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Original article

Xylem acoustic signals from mature *Pinus sylvestris* during an extended drought

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Abstract – Mature Scots pine (*Pinus sylvestris* L.) were subjected to an 11 month imposed soil drought, from November 1994. Control plots received rainfall plus irrigation. The extent of cavitation in the xylem of branches and boles was compared using ultrasonic acoustic emission (UAE) measurements. Supporting measurements of the relative water content (R_w) of the bole and shoot were recorded. Acoustic emissions were detected in both treatments. An effect of water-stress, in both boles and branches, at the height of the drought, was noted. Differences in vulnerability were found and suggest a reduced vulnerability to cavitation of remaining functional conduits, after loss of the most vulnerable conduits, in the water-stressed trees. Bole R_w was lower than branch R_w. In 1-year-old shoots of droughted trees a significant increase in xylem embolism was found. Seasonal changes in shoot R_w were interpreted as the occurrence and recovery (refilling) of emboli in xylem tissues: an active role of precipitation is hypothesised. It was estimated that 15% of tracheids were cavitated without affecting above-ground hydraulic resistances. It is suggested that runaway cavitation was avoided through stomatal closure maintaining leaf water potential (indicative of xylem tension) below the cavitation threshold, and thereby little effect of drought was evident in above ground tissues.

drought / *Pinus sylvestris* (Scots pine) / time domain reflectometry / ultrasonic acoustic emissions / xylem cavitation

Résumé – Signaux acoustiques du xylem chez des *Pinus sylvestris* matures soumis à une sécheresse prolongée. Des pins sylvestres matures (*Pinus sylvestris*) ont été soumis à partir de novembre 1994 et pendant 11 mois à une sécheresse artificielle du sol. Des placeaux témoins ont bénéficié de la pluie et d'un supplément d'eau sous forme d'irrigation. On a comparé l'importance de la cavitation dans le xylème des branches et des fûts par des mesures d'émission acoustique ultrasonique (UAE). On a également effectué des mesures de contenu relatif d'eau (Rw) dans les fûts et les branches. Les émissions acoustiques ont été détectées dans les deux traitements. L'effet du stress hydrique a été noté sur branches et fûts au stade du pic de sécheresse. Sur les arbres soumis à stress hydrique, on a mis en évidence des différences de vulnérabilité à la cavitation ; les vaisseaux encore fonctionnels apparaissent moins vulnérables après la perte des plus vulnérables. Le Rw des troncs était inférieur à celui des branches. On a constaté une augmentation du phénomène d'embolie dans les pousses de 1 an des arbres soumis à la sécheresse. Les changements saisonniers de Rw des pousses ont été interprétés comme étant une conséquence du bon rétablissement du remplissage des vaisseaux des tissus du xylème frappé d'embolie. L'hypothèse d'un rôle actif des précipitations a été avancée. On a estimé que le phénomène de cavitation a affecté 15 % des trachéides sans que les résistances hydrauliques dans les parties aériennes soient affectées. Il est suggéré que la cavitation généralisée était évitée grâce à la fermeture des stomates permettant de maintenir le potentiel hydrique des feuilles (indicateur de la tension du xylème) au dessous du seuil de cavitation, avec comme conséquence un faible effet de la sécheresse sur les tissus situés au dessous du sol.

***Pinus sylvestris* (pin sylvestre) / émissions acoustiques ultrasoniques / cavitation du xylem / TDR**

1. INTRODUCTION

The increasing incidence of summer droughts has been implicated as a factor contributing to tree decline [13, 41], yet there are few experimental studies of the physiological response of mature trees to long term soil water deficits. There is evidence that severe droughts, even in temperate climates that are generally moist, may leave trees in a weakened state and thus exhibit reduced growth in later years [23, 35]. One mechanism whereby this might occur is through the cavitation of water columns in the xylem.

According to the cohesion theory of water transport [7] the water in xylem conduits comes under tension whenever water is lost by transpiration. Many studies have shown that the water columns can be broken above a critical tension [6, 16, 17, 40, 43] and thus part of the water-transporting system becomes gas-filled and ceases to function. Embolisms are thought to be produced in woody tissues by a mechanism known as "air seeding" [45], when, under tension, water within a conducting conduit exceeds a critical tension at which the liquid water is replaced by gas. The gas is thought to enter the conduit as air micro-bubbles that are drawn into the conduit

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from outside through the pit membrane pores. The air-seeding hypothesis, has been verified experimentally [33] and this process is accompanied by an acoustic pulse, resulting from an abrupt release of tension in the elastic wall of the conduit. Detection of xylem cavitation acoustically was first demonstrated by Milburn and Johnson [28], who measured acoustic emissions in the audible range. Acoustic detection of cavitation was not new but the application to plant ecophysiology was inventive. However, interest in acoustic monitoring was limited until detectors working in the ultrasonic frequencies were applied to the study of cavitation in plants [6, 34] so allowing field portability. Dixon, Grace and Tyree [6] showed that accumulated acoustic emissions in *Thuja* were closely related to the gas-filled volume in the stem. Acoustic events can now be recorded for extended periods of time on various plant organs [20] and have been used as an index of plant water stress [1, 6, 16]. Cavitation leads to significant loss of water-conducting tissue and concomitant declines in hydraulic conductivity [24], and recovery (in gymnosperms) may be slow or incomplete [1, 9, 21, 37, 47].

Previous studies have predominantly been on laboratory or greenhouse plants or plant parts. This study aimed to quantify the effect of a prolonged drought upon a mature, commercial conifer plantation, in the UK, and was conducted in one of the driest years of the century. The primary aim of this research was to investigate the seasonal development of embolism in mature *Pinus sylvestris* L. The important questions being asked were: (1) does cavitation occur to an extent that might affect the hydraulic properties of the tree, and can this then lead to "runaway cavitation"? (cf. [43]), (2) are there any differences between plant organs in their vulnerability to cavitation? (3) is there any evidence for refilling of the xylem?

