

Stomatal regulation and xylem cavitation in Clementine (*Citrus clementina* Hort) under drought conditions

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SUMMARY

In many trees species, a strong relationship exists between stomatal closure and the onset of cavitation in xylem vessels. This relationship was investigated in a *Citrus* species. Young potted 'Clementine' (*Citrus clementina* Hort 'Clementine') grafted on 'Carrizo Citrange' (*Citrus sinensis* × *Poncirus trifoliata*) or 'Trifoliolate Orange' (*Poncirus trifoliata* Raf), and grown under greenhouse conditions, were submitted to periods of drought by withholding irrigation. Pre-dawn water potential, leaf stomatal conductance, plant transpiration, the degree of xylem embolism, and xylem vulnerability curves were measured. Transpiration and stomatal conductance were reduced to a minimum value when the pre-dawn water potential reached -1 MPa. This value corresponded to the threshold pressure below which cavitation was induced. Higher intensities of water stress provoked more cavitation, but the degree of xylem embolism, as measured by percentage loss of conductivity, always remained below 50%. 'Clementine' is therefore vulnerable to embolism, but early stomatal regulation prevents the occurrence of embolism. Under severe water stress, 'Clementine' is able to maintain functional xylem vessels, which probably enhances its survival during periods of intense drought, and favours its recovery. This suggests that xylem cavitation is a key process in understanding the response of *Citrus* to drought and, hence, is a promising criterion by which to screen for more drought-tolerant genotypes.

Water availability is a major environmental constraint that limits the expansion of fruit orchards in the Mediterranean area. There is an increasing demand for more drought-resistant genotypes and this demand will intensify if the frequency and intensity of drought episodes increase in the future. Therefore, it is essential to determine the key physiological processes that control drought resistance in order to identify genotypes that are more resistant, or to provide sound screening criteria for fruit tree breeders.

During the last two decades, research on xylem embolism has considerably improved our understanding of tree responses to drought. In trees, water is transported under tension (negative pressure) in the xylem vessels (i.e., in a physically metastable condition). Under drought conditions, the tension in the conduits increases and water vaporisation (cavitation) can occur. Cavitation causes an embolism (i.e., an interruption of the sap flow in the conduits) that limits the supply of water to leaves and other organs. Cavitation occurs only when the xylem pressure drops below a specific threshold value (ψ_{cav}) determined by pit wall porosity (Tyree and Sperry, 1989). Tree species differ widely in their susceptibility to cavitation, and it has been demonstrated that a higher resistance to cavitation confers a higher tolerance to water deficit (Pockman and Sperry, 2000). Furthermore, strong relationships

exist between stomatal function and tree hydraulics. Indeed, under drought conditions, plants respond by closing their stomata, which reduces transpiration and keeps the xylem potential above the cavitation threshold value, which therefore prevents the occurrence of embolisms (Tyree and Sperry, 1988; Jones and Sutherland, 1991; Cochard *et al.*, 1996; 2002; Cruiziat *et al.*, 2002; Salleo *et al.*, 2000; Froux *et al.*, 2004). However, such behaviour has been documented mostly in forest tree species. The information available for fruit tree species is still very limited. To our knowledge, studies on cavitation resistance in *Citrus* species have not yet been reported.

The first objective of this study was to document, for the first time, the vulnerability of xylem to cavitation in *Citrus* 'Clementine' scions. A second objective was to test the hypothesis of a correlation between xylem cavitation and stomatal conductance in 'Clementine'.

MATERIALS AND METHODS

Plant materials and experimental conditions

Two- and 3-year-old 'Clementine' saplings (*Citrus clementina* Hort) were used in this study. Clementine was grafted onto 16-month-old 'Carrizo Citrange' (*C. sinensis* × *Poncirus trifoliata*) and onto 'Trifoliolate Orange' (*P. trifoliata* Raf) seedlings, two rootstocks frequently used by growers in Corsica. Plants were grown in 18 l containers, in a substrate composed of 60:30:10 (v/v/v) peat, sand and scoria (volcanic soil), under greenhouse

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conditions at San Guiliano Research Station (Corsica, France). Pots were covered with a plastic film and an aluminium sheet to limit evaporation from the soil.

Two replicates of the same experiment each involving 16 trees were performed during the Summer period in one year. Plants were first irrigated to field capacity. Control saplings ($n = 4$ for each rootstock) were weighed every 2 d and water was added to compensate for water loss by transpiration and, hence, were maintained near field capacity. Experimental saplings ($n = 4$ for each rootstock) were exposed to drought by withholding irrigation. Experiment 1 was conducted in July 2002 on 'Clementine' saplings that had been grafted 3 years earlier. Irrigation was stopped for 9 d for the experimental plants. Experiment 2 was conducted in September 2002 on 2-year-old saplings, with irrigation stopped for 17 d for the experimental trees. The two experiments yielded similar results.

