-	-	0.7	-	-0.21	-	-0.21
	-	-	0.8	-	-0.23	-	-0.23
	-	-	0.8	-	-0.14	-	-0.14
	-	-	1.1	-	-0.25	-	-0.25
	-	-	0.7	-	-0.18	-	-0.18
	-	-	0.9	-	-0.25	-	-0.25
	-	-	1.3	-	-0.26	-	-0.26
	-	-	1.1	-	-0.26	-	-0.26

Date	Minerals K^+ (mM. dm^{-3})	Minerals Na^+ (mM. dm^{-3})	% sucrose (refractometry) (%)	Non-reducing sugars (mM. dm^{-3})	Ψ_s (solute potential) (MPa)	Ψ_p (pressure potential) (MPa)	Ψ_{xylem} ($\Psi_p + \Psi_s$) (MPa)
Apr 1st cont.	-	-	0.7	-	-0.14	-	-0.14
	-	-	1.4	-	-0.28	-	-0.28
Averages	-	-	0.9	-	-0.22	-	-0.22
May 24th	-	-	0.8	0	-0.15	-0.32	-0.47
(in leaf)	-	-	-	0	-	-0.43	-
	-	-	0.8	0	-0.14	-0.45	-0.59
	-	-	1.0	0	-0.12	-0.62	-0.73
	-	-	0.9	0	-0.16	-0.62	-0.80
	-	-	0.6	0	-0.05	-0.47	-0.53
	-	-	-	0	-	-0.37	-
	-	-	0.4	0	-0.08	-0.30	-0.38
Averages	-	-	0.8	0	-0.11	-0.45	-0.56
June 9th	6.5	0.8	0.4	0	-0.07	-0.34	-0.40
	5.5	0.5	0.2	0	-0.07	-0.37	-0.44
	6.6	0.9	0.2	0	-0.06	-0.31	-0.36
	6.4	0.4	0.4	0	-0.07	-0.25	-0.32
	5.0	0.7	0.4	0	-0.06	-0.21	-0.27
	7.8	0.6	0.5	0	-0.07	-0.29	-0.36

Date	Minerals K^+ (mM. dm^{-3})	Minerals Na^+ (mM. dm^{-3})	% sucrose (refractometry) (%)	Non-reducing sugars (mM. dm^{-3})	ψ_s (solute potential) (MPa)	ψ_p (pressure potential) (MPa)	ψ_{xylem} ($\psi_p + \psi_s$) (MPa)
June 20th	9.2	1.4	0.6	0	-0.07	-0.37	-0.44
	5.7	1.6	0.4	0	-0.05	-0.24	-0.29
	8.3	1.6	0.7	0	-0.09	-0.24	-0.34
	6.6	1.7	0.5	0	-0.07	-0.29	-0.36
	7.0	1.6	0.5	0	-0.06	-0.27	-0.33
	7.2	1.2	0.5	0	-0.08	-0.28	-0.36
June 26th	5.8	1.2	0.5	0	-0.06	-0.55	-0.61
	6.7	0.8	0.5	0	-0.06	-0.50	-0.56
	4.2	0.7	0.3	0	-0.03	-0.42	-0.45
	4.0	1.4	0.3	0	-0.03	-0.41	-0.43
	3.3	0.8	0.2	0	-0.03	-0.42	-0.45
	4.4	0.6	0.3	0	-0.03	-0.61	-0.63
Averages	6.1	1.0	0.4	0	-0.06	-0.35	-0.41
July 3rd	5.7	0.9	0.4	0	-0.05	-0.31	-0.36
	6.1	1.0	0.3	0	-0.05	-0.27	-0.32
	-	2.1	0.5	0	-0.07	-0.61	-
	5.9	1.1	0.5	0	-0.05	-0.33	-0.37

Date	Minerals K^+ (mM. dm^{-3})	Minerals Na^+ (mM. dm^{-3})	% sucrose (refractometry) (%)	Non-reducing sugars (mM. dm^{-3})	ψ_s (solute potential) (MPa)	ψ_p (pressure potential) (MPa)	ψ_{xylem} ($\psi_p + \psi_s$) (MPa)
July 3rd cont.	-	2.0	0.4	0	-0.05	-0.12	-0.17
	7.4	1.9	0.4	0	-0.06	-0.16.	-0.22
July 18th	4.2	0.8	0.2	0	-0.04	-0.55	-0.59
	7.7	1.0	0.5	0	-0.06	-0.54	-0.60
	4.2	0.5	0.4	0	-0.05	-0.69	-0.73
	6.6	0.6	0.3	0	-0.04	-0.68	-0.71
	6.1	0.8	0.3	0	-0.03	-0.62	-0.64
	6.8	1.0	0.4	0	-0.06	-0.86	-0.86
July 25th	8.8	2.2	0.3	0	-0.05	-0.37	-0.42
	4.6	1.2	0.2	0	-0.05	-0.36	-0.41
	4.3	1.4	0.2	0	-0.03	-0.32	-0.35
	3.7	1.5	0.2	0	-0.03	-0.41	-0.44
	9.6	1.1	0.3	0	-0.04	-0.41	-0.45
	6.6	0.6	0.3	0	-0.05	-0.33	-0.38
Averages	6.1	1.2	0.3	0	-0.06	-0.44	-0.50