2. MATERIALS AND METHODS

2.1. Experimental site

The experiment was in Devilla Forest, near Kincardine Bridge, Fife, Scotland. The work was carried out in a 41-year-old Scots pine stand within Compartment 474 of this commercial plantation, owned by the Forestry Commission (see Tab. I in [15]).

The soil was a homogenous sandy loam with little intra-site variation (i.e. occasional pockets of less free-draining soil). Within the forest, eight square plots of 100 m^2 were selected. Around the perimeter of each plot a 1.5 m deep trench was excavated, an impermeable plastic septum was inserted vertically into the trench and the trench was refilled. The septum minimised lateral flow of water, allowing plots to be watered (the control plots) or droughted independently, as required. Precipitation and stem flow were excluded from the four water-stressed plots by construction of clear polythene covers, which intercepted rainwater and diverted it outside the vertical soil septum. The covers permitted access to the tree boles and allowed natural aeration of the soil surface. The control treatments were without covers and received some irrigation to maintain average summer rainfall. The year of study, 1995, was exceptionally dry (3rd driest summer of the century), with March to August rainfall 128 mm lower than the 20 year average, a reduction of 40%. Over this period 125 mm was added as irrigation to the control plots.

The general conditions of the experiment are further described by Irvine et al. [15]. Site construction was complete by November 1994, after which time water was withheld from the water-stressed plots. Measurements commenced in April 1995. At the end of October 1995, after removal of the covers, the water-stressed plots were irrigated to bring the soil moisture content back to initial levels.

2.2. Continuous measurements

2.2.1. Microclimate

Sensors for air temperature and relative humidity (HMP35AC, Campbell Scientific, Loughborough, UK), net radiation (Q7, Campbell Scientific, Leicestershire, UK), photosynthetically active radiation, PAR (Skye 215, Skye Instruments, Llandrindod, Powys, Wales) and wind speed (A100R, Vector Instruments, Rhyl, Clwyd, Wales) were permanently installed on the tower, 3 m above the canopy. Hourly averages of climatological variables were recorded on a data logger (CR21X, Campbell Scientific, Leicestershire, UK). Vapour pressure deficit (VPD) was calculated from air temperature and relative humidity.

2.2.2. Cavitation by Ultrasonic Acoustic Emissions (UAE)

Counts of ultrasound acoustic emissions from xylem were taken in situ (4615 Drought Stress Monitor, Physical Acoustics Corporation, Princeton, NJ, USA). Four transducers (I15I, Physical Acoustics Corporation) were placed on branches of similar age and size for each treatment (approx. 3 cm diameter: 8–10 years old), accessed from an aerial walkway. A rectangular window in the bark was made (12 mm^2) by removing the bark, phloem and cambium. The exposed xylem surface was then smeared with petroleum jelly to aid signal conduction and prevent local water loss, and the transducer was clamped with a force of 30 N by means of a calibrated spring clamp. Acoustic events were monitored from each sensor sequentially, for 2 min, with automated switching between sensors via a multiplexer (Model SDMX50, Campbell Scientific, Leicestershire, UK). The amplification gain of the emission was set at 74 dB, a level at which background noise was less than 1 event per minute (which was measured and in situ values altered accordingly). This gain was maintained for all measurements over the experimental period. Other set-up parameters were consistent throughout the study using default instrument settings. For two periods, covering the height of the imposed drought (23rd –25th August) and during rewatering (26th–28th October), a second set of sensors were installed on tree boles at a height of 1.3 m, with one sensor per tree and three trees per treatment. The same instrument settings were used as for the branches.

Total accumulated counts for each day were calculated from average count values for each treatment, taken from every 16-min cycle of the multiplexer.

2.3. Periodic measurements

2.3.1. Soil Moisture

Soil volumetric water content (VWC) was measured using time domain reflectometry (TDR) every two weeks. A two-pin balanced design was used, measurements being taken with a cable testing oscilloscope (1502B, Tektronics Corporation, Redmond, OR, USA) fitted with an inline SDM1502 interface connected to a CR21X data logger. Probes were permanently installed in each plot: 3×20 cm and 2×50 cm (depth). Calibration and measurements were as in Irvine et al. [15].

2.3.2. Shoot relative water content

Direct measurements of embolism in current and previous year shoots were made on selected days, during the growing season. Shoot samples were collected with pole pruners. The needles were immediately removed and samples wrapped in Nescofilm™ (Nescofilm, Nippon Shoji, Osaka, Japan) to prevent sample desiccation. Samples were stored at 4 °C and analysed within 48 h of collection. Sample relative water content (R_w) was obtained using the method of Sobrado et al. [37]. R_w was calculated according to the formula:

$$R_w = 100\,(W_f - W_d) / [(\rho_w \cdot V_f)(1-(W_d / (\rho_s \cdot V_f)))],$$

where W_f is the fresh mass and W_d is the dry mass (after 48 h at 80 °C) of the debarked wood samples determined to the nearest 0.1 mg. The fresh volume (V_f) was determined by Archimedes' principle [2], and ρ_w and ρ_s are the density of water and wood, respectively. ρ_s is assumed to be a constant of 1530 kg m^{-3} [36].

2.3.3. Stem relative water content

The relative water content (R_w) of the sapwood in the bole of five trees per plot, at 0.5 m above the ground, was monitored with 0.05 m TDR probes inserted into the bole. A two-pin balanced design method was employed, measurements being taken with a cable testing oscilloscope (1502B, Tektronics Corporation, Redmond, OR, USA) fitted with an inline SDM1502 interface connected to a CR21X data logger. Calibration and measurements were as described by Irvine and Grace [14, 15].

