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Changes in stem and leaf hydraulics preceding leaf shedding in *Castanea sativa* L.

S. SALLEO*[1], A. NARDINI*, M.A. LO GULLO** and L.A. GHIRARDELLI*

*Dipartimento di Biologia, Università di Trieste, Via L. Giorgieri 10, I-34127 Trieste, Italia**
Dipartimento di Scienze Botaniche, Università di Messina,
*Salita Sperone 31, I-98166 Messina S. Agata, Italia***

Abstract

This paper describes changes in leaf water status and in stem, petiole and leaf blade hydraulics preceding leaf senescence and shedding in *Castanea sativa* L. (chestnut). Measurements of maximum diurnal leaf conductance to water vapour (g_L), minimum water potential (ψ_L), hydraulic conductance per unit leaf surface area of stems (K_{SL}), petioles (K_{PL}) and leaf blades (K_{LL}) and number of functional conduits and inside diameter distribution were performed in June, September and October 1999. In September, still green leaves had undergone some dehydration as indicated by decreased g_L (by 75 %) and ψ_L with respect to June. In the same time, K_{SL} decreased by 88 %, while K_{PL} and K_{LL} decreased by 50 % and 20 % of the conduits of stems and 10 % of the petioles (all belonging to the widest diameter range) were no longer functioning, causing a decrease in the theoretical flow by 82 % in stems and 27 % in petioles. Stem xylem blockage was apparently due to tyloses growing into conduits. We advance the hypothesis that the entire process of leaf shedding and winter rest may be initiated by extensive stem embolism occurring during the summer.

Additional key words: chestnut, xylem embolism, hydraulic conductance, leaf water status, HPFM.

Introduction

Physiological leaf shedding is the final result of a pre-programmed sequence of biochemical and anatomical events leading to the progressive disorganization of leaf tissues and to the physical isolation of leaves from the plant (*e.g.* Smart 1994, Buchanan-Wollaston 1997, Inada *et al.* 1998, 1999). Precocious leaf shedding can also be induced by some abiotic stresses like drought and freeze stress (Lin and Kao 1998, Nardini *et al.* 1998a), both of them causing cavitation-induced xylem embolism. Among the possible biophysical mechanisms that can initiate the hydraulic isolation of senescing leaves from the plant, one of the best candidates can, therefore, be expected to be xylem cavitation which is known to impair water supply to leaves, decrease the stomatal conductance (Sperry *et al.* 1993, Salleo *et al.* 2000) and photosynthesis (Tyree and Sperry 1989, Aasamaa and Sõber 2001), all these changes typically occurring in coincidence with leaf senescence. Recently, it has been recognized that any plant organ is potentially subject to cavitate including root (Sperry and Ikeda 1997, Kavanagh *et al.* 1999) and leaf veins (West and Gaff 1976, Kikuta *et al.* 1997, Salleo *et al.* 2001). The progressive hydraulic isolation of leaves from the plant is generally expected to be caused by stem, petiole or even leaf blade cavitation. However, previous studies by some of us (Nardini *et al.* 1998c) conducted on a number of forest trees like *Acer campestre* L. and *Castanea sativa* L. had showed that root hydraulic conductance (K_R) decreased in August with respect to that measured in May by over 50 %. In the presence of high transpiration rates like those typically occurring in the summertime, a significant decrease of the hydraulic conductance of shoots (K_S) was also measured which was interpreted as induced by extensive xylem cavitation.

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Abbreviations: ψ_L - leaf water potential; g_L - leaf conductance to water vapour; HPFM - high pressure flow meter; K_{LL}, K_{PL}, K_S, K_{SL} - hydraulic conductance per unit surface area of leaf blades, petioles, shoots and stems, respectively; K_R - root hydraulic conductance; VLS - visible leaf senescence.

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[1] Corresponding author; phone (+390) 40 6763875, fax (+390) 40 568855, e-mail: salleo@univ.trieste.it

The present study was aimed at investigating: *a*) whether leaf shedding is preceded by significantly altered hydraulics in the most peripheral parts of the crown like young stems and leaves; *b*) if the major changes in the efficiency of water transport preceding winter rest occur in the stem, petiole or leaf blade; *c*) the time course of hydraulic changes in the distal parts of the crown occurring between late summer and autumn. To this purpose, studies were conducted on *Castanea sativa* L. because field observations of the time course of leaf shedding had shown that leaf senescence and shedding proceeded rather gradually in this species, thus allowing an easier discrimination of the different phases.