Date	Minerals K^+ (mM. dm^{-3})	Minerals Na^+ (mM. dm^{-3})	% sucrose (refractometry) (%)	Non-reducable sugars (mM. dm^{-3})	Ψ_s (solute potential) (MPa)	Ψ_p (pressure potential) (MPa)	Ψ_{xylem} ($\Psi_p + \Psi_s$) (MPa)
Aug 1st	-	-	-	-	-	-0.44	-0.49
	-	-	-	-	-	-0.37	-0.42
	-	-	-	-	-	-0.46	-0.51
	-	-	-	-	-	-0.45	-0.50
Aug 15th	-	-	0.5	0	-	-0.29	-0.34
	-	-	0.5	0	-	-0.27	-0.32
	-	-	0.4	0	-	-0.30	-0.35
	-	-	0.3	0	-	-0.26	-0.31
	-	-	-	-	-	-0.29	-0.34
Averages	-	-	0.4	0	-	-0.33	-0.38
Sept 7th	-	-	0.5	0	-	-	-
	-	-	0.6	0	-	-	-
	-	-	0.4	0	-	-	-
	-	-	0.4	0	-	-	-
Averages	-	-	0.5	0	-	-	-

Date 1978	Minerals K^+ (mM. dm^{-3})	Minerals Na^+ (mM. dm^{-3})	% sucrose (refractometry) (%)	Non-reducing sugars (mM. dm^{-3})	Ψ_s (solute potential) (MPa)	Ψ_p (pressure potential) (MPa)	Ψ_{xylem} ($\Psi_p + \Psi_s$) (MPa)
Nov 29th	10.0	-	1.5	26	-0.18	-	-0.18
(Dormant)	6.1	-	0.8	12	-0.08	-	-0.08
	10.0	-	1.6	26	-0.18	-	-0.18
	10.0	-	1.6	37	-0.22	-	-0.22
Averages	9.0	-	1.4	25	-0.16	-	-0.16
Dec 3rd	8.2	-	4.6	112	-0.54	-	-0.54
	4.8	-	2.0	50	-0.23	-	-0.23
	6.9	-	2.2	60	-0.27	-	-0.27
	10.0	-	3.8	100	-0.44	-	-0.44
Dec 10th	5.0	1.9	1.6	31	-0.23	-	-0.23
Averages	8.0	1.9	2.8	71	-0.34	-	-0.34
Jan 28th	6.0	1.3	2.5	60	-0.26	-	-0.26
1979	5.0	1.4	2.2	47	-0.23	-	-0.23

Date	Minerals K^+ (mM. dm^{-3})	Minerals Na^+ (mM. dm^{-3})	% sucrose (refractometry) (%)	Non-reducing sugars (mM. dm^{-3})	ψ_s (solute potential) (MPa)	ψ_p (pressure potential) (MPa)	ψ_{xylem} ($\psi_p + \psi_s$) (MPa)
Jan 28th cont.	3.4	0.9	1.9	38	-0.22	-	-0.22
Averages	6.4	1.2	2.1	44	-0.24	-	-0.24
Feb 8th	3.6	2.8	2.2	50	-0.32	-	-0.32
	6.1	2.1	3.0	67	-0.40	-	-0.40
	2.6	1.7	1.8	33	-0.27	-	-0.27
Feb 9th	5.0	1.9	2.2	32	-0.32	-	-0.32
	5.5	1.6	2.4	47	-0.32	-	-0.32
	5.8	1.3	3.6	95	-0.44	-	-0.44
Feb 15th	10.7	2.3	4.4	111	-0.54	-	-0.54
	6.0	1.5	3.6	78	-0.50	-	-0.55
	6.9	1.7	2.8	59	-0.39	-	-0.39
Feb 18th	4.4	1.9	2.6	52	-0.25	-	-0.25
	3.8	1.3	2.8	58	-0.27	-	-0.27
	4.2	1.5	4.0	100	-0.44	-	-0.44
Averages	5.4	1.8	2.6	65	-0.38	-	-0.38

Date	Minerals K^+ (mM. dm^{-3})	Minerals Na^+ (mM. dm^{-3})	% sucrose (refractometry) (%)	Non-reducing sugars (mM. dm^{-3})	Ψ_s (solute potential) (MPa)	Ψ_p (pressure potential) (MPa)	Ψ_{xylem} ($\Psi_p + \Psi_s$) (MPa)
Mar 7th	2.1	1.8	2.0	51	-0.16	-	-0.16
	7.2	1.9	1.8	29	-0.14	-	-0.14
	7.4	1.9	1.8	29	-0.17	-	-0.17
	6.1	1.7	1.4	23	-0.13	-	-0.13
	9.0	1.7	1.8	31	-0.19	-	-0.19
Averages	6.4	1.8	1.8	33	-0.16	-	-0.16
Apr 9th	6.2	1.5	1.7	48	-0.26	-	-0.26
	3.2	1.5	0.8	23	-0.18	-	-0.18
	4.5	1.2	1.5	37	-0.23	-	-0.23
Apr 11th	5.3	1.5	2.5	58	-0.28	-	-0.28
	4.6	1.6	1.5	30	-0.15	-	-0.15
	9.7	2.0	1.5	28	-0.22	-	-0.22
	3.6	1.5	1.9	32	-0.25	-	-0.25
Apr 18th	-	-	1.2	-	-0.15	-	-0.15
	-	-	0.8	-	-0.10	-	-0.10
	-	-	0.8	-	-0.10	-	-0.10
	-	-	1.0	-	-0.11	-	-0.11