Pre-dawn leaf water potential, leaf stomatal conductance, and plant transpiration

Pre-dawn leaf water potential (ψ_b) was determined on a daily basis using a pressure chamber (Scholander *et al.*, 1965). Measurements were performed before sunrise, on two mature leaves per tree. Leaf stomatal conductance (g_s) was measured with an ADC Leaf Chamber Analyser LCA4 (Delta-T Devices Ltd., Cambridge, UK; Experiment 1) or with an AP4 Porometer (Delta-T Devices Ltd., Cambridge, UK; Experiment 2). The values recorded by the two instruments were comparable. Measurements were made on a daily basis, between 13.00–15.00 h, on three mature leaves per tree, marked at the start of each Experiment. Daily plant transpiration (T) was determined gravimetrically (± 5 g). Relative stomatal conductance and transpiration in the experimental trees were calculated by dividing g_s and T by the mean values for the control trees (Améglio *et al.*, 1999).

Xylem embolism and vulnerability curves

A xylem vulnerability curve represents the relation between sap tension and the degree of embolism in the xylem conduits. The curves were obtained using the air pressure technique (Cochard *et al.*, 1992) which involves dehydrating excised branches (1-year-old; 0.80 m long) in a large pressure chamber. Shoots were enclosed in the chamber with the cut-end protruding. The air pressure in the chamber was set to a predefined value (between 0.4–4.0 MPa) and kept constant for 1–4 h until sap stopped exuding from the cut-end. The pressure was then released and the dehydrated shoots were kept for at least 4 h in airtight bags to allow air equilibration in the embolised conduits. The degree of embolism in the shoot was then measured as described below.

Embolism was measured *via* its effect on the loss of xylem conductance (Sperry *et al.*, 1988) using a XYL'EM apparatus (Bronkhorst, Montigny les Cormeilles, France). Shoot segments (3 cm long) were excised under water, to prevent air entering into the vessels, and connected to the XYL'EM apparatus. Using the air infiltration method (Cohen *et al.*, 2003) we determined that the maximum vessel length was about 45 cm in *Citrus*. Therefore, segments were excised at least 45 cm from the shoot base. For each shoot, embolism levels

were obtained for five segments. The initial conductivity (K_i ; $\text{mmol m s}^{-1} \text{MPa}^{-1}$) of each segment was measured with a hydrostatic pressure gradient of 3 kPa using deionised, degassed and ($0.2 \mu\text{m}$) filtered water. Segments were then perfused at a pressure of 0.15 MPa for 5 min to dissolve and expel bubbles. Segment hydraulic conductivity was determined again, and flushes were repeated until a maximum conductivity, K_m , was obtained. The percentage loss of xylem conductivity (PLC) was calculated according to the formula:

$$PLC(\%) = 100(K_m - K_i)/K_m$$

Similar segments from the same dehydrated shoots were sampled as described above and perfused with 1 g l^{-1} phloxine B (Sigma-Aldrich, Saint Quentin Fallavier, France) at 3 kPa in order to stain the remaining functional vessels.

RESULTS

Drought treatment had a strong effect on water relations and gas exchange in 'Clementine'. The two rootstock varieties that we evaluated yielded similar results in this study. The mean time-courses of pre-dawn leaf water potential (ψ_b), and transpiration rate are reported in Figure 1 for Experiment 2. The results of