Materials and methods

All the experiments were conducted on stems and leaves of the current year growth, collected on a tree of *Castanea sativa* L. over 30 years of age, growing in the Botanical Garden of the University of Trieste (Northeastern Italy) at about 120 m above the sea level. This plant was about 7 m tall with a trunk diameter of about 0.4 m and received no irrigation. Measurements were performed during the second half of June 1999, when leaf expansion and stem growth had been completed, and were repeated during the second half of September and October of the same year. In September, all the leaves appeared to be green and healthy. One month later, about one third of the leaves showed yellowish spots on increasing areas of the leaf blade. Leaf shedding started by the second half of November and was completed the early days of December (see Fig. 1 for year course of mean air temperatures and precipitation, data from the Oceanography and Meteorology Section of the Department of Earth Sciences, University of Trieste). When leaves began to discolour, *i.e.*, yellowish spots appeared on the leaf blade (we will call this phenomenon "visible leaf senescence", VLS, hereafter), the different levels of VLS were estimated by covering leaves with transparent millimeter paper. The entire leaf surface area was divided into 10 × 10 mm squares which were considered as green or yellow depending on the prevailing leaf colour. Leaves were then measured for total leaf surface area (see below) and the discoloured fraction of the leaf surface area was expressed in percentage of the total.

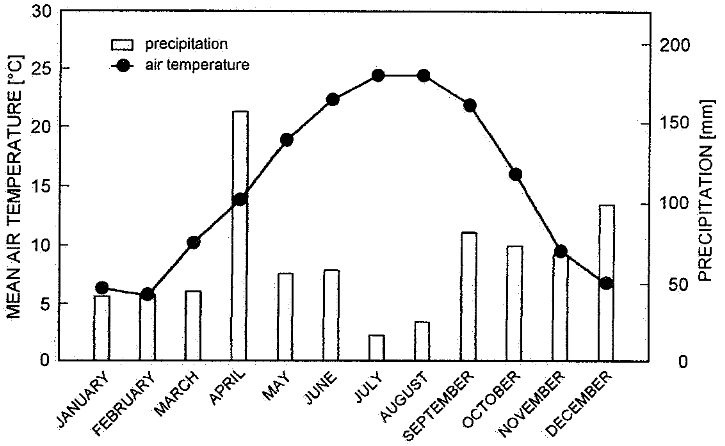

Fig. 1. Precipitation and mean air temperatures recorded during the year 1999 in Trieste. Data from the Oceanography and Meteorology Section of the Department of Earth Sciences, University of Trieste.

To estimate changes in the leaf water status before and at the beginning of VLS, leaf conductance to water vapour (g_L) and water potential (ψ_L) were measured in the field using a steady state porometer (*LI-1600*, *LiCor*, Lincoln, USA) and a portable pressure chamber (*Soilmoisture mod. 3050*, *Soilmoisture Equipment Corp.*, Santa Barbara, USA) for g_L and ψ_L measurements, respectively. Maximum diurnal g_L and minimum ψ_L were measured between 10:00 and 14:00 in two different days (the 16th and 18th of June, September and October) on green leaves (Vertovec *et al.* 2001). The average g_L and ψ_L values were obtained from at least 10 measurements, *i.e.* two measurements per hour within the pre-established time interval (see above).

Hydraulic measurements were all performed using the High Pressure Flow Meter (HPFM) technique, first introduced by Tyree *et al.* (1993) and described in detail elsewhere (Tyree *et al.* 1995). Briefly, the HPFM consists of an apparatus allowing the perfusion of distilled water filtered to 0.1 µm under pressure into the base of a cut leafy shoot while measuring the corresponding flow

(Nardini and Tyree 1999). In our case, leafy shoots were cut off in the field while immersed in distilled water. They were transported to the laboratory with their cut end immersed in water and immediately connected to the HPFM. The instrument was used in the "steady-state mode", *i.e.*, maintaining constant the pressure applied and measuring the corresponding flow. The pressure was increased to 0.3 MPa at a rate of 5 kPa s^{-1}. After about 30 min, leaves appeared to be completely infiltrated with water as indicated by their change in colour (leaves became dark green) and water dripping from the stomata. Under these conditions, the flow was fairly stable.