2.3.4. Leaf water potential

Leaf water potential (Ψ_L) was assessed at 3-h intervals, on selected days, from pre-dawn to dusk. Shoots were obtained from the mid-canopy using pole pruners, a random sample being taken from each plot (four per treatment). To minimise water loss samples were immediately placed in polythene bags containing moist towels. From each sample two Ψ_L measurements were taken using a Scholander pressure bomb (Model 1400, Skye Instruments Ltd., Landrindod Wells, UK.) on fully expanded (last year's) needles. Measurement was complete within 20 min of sample collection.

2.4. Statistical analysis

Where a particular measured variable showed a clear treatment effect (graphically) *t*-tests were performed to quantify the significance of treatment at each date or time. Where no clear interaction was observed a two-way ANOVA with one "repeat measures" factor was performed (SAS Institute Inc., Cary, NC). Regression analysis was performed using Sigmaplot™.

3. RESULTS

3.1. Seasonal changes of soil and plant water status

Soil moisture content integrated over the top 20 and 50 cm depth in the water-stressed treatment declined to a minimum of 0.05 and 0.11 respectively, at the end of August 1995. The soil moisture in the control treatment never fell below 20% (50 cm depth). This confirmed that a statistically significant difference occurred from April 1995 (Fig. 1a). The pre-dawn needle water potential (Ψ_{LPD}) of the water-stressed treatment was lower than in controls throughout the season (Fig. 1b).

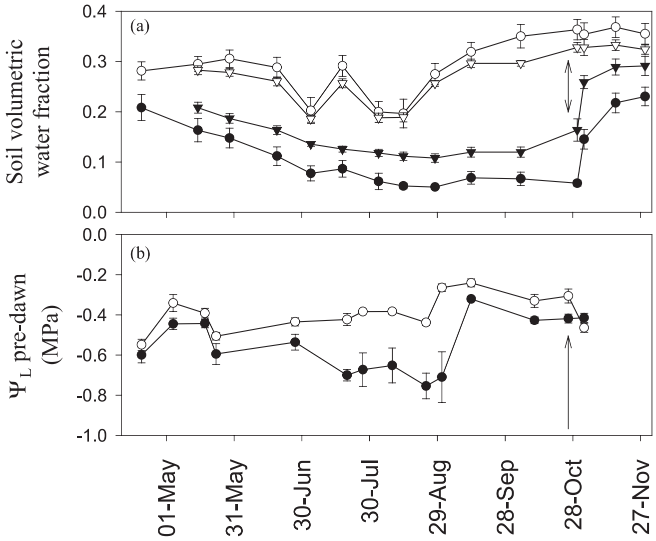

Figure 1. Seasonal variation in (a) soil volumetric water fraction, integrated over the top 20 cm soil depth (circles) and 50 cm soil depth (triangles) (n = 12), and (b) the pre-dawn needle water potential (Ψ_{LPD}) (n = 4). The arrows indicate the rewatering date when the water-stress plot was returned to field capacity. Closed symbols: water-stressed trees, open symbols: controls. Points are means ± 1 standard error.

From 27th July until the end of October (when the plots were rewatered) the difference between treatments was statistically significant ($p < 0.05$). The minimum daily leaf water potential experienced in the two treatments did not differ significantly except at the height of the drought period, at the end of August, 1995 (see Tab. II in [15]).

3.2. Ultrasonic Acoustic Emissions (UAE)

The relationship between cavitation events (i.e. ultrasonic acoustic emissions, UAE) and tree physiological variables is shown in Figure 2. UAE coincided with extreme saturation vapour pressure deficit (VPD), and occurred when Ψ_L was at its most negative. Cavitation rate peaked during the daytime and was very low at night, and no relationship with wind speed was evident. On May 23rd, 1995, there was no significant difference between treatments for both branch UAE or leaf water potential (Ψ_L). The VPD on this day was relatively high for the time of year in central Scotland. On August 9th, 1995, a significant difference was observed between treatments for branch UAE (Fig. 2) ($p < 0.01$) though differences in Ψ_L measured at mid-day were not statistically significant (Tab. II in [15]). High VPD on August 9th coincided with very high values of branch UAE being recorded. Total accumulated counts of 16 000 and 8 400 in the water-stressed and control treatments respectively, were recorded over the day. August 31st, 1995, was a dull and overcast day with low VPD and PAR, however significant differences in Ψ_L ($p < 0.05$) and branch UAE were again evident ($p < 0.05$). Total accumulated counts were 12 250 in the water-stressed tree branches and 9 200 in the control trees. Diurnal data for 11th October represents the period