Date	Minerals K^+ (mM. dm^{-3})	Minerals Na^+ (mM. dm^{-3})	% sucrose (refractometry) (%)	Non-reducing sugars (mM. dm^{-3})	Ψ_s (solute potential) (MPa)	Ψ_p (pressure potential) (MPa)	Ψ_{xylem} ($\Psi_p + \Psi_s$) (MPa)
Apr 18th cont.	-	-	0.7	-	-0.09	-	-0.09
	-	-	0.7	-	-0.09	-	-0.09
	-	-	0.7	-	-0.09	-	-0.09
	-	-	0.7	-	-0.09	-	-0.09
Averages	5.3	1.5	1.2	37	-0.16	-	-0.16
June 6th	-	-	0.5	0	-0.09	-	-
(in leaf)	-	-	0.5	0	-0.07	-	-
	-	-	0.4	0	-0.04	-	-
	-	-	0.3	0	-0.04	-	-
Averages	-	-	0.4	0	-0.06	-	-

Table Ib) Bark sap analyses for samples taken from sycamore seedlings at various times throughout 1978 & 1979.

Date 1978	ψ_s Solute potential (MPa)	% sucrose (refractometry) (%)
Jun 9th	-1.17	10.2
	-1.26	10.0
	-1.00	7.8
	-1.08	9.7
	-1.29	12.1
	-1.30	11.0
Jun 20th	-0.99	9.6
	-0.98	8.9
	-1.15	9.2
	-1.14	10.1
	-0.96	8.5
	-1.11	10.6
Jun 26th	-1.01	9.6
	-0.92	9.6
	-1.04	9.8
	-1.22	12.2
	-1.16	12.2
	-1.12	10.3
Averages	-1.12	10.1
Jul 3rd	-1.20	11.2
	-1.17	11.4
	-1.14	12.8
	-1.24	14.0
	-1.10	10.0
	-0.97	8.6
Jul 18th	-1.30	13.9
	-1.24	13.9

Date 1978	ψ_s solute potential (MPa)	% sucrose (refractometry) (%)
Jul 18th	-1.07	12.6
cont.	-1.27	14.0
	-1.31	15.0
	-1.18	14.6
Jul 25th	-0.98	10.3
	-1.05	10.3
	-1.07	12.6
	-0.86	8.6
	-0.89	9.7
	-0.96	10.2
Averages	-1.12	11.8
Aug 1st	-0.85	9.8
	-0.91	10.7
	-0.82	7.8
	-0.95	9.5
	-0.94	9.5
Dec 8th	-2.06	19.0
	-1.75	16.8
	-1.91	16.8
	-1.75	16.0
Dec 10th	-1.86	17.2
Averages	-1.95	17.2
Feb 18th	-2.48	18.9
1979	-2.53	20.2
	-2.37	21.7
	-2.48	20.9
Averages	-2.54	20.4

Date	ψ_s Solute potential (MPa)	% sucrose (refractometry) - (%)
Mar 7th	-1.45	13.6
Mar 26th	-1.47	13.9
	-1.39	13.1
	-1.37	13.2
	-1.50	13.4
Averages	-1.45	13.4
Apr 9th	-1.30	12.4
	-1.37	14.9
	-1.32	12.4
Apr 11th	-1.55	13.7
	-1.29	12.0
	-1.68	14.2
	-1.70	15.0
	-1.75	14.3
Apr 18th	-1.37	13.3
	-1.25	11.8
	-1.12	10.6
	-1.30	12.6
	-1.37	13.2
	-1.24	11.2
	-1.79	16.4
	-1.45	14.9
Averages	-1.47	13.3