The hydraulic conductance (K) of leafy shoots was calculated as the ratio of the recorded flow (F) to the pressure difference applied (ΔP). Then, all the leaf blades were removed and the new K (of stems + petioles) was remeasured. Finally, the petioles were cut off at the petiole-to-stem junction and K of the leafless stem was measured. Because stems, petioles and leaves are serial to one another, their resistances (R = 1/K) are additive:

$$R_{\text{leaves}} = R_{\text{leafy shoot}} - R_{\text{shoot minus leaves}}$$

$$R_{\text{petiole}} = R_{\text{shoot minus leaves}} - R_{\text{shoot minus leaves and petioles}}$$

After each experiment, total leaf surface area (ΣA_L) of each shoot was measured using a Leaf Area Meter (*LI-3000A*, *LiCor*, Lincoln, USA). All K values were normalized by dividing them by ΣA_L, thus obtaining K per unit leaf surface area (Tyree *et al.* 1993, 1998). Five to seven leafy shoots were measured for K in June and September. In October, hydraulic measurements were repeated on about 30 shoots bearing leaves at different levels of VLS (see above).

Anatomical measurements included countings of the total number of conduits per section and of conduit inside diameters in stems and petioles collected in June and September 1999 *i.e.* on leaves without any visible symptom of senescence. These were aimed at getting information of eventual changes in the diameter distribution of functioning xylem conduits just before VLS. Five leafy shoots were selected in the two pre-established months (June and September). The third and fourth most proximal internodes of stems and at least two petioles per shoot were cut off and fixed in glutaraldehyde (4 %), embedded in resin and cross-sectioned using an ultramicrotome (*Ultratome III*, *LKB Vertriebs*, Vienna, Austria). Internodes and petioles were cross-sectioned at their middle plane and sections 1 μm thick were observed under a light microscope. The number of potentially conducting conduits (*i.e.* conduits not containing any solid material) was also recorded, together with their inside diameters. Conduit diameters were measured either directly if they appeared circular in shape or by averaging their axes if they were elliptical. Conduits below 10 μm in diameter were excluded from recordings because they looked very similar to fibres and contribute very little to the total flow, according to the Hagen-Poiseuille's equation. In September, several conduits appeared to contain tyloses either just entering conduits or already occluding them. Because tyloses are known to enter xylem conduits after they remain embolized for several hours (Zimmermann 1983, Cochard and Tyree 1990), all conduits containing tyloses were considered as no longer conducting even if they were not completely clogged. At least two sections per internode and per petiole were observed and measured for conduit number and diameters.

Results

Changes in leaf water status and in leaf and stem hydraulics: Leaves measured for g_L in September, showed a pronounced drop in this variable with respect to June although they appeared seemingly healthy and without any visible symptom of senescence (Fig. 2). In particular, g_L measured in June was of the order of about 195 mmol m^{-2} s^{-1} while in September g_L was as low as about 50 mmol m^{-2} s^{-1} and one month later, g_L remained at an approximately similar level. Extensive stomatal closure was apparently due to some leaf dehydration as suggested by the corresponding decrease in the leaf minimum diurnal ψ_L (from about -1.3 to -2.0 MPa, Fig. 2). In October, ψ_L was higher (less negative) than in September, probably due to several rainy days preceding measurements (Fig. 1). The recorded decrease in the leaf water status was paralleled by a dramatic drop in the hydraulic conductance (K) of stems (K_{SL}, Fig. 3) which was only about 2.5 × 10^{-4} kg s^{-1} m^{-2} MPa^{-1} in September in comparison with that measured in June when K_{SL} was of the order of 21.5 × 10^{-4} kg s^{-1} m^{-2} MPa^{-1}. In October, K_{SL} decreased further to about 1.2 × 10^{-4} kg s^{-1} m^{-2} MPa^{-1}. A similar but less impressive drop in K was recorded in petioles (K_{PL}) and leaf blades (K_{LL}) *i.e.* from 46 to 21 × 10^{-4} kg s^{-1} m^{-2} MPa^{-1} in the former and from 0.8 to 0.4 × 10^{-4} kg s^{-1} m^{-2} MPa^{-1} in the latter (Fig. 3).