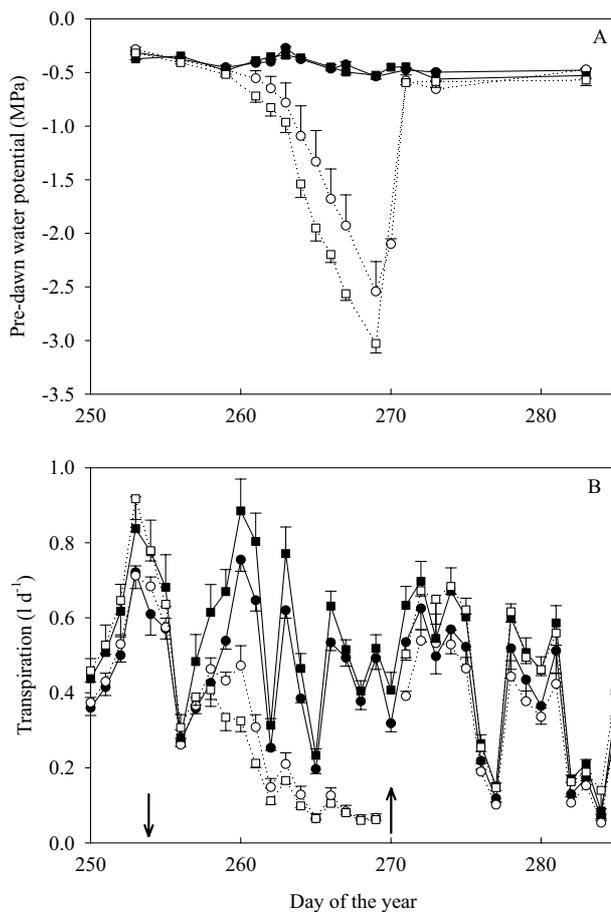


FIG. 1 Time-course of pre-dawn leaf water potential (Panel A), and transpiration rate (Panel B) of control plants (closed symbols) and water-stressed plants (open symbols) of 'Clementine' grafted onto 'Trifoliolate Orange' (circles) or 'Carrizo Citrange' (squares). Error bars represent \pm SE. Arrows indicate the onset and end of the drought period.

Experiment 1 were similar. For control plants, ψ_b was constant and remained above -0.5 MPa for the whole period. Transpiration in control plants remained high, but was substantially reduced during cloudy days. One week after irrigation was stopped, the ψ_b of stressed plants decreased steadily, down to -2.5 MPa. Simultaneously, plant transpiration was strongly reduced. At the end of the drought period, transpiration in the stressed plants was less than 20% of that in control plants. Relative stomatal conductance was highly correlated with relative plant transpiration in this study (Figure 2). Hence, g_s followed very similar patterns to T . At the end of the drought period, the plants were wilted and the leaves had started to roll. ψ_b , g_{ss} and T values for control and stressed plants were similar 1–2 d after the droughted plants were re-watered.

The natural level of xylem embolism in control plants was between PLC values of 15–20%. A significant increase in PLC values was detected for xylem pressures below -1.0 MPa (Figure 3). Lower pressures significantly increased PLC values, but a distinct plateau at 45% was observed for xylem pressure in the range of -2 MPa to -4.0 MPa. ‘Clementine’ grafted on the two different rootstocks showed similar vulnerability curves. Dye staining of samples pressurised at 2.5, 3.75 or 4 MPa showed distinct areas with coloured (functional) and uncoloured (embolised) vessels. However, coloration was not associated with any particular position in the ring (i.e., early-wood vs. late-wood), or correlated with vessel size.

DISCUSSION

The main objectives of this study were to document the vulnerability of xylem to embolism in grafted *Citrus* plants and to test the hypothesis of a correlation between cavitation and stomatal regulation during water stress. The effects of soil drought on pre-dawn leaf water potential and stomatal conductance reported in this study were consistent with previous observations in other *Citrus* species such as orange (Ferreira *et al.*, 1979; Savé *et al.*, 1995), tangor (Savé *et al.*, 1995), lemon (Ruiz-Sanchez *et al.*, 1997), and mandarin (Yakushiji and

Morinaga, 1998). Stomatal closure during water stress in *Citrus* species seems to occur for ψ_b values between -1.0 MPa and -2.5 MPa, which may classify these species as being relatively sensitive to drought. This can be related to the sub-tropical and tropical areas of native *Citrus* in eastern Asia.

As far as we know, the vulnerability of xylem to cavitation has not yet been reported in *Citrus* species, therefore direct comparisons are not possible. Compared to other woody species (Maherali *et al.*, 2004), xylem vessels in ‘Clementine’ are relatively sensitive to cavitation. Similar sensitivities have been reported for hygrophilous species such as poplars or willows (Cruziat *et al.*, 2002). The pattern of xylem cavitation in ‘Clementine’ was consistent with its stomatal behaviour during water stress. When the cavitation and stomatal data were plotted on the same graph (Figure 3), it was clear that the stomata were almost closed at the onset of xylem cavitation. This validates our hypothesis of a correlation between xylem cavitation and stomatal closure in ‘Clementine’.

As ‘Clementine’ is highly sensitive to cavitation, it is likely that genotypes with xylem vessels more resistant to embolism would perform better under drought conditions. It can be predicted that such genotypes would either close their stomata at a higher water stress level, which could improve their growth performance under drought conditions, or they would be less exposed to the damaging effects of cavitation. The screening of a larger number of *Citrus* genotypes for cavitation resistance, and experimental confirmation under field conditions are required to substantiate these hypotheses.