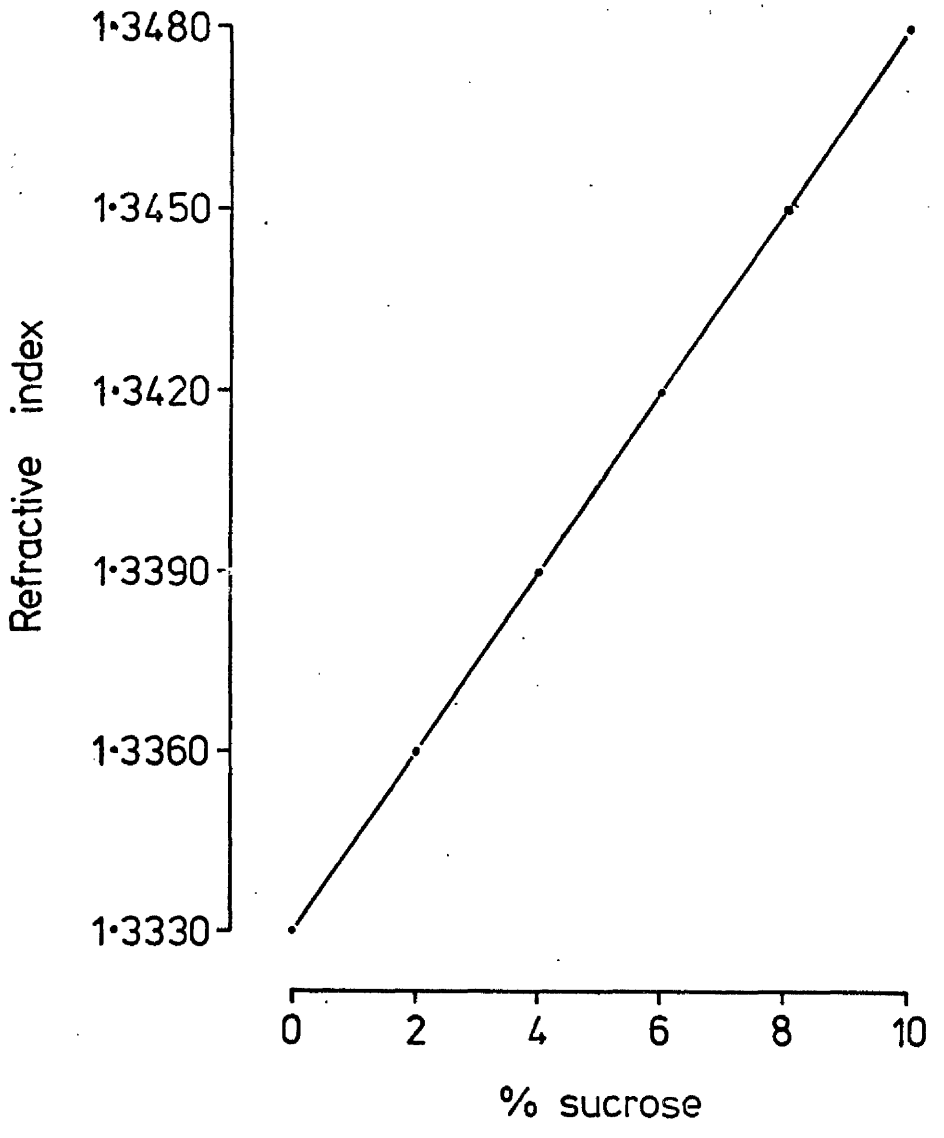


Fig. I. Relationship between refractive index (R.I) and % sucrose by weight (pure solution) for refractometer.

APPENDIX II

Investigation of freezing in sycamore wood samples using nuclear magnetic resonance (NMR) spectroscopy.

Considerable interest has developed regarding investigation of freezing processes within living tissue and food stuffs. Several general reviews of the topic have been published (eg. Olien 1967, Mazur 1969, Burke et al 1976). Frequent attempts have been made to quantify (eg. estimate the ratio of liquid water to ice) the effects of freezing for different temperatures, using various techniques. Calorimetry has been used (eg. Krasavtsev 1968, 1970, Johansson 1970). More detailed information regarding extra- and intra- cellular freezing may be obtained by the electropheretic method developed by Olien (1961, 1964 and Olien & Chao 1973). Increasingly popular however is the use of N.M.R. spectroscopy. (eg. Toledo et al 1968, Burke et al 1974, George et al 1974 a & b, Gusta et al 1975, George & Burke 1976 George & Burke 1977 a & b.) This is a very convenient tool providing detailed information of subject material without its destruction.

The principle behind N.M.R is the fact that rotating charged nuclei produce magnetic fields, and hence may be aligned when influenced by an external field. Allignment with or against the direction of the field can be altered by stimulation with electromagnetic radiation of radio wave frequencies. The energy required for alteration of state is influenced by the immediate environment of the nucleus (eg. neighbouring atoms, strength and type of bond). Energies of excitation or decay are precise and can be monitored to estimate presence and abundance of any substance that exhibits magnetic spin quantum effects (eg. protons).

To relate this to investigation of freezing within plant tissues, frequencies for aqueous phases are typically recorded in the range of $\leqslant$8,000 Hz. The corresponding line width for ice however, is near 100,000 Hz (reflecting increased crystal structure). Distinct changes in spectroscopic properties are therefore readily detected during ice formation. These changes can be quantified with ease if overall water content is constant. In practice, changes due to freezing correspondingly reduce the N.M.R. output, as the line width for ice is much greater than that for liquid water. Hence the output from the former broadens and its contribution becomes insignificant. (See Burke et al 1974).

Materials & Methods.

Dormant sycamore seedlings, (described p 14) were used as the source of wood. Stem segments were individually prepared for treatment as described on p 22 except no probes were embedded in the basal ends. Standard pretreatment (ie. supplying of distilled water) was imposed (12 hours). The basal 5cm. of stem was then excised, (with secateurs), encased in polythene, (to reduce water loss), and transported to the N.M.R. laboratory. A core of wood (length 3cm., diameter 0.4cm.), was then bored, longitudinally, from the peripheral region of the xylem core. A No.1 cork borer was used in this procedure. Bark was not included in the sample. The core of wood was then quickly transferred and inserted into a previously weighed, clean, glass N.M.R sample tube, (bore 0.4cm., O.D. 0.5cm). Reweighing (on a Stanton Balance, Model CL1.) allowed calculation of tissue fresh weight by subtraction. A thermistor temperature probe of catheter type construction (type FF, Grant Instruments (Cambridge) Ltd., Barrington , U.K.) was inserted into the N.M.R sample tube. The sensitive thermistor tip was positioned in direct contact with the wood sample, and the N.M.R tube then plugged, (plastic around the probe lead). Temperature was recorded using a Grants miniature temperature recorder.