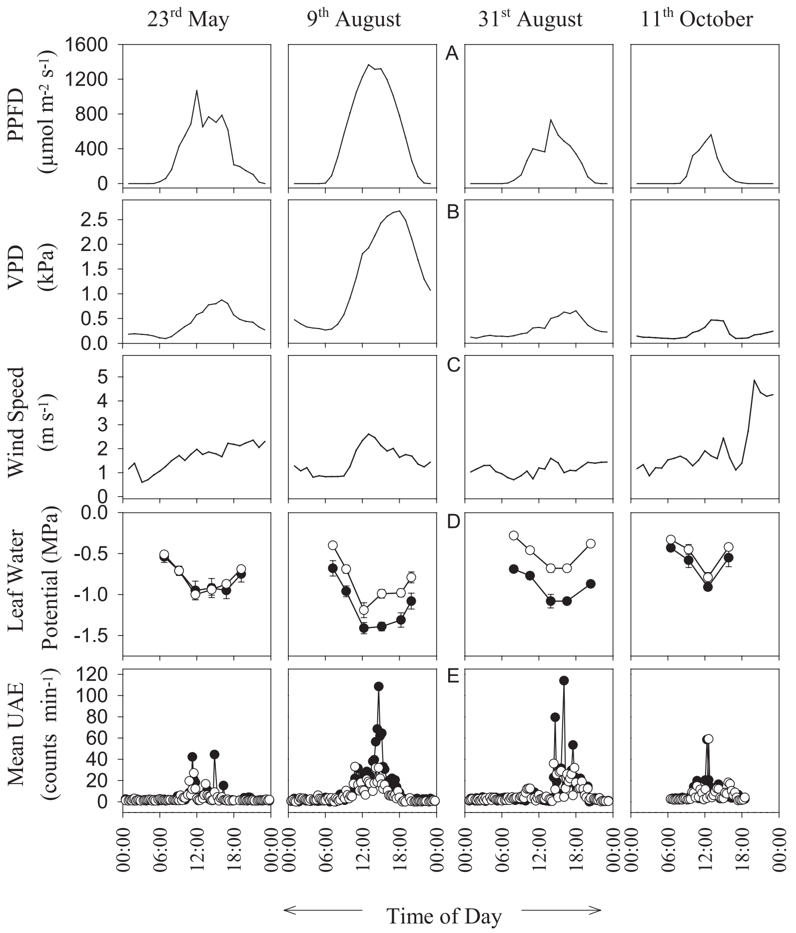

Figure 2. Diurnal variation of physiological and meteorological variables during four days over the experimental period, in 1995: (A) photosynthetically active radiation (PAR), (B) air vapour pressure deficit (VPD), (C) wind speed, (D) leaf water potential (Ψ_L), and (E) ultrasonic acoustic emissions in branches (UAE). The first three columns represent days during the progression of the drought, the final column is after the end of the drought period. Closed symbols: water-stressed trees, open symbols: controls. Error bars indicate ± 1 standard error.

after a recovery of leaf water potential occurred. Despite low VPD on this day some UAE's were detected, however, no significant treatment differences were observed in the rate. Total accumulated counts reached 4 000 between 06:00 and 18:00, the period of measurement.

The apparent vulnerability to cavitation at particular Ψ_L was investigated on the four days for which diurnal fluctuations were measured and branch cavitation data were available. This was possible by plotting the count rate (log, y-axis) against the water potential (x-axis) (Fig. 3). A linear negative correlation below a threshold Ψ_L was found for all dates. On the 23rd May and 11th October there was no observable difference in the apparent vulnerability to cavitation between treatments. Data from 23rd May suggests an apparent threshold to cavitation of –0.55 MPa (Fig. 3a), which was maintained until 9th August in the control trees. Under the relatively extreme meteorological conditions of the 9th August apparent vulnerability in the water-stressed trees decreased to –0.82 MPa (Fig. 3b). On 31st August, apparent vulnerability to cavitation increased to –0.71 MPa in the water-stressed trees (Fig. 3c). The observed variation between these two dates at the height of the imposed drought is symptomatic of the varying VPD experienced on the two days. However, the differences between treatments were broadly consistent on both dates. By 11th October the threshold to cavitation was –0.41 MPa (Fig. 3d).

Diurnal behaviour in both branch UAE and bole UAE and meteorological variables was recorded over two three-day periods, at the height of the drought (late August) and after re-watering. For both periods, bole cavitation occurred when VPD was high and was of the same order of magnitude in boles as in branches (data not shown). There was no significant statistical difference in UAE counts between boles and branches on 23rd August 1995 (Figs. 4a and 4b). Cumulative UAE over this 24 h period was 16 000 and 8 000 counts in water-stressed and control treatments, respectively. When VPD was low i.e. on 24th August and 26th October, cavitation occured preferentially in the branches (data not shown). On the 25th October UAE counts in the bole and branch were not significantly different ($p < 0.05$), with total accumulated counts in the water stressed trees reaching 7 500 and 6 800 respectively and in the control trees 5 500 and 4 800 (Figs. 4c and 4d).

3.3. Xylem embolism

The relative water contents (R_w) of the xylem of current and previous year twigs and of the sapwood in the trunk over the experimental period, and extending into the following year, are presented in Figure 5. Over the course of the drought, R_w of the xylem of the previous-year shoots was significantly reduced by drought, when grouped over all dates ($p < 0.05$)

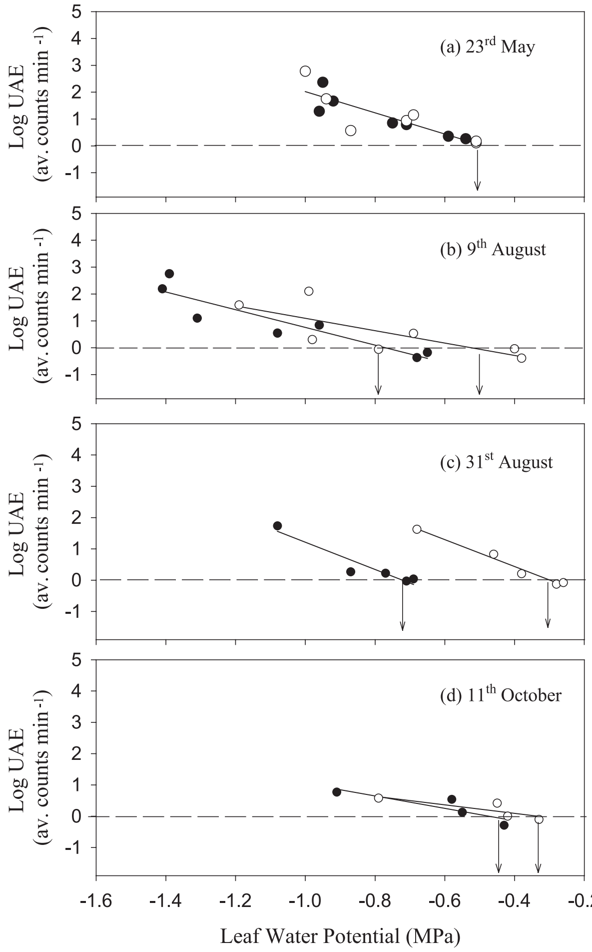

Figure 3. The relationship between mid-day leaf water potential and the log of cavitation rate (data averaged for all plots taken from a 45 min period, corresponding with the time of Ψ_L measurement), indicating vulnerability to cavitation, on four days, during 1995: (a) 23rd May (treatments combined, $r^2 = 0.28$), (b) 9th August (water-stressed, $r^2 = 0.34$, watered, $r^2 = 0.28$), (c) 31st August (water-stressed, $r^2 = 0.39$, watered, $r^2 = 0.40$), and (d) 11th October (treatments combined, $r^2 = 0.31$). All regressions were significant at $\alpha = 0.05$. Closed symbols: water-stressed trees, open symbols: controls. The broken horizontal lines correspond to one cavitation event per minute below which UAE's are deemed indistinguishable from noise.