When leaf colour started to switch from green to yellowish (October), K was measured of stems, petioles and leaf blades at increasing levels of VLS estimated as described above, so that further changes in K_{SL}, K_{PL} and K_{LL} could be related to the degree of leaf senescence (Fig. 4). Both stems and leaf blades did not undergo any further significant change in their K during progressive chlorophyll breakdown. On the contrary, petioles whose K_{PL} was still about 21 × 10^{-4} kg s^{-1} m^{-2} MPa^{-1} until leaves were green, showed a further critical drop in this variable that tended to near zero when 20 to 30 % of leaf surface area became discoloured (Fig. 4). The correlation coefficient (r^2) of the relationship of K_{PL} to the degree of VLS was in fact, as high as over 0.95. In other words, the complete blockage of the petiole as a water path occurred in coincidence with the beginning of chlorophyll breakdown.

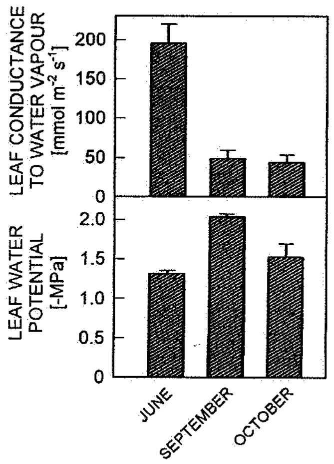

Fig. 2. Maximum diurnal leaf conductance to water vapour (g_L) and minimum diurnal leaf water potential (ψ_L) of still green leaves of *C. sativa*, collected in June, September and October 1999. Means ± SE (*vertical bars*), $n = 10$.

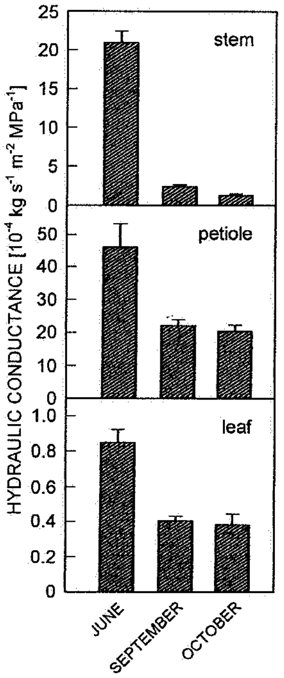

Fig. 3. Hydraulic conductance (K) per unit leaf surface area of stems, petioles and leaves of *C. sativa*, collected in June, September and October 1999. Means ± SE, $n = 7$. Leaves measured for K were all green and without visible symptoms of senescence.

Anatomical changes: In June, about 500 conduits per section were counted in stems and petioles (Table 1). In September, about 20 % of stem conduits and about 10 % of conduits in petioles contained tyloses (Fig. 5) either just entering conduits or clogging them completely and containing solid materials. Because the number of conduits per section resulted to be very different even in seemingly similar stems and petioles, differences in this variable recorded between June and September, resulted to be not statistically significant. Much more significant, however, were differences in the conduit diameter distribution recorded in September with respect to June in stems (Fig. 6). In June, conduits wider than 40 μm in diameter were about 13 % while in September such a fraction decreased to only about 5 % and conduits between 31 and 40 μm in diameter decreased from 16 to 10 % from June to September. In the case of petioles (Fig. 6), changes in the conduit diameter distribution were less evident but still detectable. Widest conduits in petioles (31 to 40 μm in diameter), in fact, decreased from 4 % in June to about 2 % in September i.e. from about 17 to 8 conduits per section. On the basis of the number of potentially functioning conduits per section and of their inside diameters, it was possible to estimate the theoretical hydraulic conductance of stems and