The sample tube (with sample) was placed in the N.M.R. spectrometer, (model C60 HL, Japan Electron Optics Ltd., Colindale, London, U.K.) and its position unaltered throughout investigation. Tuning of the continuous wave spectrum was undertaken (operating at 60MHz for protons). The sample was then first equilibrated at $+2^{\circ}C$, before cooling ($1^{\circ}C/5min$) was effected by careful alteration of the thermostat on the cooling unit (model JNM - VT - 3B). N.M.R spectra were recorded every $2^{\circ}C$ change in temperature, and curves integrated automatically. Sample temperature was recorded intermittantly, and not during curve integration. Once freezing had occured, (usually approx. $-10^{\circ}C$), the sample temperature was maintained constant and equilibration allowed (usually 0.5 hours). The thawing cycle was then induced. Sample temperature was raised by $2^{\circ}C$ and allowed to equilibrate, (usually 20-30 minutes). This was repeated until the temperature reached $+2^{\circ}C$. (NB. frequently changes of only $1^{\circ}C$ were induced in the critical temperature range $-4 \rightarrow 0^{\circ}C$). Again N.M.R spectra were recorded and integrated at each sample temperature. Curve area when fully thawed represented total liquid water content. Changes in curve area represented relative change in liquid water content - and hence ice content. Boltzmann

temperature correction was not considered necessary for the temperature range investigated.

After experimentation the thermistor probe was removed and the sample sealed into the N.M.R. tube with a tight-fitting plastic stopper. The whole was then immersed in liquid nitrogen (T = -196°C) for 15mins. to kill the plant tissue. After storage overnight (in a deep freeze T = -15°C), the sample was thawed and equilibrated at +2°C for approx. 1 hour. Repetition of the experimentation described above was then undertaken. Finally, the tube and sample were reweighed to ensure no loss or gain during investigation, before drying (90°C) in an oven (model OV100, Gallenkamp Ltd., London, U.K.). When no further change in weight was detected ($\leqslant$ 1 week), the sample was weighed again.

In total, 5 samples were investigated, from different seedlings throughout the period November 1978 - April 1979. Further experimentation was intended but not undertaken due to mechanical problems. Figs II a and b show typical responses from a sample when alive and dead. Table II shows % liquid water at -10°C (after equilibration), compiled from data collected.

Results & Discussion.

Figs II a and b illustrate that considerable supercooling of sap occured in both living and dead tissue, before freezing. (eg. sap of living tissue, melting pt. $\geqslant$ -0.5°C). Once ice formation has been initiated, however, freezing is rapid - although it must be stated here that fast rates are predicted due to the small thermal capacity of the sample. Also of interest is the observation that after freezing and equilibration at -10°C both living and dead samples show high proportions of stem water still unfrozen. (av. 73% & 63% respectively). This is presumed to be a large relatively stable fraction of supercooled water (Burke et al 1976, George & Burke 1977). In the living state, most of this water is considered to be bounded by membranes, (ie. intracellular), the remainder presumably within the capillaries of the tissue cell walls.

Thawing of the samples revealed a region of temperature (above approx. -4°C), where liquid:ice ratio altered quite rapidly - more so for dead tissue. This early thawing may be associated with competition between ice and hydrophilic interfaces, (Olien 1973, 1974, Olien & Smith 1977). Such a phenomenon may indeed be of significance during conditioning and sap replenishment, (see chapter 5).