(Fig. 5b). There was no significant treatment effect on any single day of measurement. Current-year shoots showed no significant treatment differences, although there was a similar trend in the data observed in August, the height of the drought (Fig. 5a). In shoots a recovery in R_w in the water-stressed trees was observed in early October. This recovery occurred approximately one month after the recovery noted in Ψ_{LPD}. A significant treatment × date interaction in R_w of the sapwood in the trunk, over the drought period, was found ($p < 0.01$) (Fig. 5c). This was, however, due to a significant increase in R_w of the control trees over this period [15]. R_w of bole was considerably lower (5–25%) than that found in the shoots. This is most likely indicative of a higher air volume fraction in bole sapwood, in contact with the embedded TDR pins.

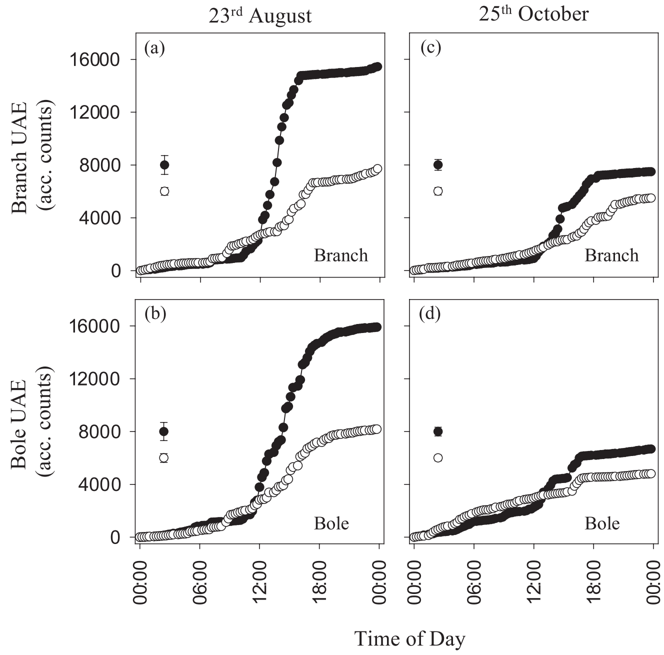

Figure 4. Daily accumulated counts of ultrasonic acoustic emissions in *Pinus sylvestris* (L.) on two dates over the season: 23rd August in (a) branches, and (b) boles, 25th October in (c) branches, and (d) boles. Closed symbols: water-stressed trees, open symbols: controls. Error bars indicate ±1 standard error (grouped data).

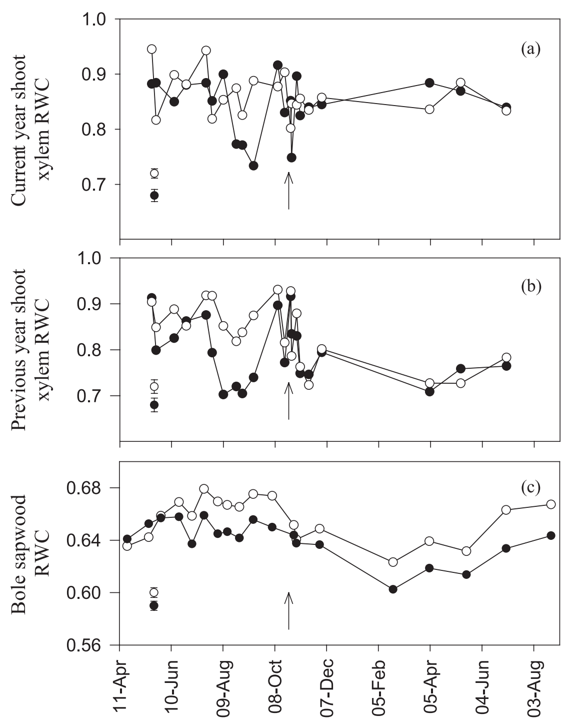

Figure 5. Seasonal variation in the relative water content (R_w): (a) current year shoots, (b) previous year shoots, and (c) bole sapwood (to 5 cm depth), during the drought year (1995). Closed symbols: water-stressed trees, open symbols: controls. Points are mean values. The arrows indicate the rewatering date when the water-stress plot was returned to field capacity. Error bars indicate ±1 standard error, $n = 4$.

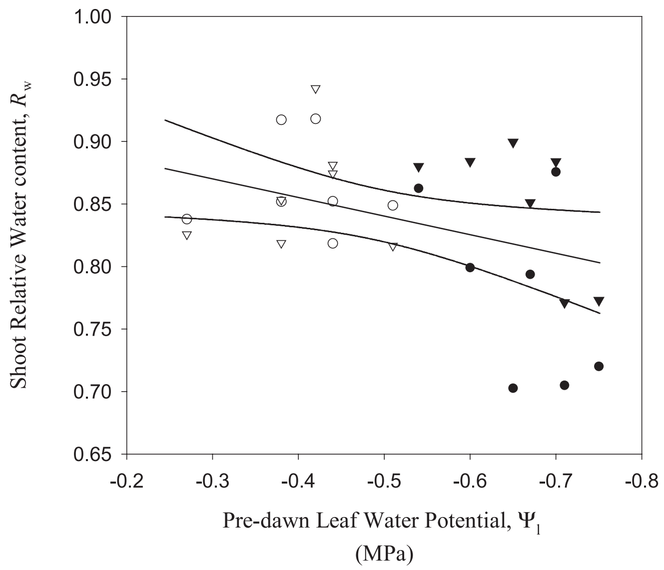

Figure 6. Relative water content (R_w) in the twig xylem as a function of pre-dawn leaf water potential (Ψ_{LPD}) ($R_w = 0.94 + 0.19\ \Psi_{LPD}$), 95% confidence intervals are reported ($r^2 = 0.21$, $p < 0.05$). Data presented is up to and inclusive of 31st August. Closed symbols: water-stressed trees, open symbols: control trees, circular symbols: current year shoots, triangular symbols: previous year shoots.