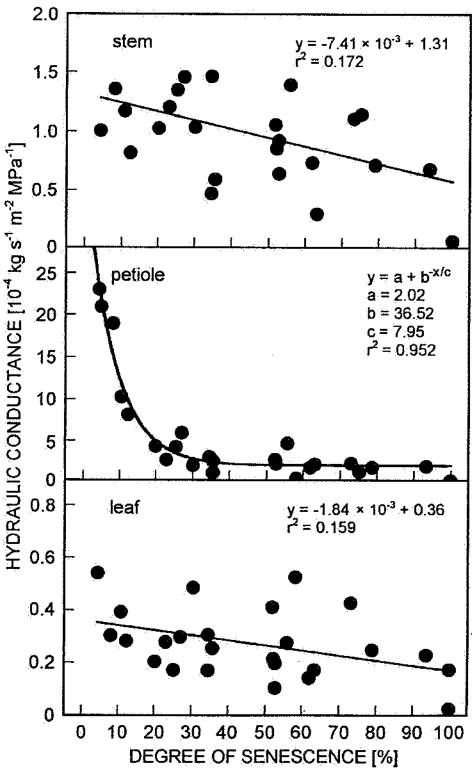

Fig. 4. Hydraulic conductance (K) per unit leaf surface area of stems, petioles and leaves of *C. sativa*, collected in the second half of October 1999 at increasing degrees of leaf senescence as estimated in terms of percentage of the area of yellowish spots on leaves, with respect to total leaf surface area. The *solid lines* and *curve* are regressions (equation and regression are reported in figures).