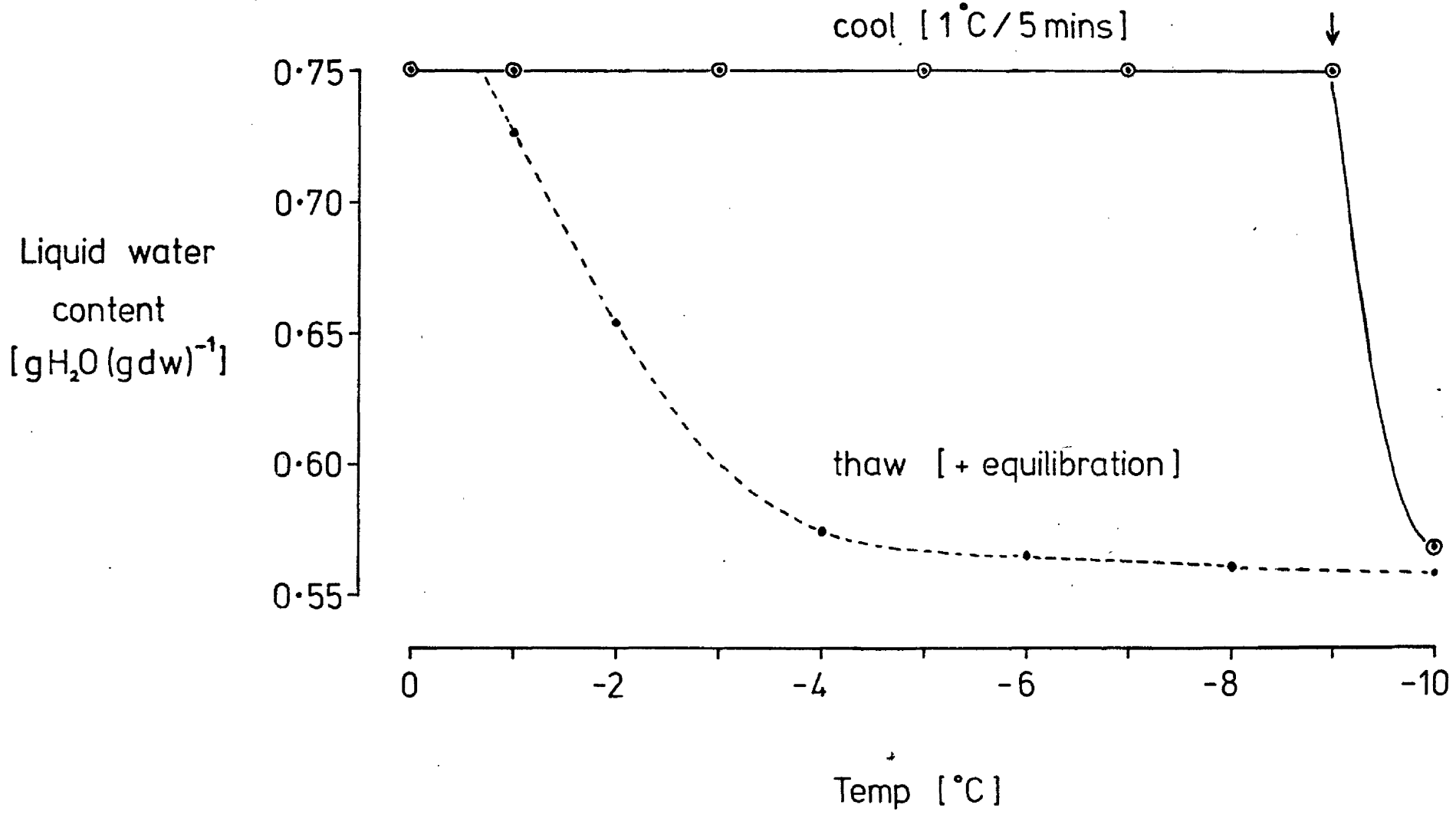


Fig II a

Ratio of liquid water : ice in a <u>living</u> wood sample of a sycamore seedling during cooling and warming phases. (↓ = freezing)