A positive linear relationship was found between leaf water potential and R_w of shoots (Fig. 6). The three points of low R_w noted for the previous-year shoots are indicative of an increase in cavitation and embolism during the height of the drought (July–August, 1995).

4. DISCUSSION

Estimates of xylem cavitation, obtained by the ultrasonic acoustic emission method, are advantageous in that the technique is non-destructive and can be performed continuously, for extended periods, on the same plant organ. Thus it is able to reveal how real-time environmental variability affects plant water relations [17, 20]. However, the disadvantage of this technique is that it provides only semi-quantitative data as the effect of the number of emissions on hydraulic conductance and, hence, water transport sufficiency is difficult to estimate [17, 43]. Nevertheless, the UAE technique has previously been shown to measure the same phenomenon (xylem cavitation and embolism) both qualitatively and quantitatively [24] as the destructive hydraulic technique of Sperry et al. [38] for several species, including *Pinus sylvestris* [5]. Further evidence supporting the contention that UAE's originate from cavitation is the close agreement between total embolism, as measured by γ-radiation, and accumulated UAE's [6]. It is possible for acoustic signals to be produced from non-conducting tissues, such extraneous acoustic emissions most likely at the first stage of dehydration. However, this comparative study details the cumulative and peak (August) effects of drought, after 9 months of water deficit (Fig. 4), when diurnal refilling is least likely to have occurred and significant effects of water deficit were evident between treatments.

Two studies of acoustically monitored cavitation, in conifers under field conditions, have previously been reported: (1) Ikeda and Ohtsu [12] on 6-year-old *Pinus thunbergii*, and, (2) Jackson et al. [16] on a 39 year old *Pinus sylvestris*. In the present study, significant acoustic emissions in the branches began when the needle water potential (Ψ_L) was –0.7 to –1.0 MPa (Fig. 3), which is similar to the value of –1.1 to –1.2 for the lower bole reported by Jackson et al. [16], but is greater than –0.7 MPa reported for Scots pine seedlings by Peña and Grace [30]. No discernible lag was noted between branch and bole cavitation in this study. Cavitation was also found to occur throughout the season.

It is possible to estimate the loss of conducting pathway from the accumulated emissions, with some assumptions: a sensor listening volume of 3 cm^3 and a counting efficiency of 10% [34], and a value for tracheid packing of 13×10^6 cm^{-3} [26]. Over the course of the drought we recorded 0.6 million events (extrapolating for the periods when acoustic emissions were not monitored) which equates to 6 million actual events, from a listening volume containing 40 million tracheids. Over the period of the imposed drought this would result in a 15% reduction in the number of functional conduits, if no refilling occurred. There was independent evidence of a three-fold increase in the soil-to-leaf hydraulic resistance of the drought trees, in this study, at the height of the water-stress [15]. However, hydraulic resistance values from this site suggest that the majority of increased resistance occurred below ground. Root xylem is considered more vulnerable to cavitation [21] and, in conifers, it has been suggested that the majority of embolisms occur in small roots [39]. However, it is equally feasible that the increase in below ground resistance was a result of fine root mortality in the upper soil horizons. Therefore, it appears that although substantial numbers of cavitation events were recorded, a significant reduction in hydraulic conductivity, of above ground tissues, is unlikely to have occurred.

In conifers changes in R_w of tissues are predominantly as a result of cavitation in tracheids causing the formation of embolisms [2, 10]. Recent studies in Birch [42] and Douglas-fir have shown measurements of changes in R_w by Archimedes' are closely coupled to changes in hydraulic conductivity as evidenced by the "sperry" technique [38]. Furthermore, the storage capacity contribution to water status (in the crown) is less than 1% of RWC [8]. Previously a model of "hydraulic sufficiency", which accounts for tree hydraulic architecture and xylem vulnerability to water-stress induced cavitation, has predicted that xylem tensions could lead to a 5–30% loss of transport capacity without adverse affects on the soil-to-leaf hydraulic resistance [43]. This may be the result of spare conducting tissue capacity [18], be indicative of refilling or could be because, in this study, the soil resistance plays a dominant role in determining increases in the soil-plant-atmosphere-continuum (SPAC).

The apparent vulnerability to cavitation (Fig. 3) was essentially the same for the two treatments at the beginning and end of the drought period, but in August a significant treatment difference was observed. This suggests that water-stress had resulted in cavitation of the most vulnerable conduits, and led to a subsequent increase in the apparent vulnerability threshold of the system (i.e. remaining functional conduits had a higher threshold to cavitation). Previous experiments have shown that, within an individual, vulnerability to cavitation is a function of the maximum pit membrane pore dimensions [43]. This contrasts with the findings of Jackson et al. [15] and underlines that the response of trees may vary with environmental conditions and change with the severity and duration of the soil water deficit experienced.

Sapwood R_w was consistent with the previously reported values for *Pinus sylvestris* of Waring et al. [44], though lower values have been reported under dry summer conditions [16]. Whilst consistently lower in the water-stressed trees, no variation over the drought period was evident.