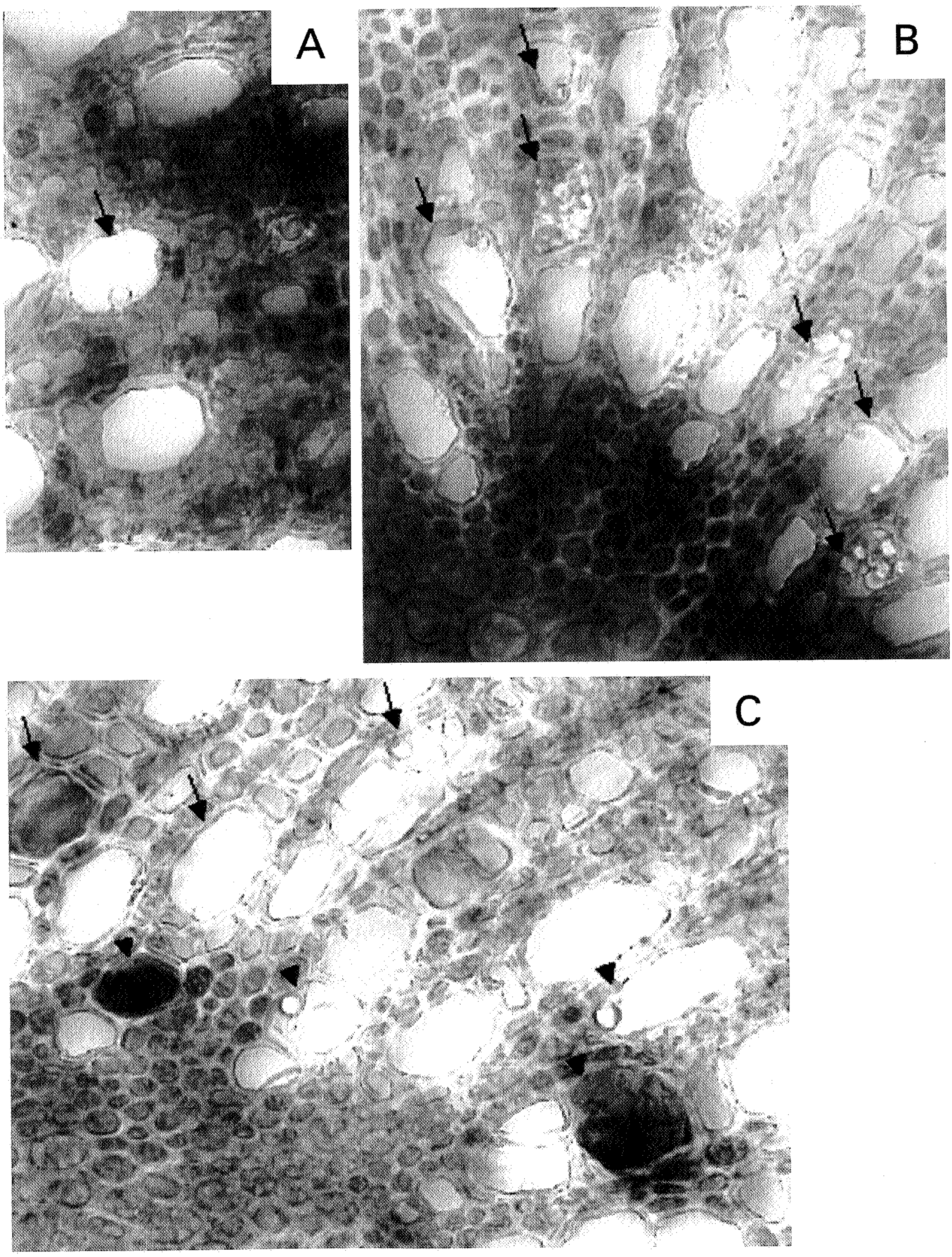

Fig. 5. Cross-sections of xylem in stems of the current year growth of *C. sativa* collected in September 1999. *Arrows* indicate vessels containing one (*A*) or more (*B*) tyloses as well as vessels completely clogged by tyloses containing solid materials (*C*) (×240).

petioles by calculating the theoretical flow as proportional to $\Sigma\pi r^4$ (where r is the conduit inside radius), according to the Hagen-Poiseuille's equation. In June, $\Sigma\pi r^4$ (Fig. 7) as calculated for stems was of the order of 7.8×10^7 µm^4 while in September it decreased to about 1.4×10^7 µm^4 *i.e.* by about 82 %. Also petioles showed a smaller but still significant decrease in $\Sigma\pi r^4$ between June and September, from 0.55 to 0.4×10^7 µm^4 *i.e.* by about 27 %.

Table 1. Number of conduits per section with diameter more than 10 µm as counted on stems and petioles of the current year growth. Means ± SE (*n* = 10).

	Stem	Petiole
June	499 ± 70	440 ± 57
September	403 ± 58	394 ± 40

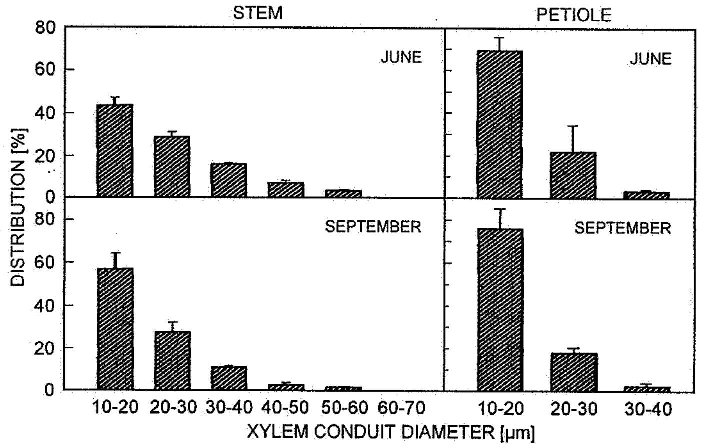

Fig. 6. Xylem conduit diameter distribution in stems and petioles of *C. sativa* collected in June and September 1999. Means ± SE, *n* = 5.

Discussion

In September, chestnut leaves showed no visible change in their general appearance. Nonetheless, they had already undergone pronounced alterations in their water status and kinetics of gas exchange. Leaf water potential had become substantially more negative and stomatal opening was strongly reduced (by 75 %, Fig. 2). Such a decrease in the leaf water status was apparently caused by the extensive blockage of the water conducting system of stems whose hydraulic conductance (K_{SL}) had undergone a loss of about 88 % (Fig. 3). This was apparently due to conduit clogging by tyloses (Fig. 5) that had invaded the widest conduits (Fig. 6) that contribute most to the xylem flow (Fig. 7). *C. sativa* is well known to produce tyloses under a variety of environmental stimuli including winter rest and different stresses (*e.g.* Fahn 1990). This species is a ring-porous tree whose early-wood conduits are lost every year (Zimmermann 1983). New early-wood is produced next spring somewhat later than it is in diffuse-porous species (Zimmermann 1983, Carlquist 1988) so that the early phases of sprouting and leaf expansion are sustained by the water and nutrient supply provided by the late-wood produced during the two to three previous years. This, however, is not enough conductive to supply fully expanded transpiring leaves. In a similar way, the clogging of a fraction of the stem widest conduits like that recorded in September, was likely to have caused the recorded leaf dehydration and stomatal closure. The hydraulic conductance of petioles as measured in September, was about 50 % of that measured in June (Fig. 3), *i.e.* the their K loss was less large than that measured in stems. This could be due to the fact that conduits in the petioles are, on average, much narrower than those in the stem (Fig. 6). A clear relationship has been reported to exist between conduit diameter and vulnerability to cavitation (*e.g.* Salleo and Lo Gullo 1986) at least in one species. Our interpretation of the