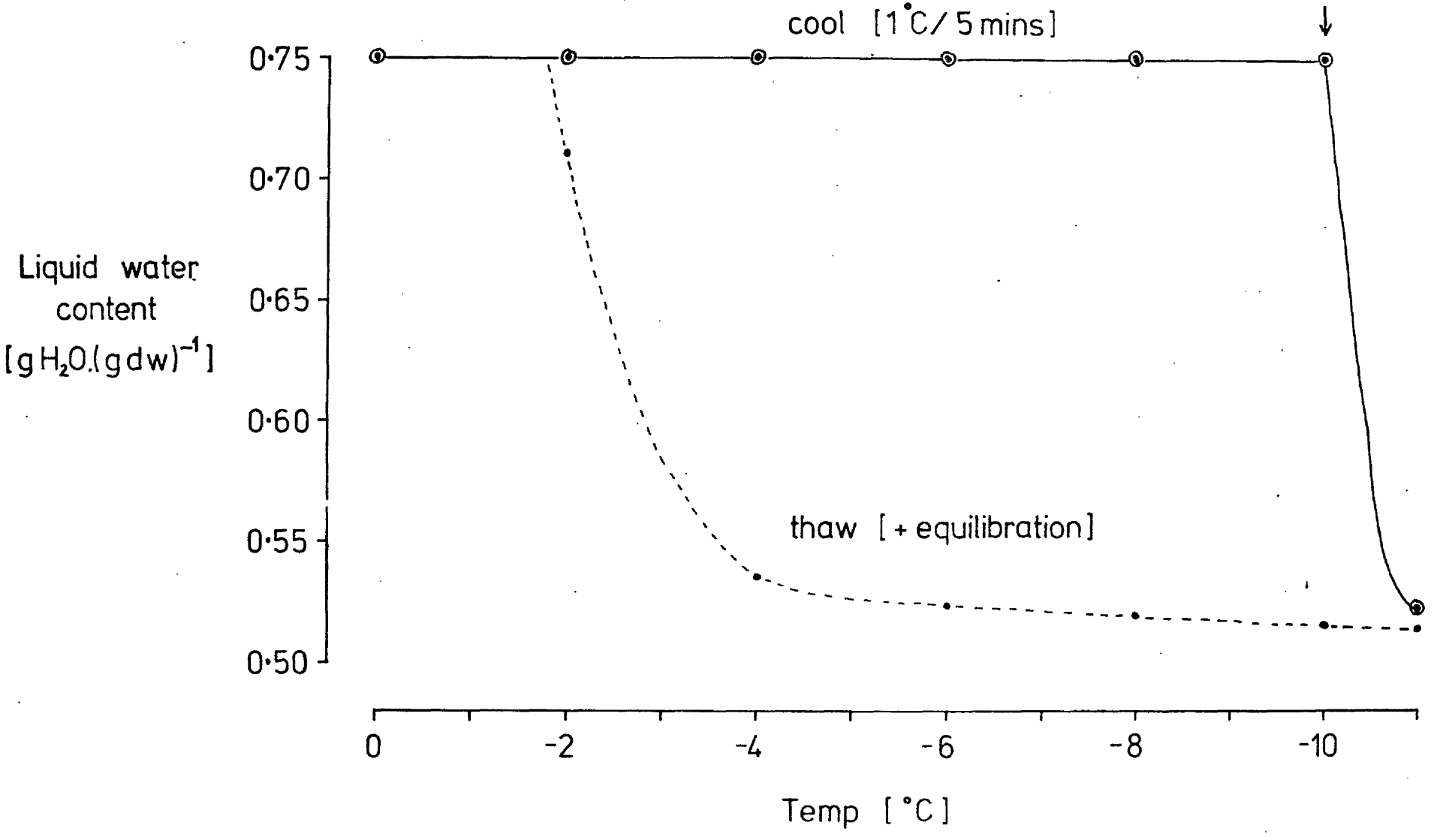


Fig. II b Ratio of liquid water : ice in a dead wood sample of a sycamore seedling during cooling and warming phases. (↓ = freezing)

Table II % liquid water (of total water content) at -10°C in wood of dormant sycamore seedlings (from N.M.R. studies)

Sample No.	living % liq H_2O ($g.H_2O\ (g.fw)^{-1}$)	dead % liq H_2O ($g.H_2O\ (g.fw)^{-1}$)
1	72	65
2	72	64
3	68	62
4	76	-
5	75	60
Averages	73%	63%

APPENDIX III

Investigation of wood anatomy.

The presence of abundant gas-filled fibres in close association with xylem vessels is a notable property of maple wood, (Jones 1903, Wiegand 1906, Sauter 1974). Anatomical investigation of sycamore wood was undertaken to establish that fibres therein were similarly numerous.

Materials & Methods

Small portions of the xylem core were taken from sycamore seedlings in stock. Samples were collected from a region approx. 40cm. from each apex, and hence tissue was usually 2-3 years old. Processing for microscopy involved fixation by submersion in gluteraldehyde (buffered, p.H 7.2) for 3 hours at room temperature. The tissue was then washed three times (each of 1 hour duration) in buffer (veronal acetate-HCl, p.H 7.2), and post-fixed in 2% (w/v) osmic acid, in buffer, for at least 2 hours, (max. 24 hours). After further washing in buffer (as above), material was dehydrated in a graded series of acetone. This was followed by infiltration/embedding in epon/resin (araldite) mixture (1:1, v/v) under vacuum. The resin was hardened by polymerisation at 60°C for 24-72 hours. Sample blocks were trimmed to shape and sections (1-2 µm thick) cut using an LKB 11800 Pyramitome (LKB - Produkter AB, Stockholm, Sweden). Sections were dried onto a gelatine coated slide, and made permanent by addition of a drop of resin as mounting medium. Sections were examined using a Zeiss Photomicroscope II, (Carl Zeiss, Oberkochen, F.R.G). Ilford Pan-F film (Kodak Ltd., Hemel Hempstead, Hertfordshire, U.K.) was used for photography. Anatomy shown in plates III a & b is representative of the tissue studied.

Results and Discussions

The cross sectional views of sycamore wood presented in plates III a & b clearly show the presence of fibres (F) in close proximity with vascular tissue (XC). That such fibres of sycamore wood are usually gas-filled has been determined by Milburn (unpublished). These observations accord well with previous investigations of sugar maple (Wiegand 1906, Sauter 1974).