Vulnerability curves for ‘Clementine’ cultivars exhibited an unusual pattern. Most woody species documented to date have clear sigmoid-shaped curves, with PLC values steadily reaching 100% with decreasing xylem pressure. In *Citrus*, we showed that PLC values levelled-off at 45% at -2 MPa, with no visible variation when the xylem pressure was decreased further to -4.0 MPa. It is probable that PLC values increase at more negative values, but we were not able to document this part of the curve with our equipment. A similar

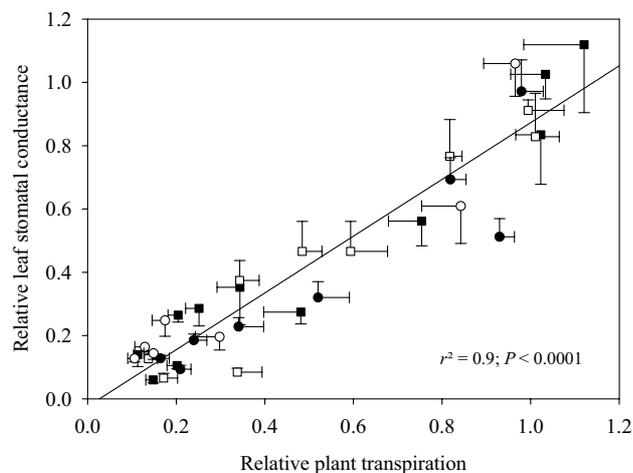


FIG. 2

Relationship between relative stomatal conductance and relative transpiration in ‘Clementine’ grafted onto ‘Trifoliate Orange’ (open symbols) or ‘Carrizo Citrange’ (closed symbols) during Experiment 1 (circles) and Experiment 2 (squares). Error bars represent \pm SE.

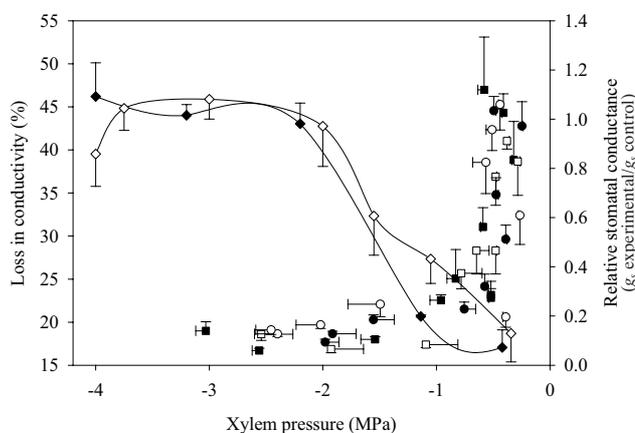


FIG. 3

Vulnerability curves (plain lines), and relative transpiration (points) vs. xylem pressure, measured on ‘Clementine’ grafted on ‘Trifoliate Orange’ (open symbols) and ‘Carrizo Citrange’ (closed symbols) during Experiment 1 (circles) and Experiment 2 (squares). Error bars represent \pm SE.

vulnerability curve was obtained using the centrifuge technique described by Cochard *et al.* (2005) on *Citrus* plants grown in a greenhouse in Clermont-Ferrand, Central France (data not shown). This would suggest that xylem tissue in 'Clementine' consists of two compartments having similar hydraulic conductances, but with different vulnerabilities to cavitation. A similar structure exists in ring-porous species such as oak (Cochard and Tyree, 1990) and many gymnosperms (Sperry and Tyree, 1990), where large conduits in the early-wood tend to cavitate before smaller conduits in the late-wood. However, for these species, the difference in cavitation is small between compartments and the whole xylem vulnerability curve remains sigmoid. Unexpectedly, dye staining of *Citrus* samples with a PLC value of 45% did not reveal any striking compartmentation. This behaviour could therefore represent a new plant strategy to cope with drought. A highly vulnerable compartment would trigger stomatal closure during moderate water stress, while a more cavitation-resistant compartment would help the plant to

survive episodes of severe drought, or to recover more rapidly. In our experiments, water-stressed plants showed substantial symptoms of drought such as leaf-rolling or wilting. However, the plants recovered well from the effects of drought in less than 2 d after rehydration. Similar results have been reported in orange by Fereres *et al.* (1979). These results suggest that 'Clementine' can resist cavitation under severe water stress. The fact that half of the xylem conduits were presumably still functional at the end of the drought period probably contributed to this rapid recovery.

In conclusion, some xylem conduits in 'Clementine' are highly vulnerable to cavitation. During water stress, early stomatal closure was observed, which had the effect of preventing xylem embolism. More severe drought conditions induced cavitation events, but the degree of embolism (PLC) always remained below 50%. Our results suggest that xylem cavitation is a key process to understand the response of *Citrus* to drought and, hence, is a promising criterion for screening for more drought-tolerant genotypes.

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