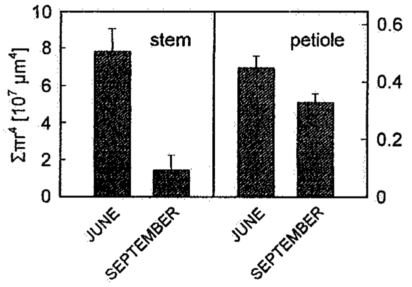

Fig. 7. Theoretical flow measured in stems and petioles collected in June and September 1999, as proportional to $\Sigma\pi r^4$ where r is the conduit radius. Means ± SE, *n* = 5.

lower hydraulic damage recorded in petioles and leaf veins with respect to stems is that water potentials developing in the formers were not enough negative for causing substantial cavitation and embolism in the majority of their conduits whose diameters were less than 20 μm (Fig. 6). As a consequence, the K loss recorded in petioles (and leaf veins) in September, was reasonably only a fraction of that measured in stems. It is worth noting that chlorophyll breakdown as revealed by changes in leaf colour started only the second half of October (about one month later than the first significant drop in K). Since this date, an overall blockage of the xylem conduits of petioles took place so that K_{PL} dropped to near zero (Fig. 4). Leaf blades, on the contrary, did not undergo any further decrease in their K (K_{LL}, Fig. 4), probably due to the redundancy of the vein network that allowed to maintain a sufficient efficiency of the overall water path in the leaf blade (Nardini 2001, Nardini *et al.* 2001).

A substantial agreement between the measured loss in K_{SL} (Fig. 3) and that in $\Sigma\pi r^4$ (Fig. 7) was observed in stems, *i.e.* K_{SL} decreased by about 88 % and $\Sigma\pi r^4$ by about 82 %. Some discrepancy, on the contrary, existed between the measured loss in K for petioles (K_{PL}, Fig. 3) and the calculated loss in $\Sigma\pi r^4$ (Fig. 7), *i.e.* K_{PL} decreased by about 51 % while $\Sigma\pi r^4$ decreased by only about 27 %. As anatomical measurements were made at the middle plane of petioles (see above), such a discrepancy strongly suggests that the major blockage of the water conducting system had occurred at the junction plane between petiole and stem, maybe at the abscission layer that usually develops just distally to the petiole-to-stem junction (Fahn 1990).

The extensive blockage of xylem conduits due to tyloses and/or other solid materials is known to be preceded by conduit embolism. In turn, cavitation-induced xylem embolism has been recognized to be a much more common event in plant life than previously thought (*e.g.* Tyree and Sperry 1988, Cochard *et al.* 1992, Zwieniecki and Holbrook 1998, Nardini and Salleo 2000). We did not measure xylem cavitation in stems or petioles, directly. Nonetheless, it can be noted that: *1*) most conduits blocked by solid materials (tyloses) belonged to the widest diameter ranges (Fig. 6) both in stems and petioles; *2*) several studies (*e.g.* Tyree *et al.* 1994, Lo Gullo *et al.* 1995) report a positive relationship existing between vulnerability to cavitation and embolism to conduit diameter. On the basis of the above, we feel that our data provide more than a circumstantial evidence showing that leaf shedding occurring at the beginning of the winter rest is preceded by cavitation-induced xylem embolism that would take place in the stem long before leaf senescence becomes evident. In fact, our September measurements showed a large reduction in the stem hydraulic conductance which was likely to be caused by previous reduction in root hydraulic conductance as reported by Nardini *et al.* (1998b). A convincing body of evidence exists, showing that roots of several species are more vulnerable to cavitation than stems (*e.g.* Mencuccini and Comstock 1997, Sperry and Ikeda 1997, Kavanagh *et al.* 1999). It is not unreasonable, therefore, to conceive that the entire process of leaf senescence and shedding is initiated by the summer decrease in the hydraulic efficiency of the root system. More extensive studies addressed to monitoring seasonal changes in root anatomy and hydraulics and their impact on whole plant hydraulics appear to be promising enough for deserving further research.

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