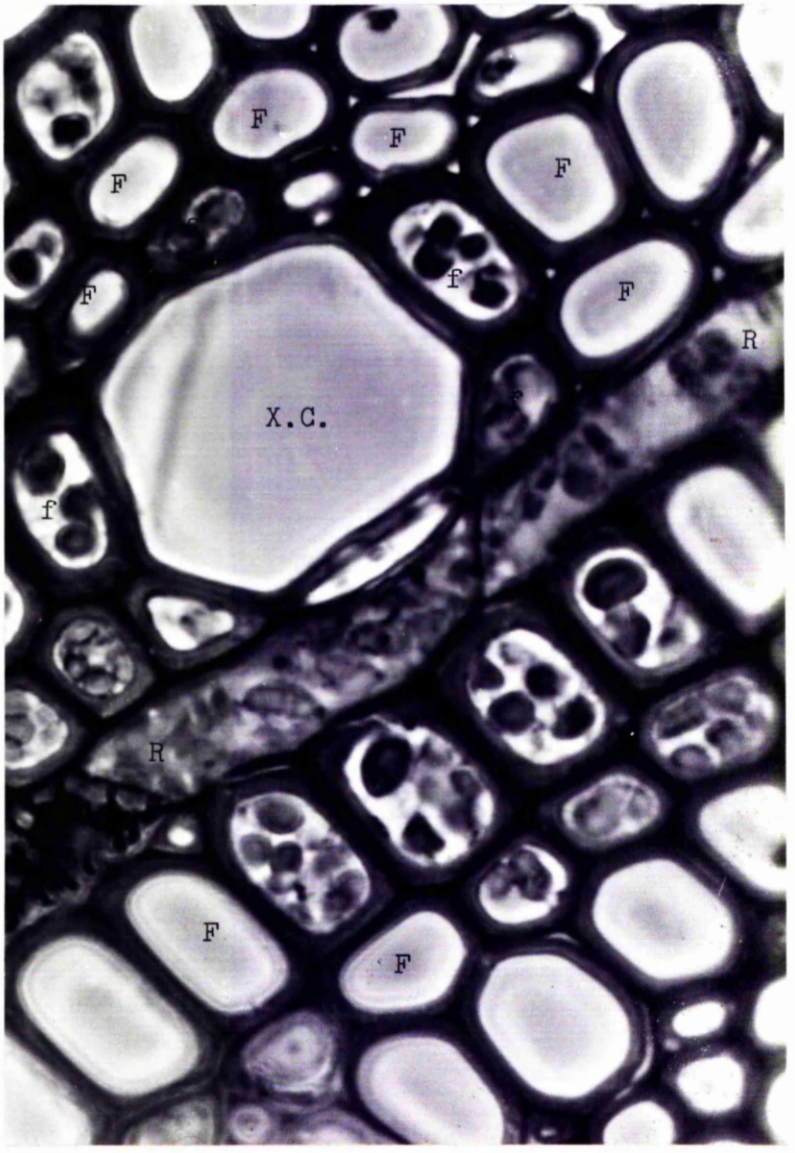


Plate II a

Cross sectional view of sycamore wood showing relative position of xylem conduits (eg. X.C.) and fibres (eg. F). Some fibres appear to have inclusions, and may be living (eg. f). A vascular ray is also visible (R-R).

(Magnification x 2500)

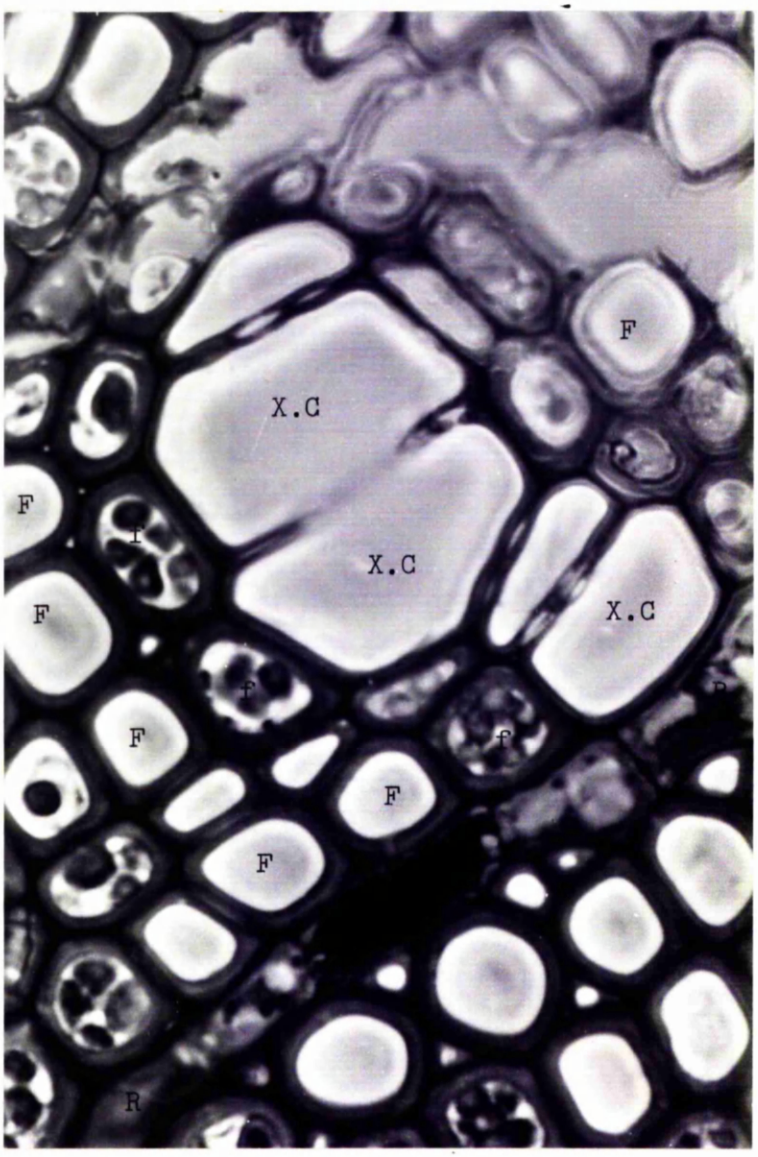


Plate II b

Similar view to that of Plate II a.

X.C. = xylem conduit

F = fibre (dead)

f = fibre (possibly living)

R-R = vascular ray

(Magnification x 2500)

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