Although Ψ_{LPD} recovered in September, only a slight inflection was noted in soil moisture (Fig. 1) and it was a month later that R_w in the shoots was seen to recover (Fig. 5). Recently, several papers have shown that water uptake across the cuticle (*i.e.* aerial absorption) can be sufficient for a major increase of leaf and/or plant water potential (Ψ_L) [3, 19, 29]. In *Pinus sylvestris* the likely route for absorption is through the non-cuticularised surface below the needle sheath [22]. Our data, we tentatively suggest, support this theory, although the observed increase in Ψ_L could also be expected from a simple response to a reduction in transpiration (E), via stomatal closure.

Refilling of the shoot xylem was observed in September, when Ψ_{LPD} was –1.11 MPa. The mechanism of refilling is still unclear. In *Pinus sylvestris* studies have demonstrated embolism reversal in the laboratory [2, 9] and recovery of stem water content over winter has been demonstrated in the field [44]. The refilling of embolized vessels may be by osmotic pressure of the xylem sap from the adjoining parenchyma, the so called "vitalistic theory", first purported by Grace [10] and given weight by the results of Salleo et al. [33]. However, alternative hypotheses involving reverse osmosis [4] and non-osmotic active secretion of water [25] have been proposed. An active mechanism for refilling has, however, been disproved experimentally in *Pinus sylvestris* [2]. Several authors have challenged the cohesion-tension theory [4, 46] but it is generally supported by recent papers [14–16, 32] and several alternative mechanisms, supporting cohesion-tension theory whilst providing a theoretical construct for refilling, have been proposed [27, 48], which would allow reversal of embolism under conditions of tension within the soil-plant-atmosphere continuum (SPAC). Whatever the mechanism of refilling/recovery this study suggests that, in *Pinus sylvestris*, cavitation was not sufficient to perturb stem R_w, or affect overall plant hydraulic resistance, even after an extended period of soil water deficit. However, sequential dry years may result in the continuation of a decline in plant water status (G.E. Jackson, unpublished data). Thus the capacity of a tree species for avoidance of, or recovery from severe water deficits or successive years of drought may be of particular importance [11, 23]. It has been suggested that the cavitation of a proportion of the conducting tissue may be beneficial, with water being released to rehydrate leaves, and the associated increase in resistance inducing stomatal closure and reducing transpiration [18, 40, 45]. Scots pine avoided the occurrence of substantial xylem embolism by the regulation of stomatal conductance (through stomatal closure) thus limiting a significant decline in plant conductivity [15], and the mechanism did not involve long distance (root sourced) chemical signals [31]. We presume that the tree will respond in subsequent years to any loss of conducting tissue through altering resource partitioning.

In conclusion, cavitation, as measured by the ultrasonic acoustic technique, was found to be a regular occurrence, however, despite a prolonged period of imposed drought no "runaway cavitation" was observed [cf. 43]. From this study we suggest that any future change in precipitation levels and/or increases in radiation, if over a 12-month period, are unlikely to impose a serious threat to the success of conifer species. We cannot, however, discount the potential of several sequential years of drought having carry-over effects upon conifer health.

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REFERENCES

[1] Borghetti M., Raschi A., Grace J., Ultrasound emission after cycles of water stress in *Picea abies*, Tree Physiol. 5 (1989) 229–237.

[2] Borghetti M., Edwards W.R.N., Grace J., Jarvis P.G., Raschi A., The refilling of embolized xylem in *Pinus sylvestris*, Plant Cell Environ. 14 (1991) 357–369.

[3] Boucher J.-F., Munson A.D., Bernier P.Y., Foliar absorption of dew influences shoot water potential and root growth in *Pinus strobus* seedlings, Tree Physiol. 15 (1995) 819–823.

[4] Canny M.J., A new theory for the ascent of sap: cohesion supported by tissue pressure, Ann. Bot. 75 (1995) 343–357.

[5] Cochard H., Vulnerability of several conifers to air embolism, Tree Physiol. 11 (1992) 73–83.

[6] Dixon M.A., Grace J., Tyree M.T., Concurrent measurements of stem density, leaf water potential and cavitation on a shoot of *Thuja occidentalis*, Plant Cell Environ. 7 (1984) 615–618.

[7] Dixon H.H., Joly J., On the ascent of sap, Philos. Trans. R. Soc. Lon. Ser. Biol. Sci. 186 (1896) 563–576.

[8] Domec J.-C., Gartner B.L., Cavitation and water storage capacity in bole xylem segments of mature and young Douglas-fir trees, Trees 15 (2001) 204–214.

[9] Edwards W.R.N., Jarvis P.G., Grace J., Moncrieff J.B., Reversing cavitation in tracheids of *Pinus sylvestris* under negative water potentials, Plant Cell Environ. 17 (1994) 389–397.

[10] Grace J., Refilling of embolised xylem, in: Borghetti M., Grace J., Raschi A. (Eds.), Water transport in plants under climatic stress, Cambridge University Press, UK, 1993, pp. 52–62,

[11] Grace J., Consequences of xylem cavitation for plant water deficits, in: Smith J.A.C., Griffiths H. (Eds.), Water deficits: plant responses from cell to community, BIOS Scientific Publishers Ltd., Oxford, UK, 1993, pp. 109–127.

[12] Ikeda T., Ohtsu M., Detection of xylem cavitation in field-grown pine trees using the acoustic emissions technique, Ecol. Res. 7 (1992) 391–395.

[13] Innes J.L., Forest health: its assessment and status, CAB International, Wallingford, 1993, 677 p.

[14] Irvine J., Grace J., Non-destructive measurement of stem water content by time domain reflectometry using short probes, J. Exp. Bot. 48 (1997) 813–818.

[15] Irvine J., Perks M.P., Magnani F., Grace J., The response of *Pinus sylvestris* to drought: stomatal control of transpiration and hydraulic conductance, Tree Physiol. 18 (1998) 393–402.

[16] Jackson G.E., Irvine J., Grace J., Xylem cavitation in two mature Scots pine forests growing in a wet and a dry area of Britain, Plant Cell Environ. 18 (1995) 1411–1418.

[17] Jackson G.E., Grace J., Field measurements of xylem cavitation: are acoustic emissions useful? J. Exp. Bot. 47 (1996) 1643–1650.

[18] Jones H.G., Sutherland R., Stomatal control of xylem embolism, Plant Cell Environ. 14 (1991) 607–612.

[19] Katz C., Oren R., Schulze E.-D., Milburn J.A., Uptake of water and solutes through twigs of *Picea abies*, Trees 3 (1989) 33–37.

[20] Kikuta S.B., Lo Gullo M.A., Nardini A., Richter H., Salleo S., Ultrasound acoustic emissions from dehydrating leaves of deciduous and evergreen trees, Plant Cell Environ. 20 (1997) 1381–1390.

[21] Kolb K.J., Sperry J.S., Lamont B.B., A method for measuring xylem hydraulic conductance and embolism in entire root and shoot systems, J. Exp. Bot. 47 (1997) 1805–1810.

[22] Leyton L., Juniper B.E., Cuticle structure and water relations of pine needles, Nature 198 (1963) 770–771.

[23] Lebourgeois F., Becker M., Dendroecological study of Corsican pine in western France. Growth potential evolution during the last decades, Ann. Sci. For. 53 (1996) 931–946.

[24] Lo Gullo M.A., Salleo S., Three different methods for measuring xylem cavitation and embolism: a comparison, Ann. Bot. 67 (1991) 417–424.

[25] McCully M.E., Huang C.X., Ling L.E.C., Daily embolism and refilling of xylem vessels in the roots of field-grown maize, New Phytol. 138 (1998) 327–342.

[26] Mencuccini M., Grace J., Fioravanti M., Biomechanical and hydraulic determinants of tree structure in Scots Pine: anatomical characteristics, Tree Physiol. 17 (1997) 105–113.

[27] Milburn J.A., Sap ascent in vascular plants: challengers to the cohesion theory ignore the significance of immature xylem and the recycling of Munch water, Ann. Bot. 78 (1996) 399–407.

[28] Milburn J.A., Johnson R.P.C., The conduction of sap. II. Detection of vibrations produced by sap cavitation in *Ricinus* xylem, Planta 69 (1966) 43–52.

[29] Munné-Bosch S., Nogués S., Alegre L., Diurnal variations of photosynthesis and dew absorption by leaves in two evergreen shrubs growing in Mediterranean field conditions, New Phytol. 144 (1999) 109–119.

[30] Peña J., Grace J., Water relations and ultrasound emissions of *Pinus sylvestris* before, during and after a period of water stress, New Phytol. 103 (1986) 515–524.

[31] Perks M.P., Irvine J., Grace J., Canopy stomatal conductance and xylem sap abscisic acid (ABA) in mature Scots pine during a gradually imposed drought, Tree Physiol. 22 (2002) 877–883.

[32] Richter H., Water relations of plants in the field: some comments on the measurement of selected parameters, J. Exp. Bot. 48 (1997) 1–7.

[33] Salleo S., Lo Gullo M.A., De Paoli D., Zippo M., Xylem recovery from cavitation-induced embolism in young plants of *Laurus nobilis*: a possible mechanism, New Phytol. 132 (1996) 47–56.

[34] Sandford A.P., Grace J., The measurement and interpretation of ultrasound from woody stems, J. Exp. Bot. 36 (1985) 298–311.

[35] Sellin A., Hydraulic and stomatal adjustment of Norway spruce trees to environmental stress, Tree Physiol. 21 (2001) 879–888.

[36] Siau F., Flow in wood, Syracuse University Press, NY, USA, 1971.

[37] Sobrado M.A., Grace J., Jarvis P.G., The limits to xylem embolism recovery in *Pinus sylvestris* (L.), J. Exp. Bot. 43 (1992) 831–836.

[38] Sperry J.S., Donnelly J.R., Tyree M.T., A method for measuring hydraulic conductivity and embolism in xylem, Plant Cell Environ. 11 (1987) 35–40.

[39] Sperry J.S., Ikeda T., Xylem cavitation in roots and stems of Douglas-fir and white fir, Tree Physiol. 17 (1997) 275–280.

[40] Sperry J.S., Sullivan J.E.M., Xylem embolism in response to freeze-thaw cycles and water stress in ring-porous, diffuse-porous and conifer species, Plant Physiol. 100 (1992) 605–613.

[41] Spiecker H., Mielikäinen K., Köhl M., Skovsgaard J. (Eds.), Growth trends in European forests – studies from 12 countries, Springer-Verlag, Berlin, 1996, 372 p.

[42] Strati S., Patiño S., Slidders C., Cundall E.P., Mencuccini M., Development and recovery from winter embolism in silver birch: seasonal patterns and relationships with the phenological cycle in oceanic Scotland, Tree Physiol. 23 (2003) 663–673.

[43] Tyree M.T., Sperry J.S., Do woody plants operate near the point of catastrophic xylem dysfunction caused by dynamic water stress? Plant Physiol. 88 (1988) 574–580.

[44] Waring R.H., Whitehead D., Jarvis P.G., The contribution of stored water to transpiration in Scots pine, Plant Cell Environ. 2 (1979) 309–317.

[45] Zimmerman M.H., Xylem structure and the ascent of sap, Springer-Verlag, Berlin, 1983, 143 p.

[46] Zimmerman V., Meinzer F., Bentrup F.W., How does water ascend in tall trees and other vascular plants? Ann. Bot. 76 (1995) 541–551.

[47] Zwieniecki M.A., Holbrook N.M., Diurnal variation in xylem hydraulic conductivity in white ash (*Fraxinus americana* L.), red maple (*Acer rubrum* L.) and red spruce (*Picea rubens* Sarg.), Plant Cell Environ. 21 (1998) 1173–1180.

[48] Zwieniecki M.A., Melcher P.J., Holbrook N.M., Hydrogel control of xylem resistance in plants, Science 291 (2001) 1059–1063.