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Diurnal cycles of embolism formation and repair in petioles of grapevine (*Vitis vinifera* cv. Chasselas)

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Abstract

The impact of water deficit on stomatal conductance (g_s), petiole hydraulic conductance ($K_{petiole}$), and vulnerability to cavitation (PLC, percentage loss of hydraulic conductivity) in leaf petioles has been observed on field-grown vines (*Vitis vinifera* L. cv. Chasselas). Petioles were highly vulnerable to cavitation, with a 50% loss of hydraulic conductivity at a stem xylem water potential (Ψ_x) of –0.95 MPa, and up to 90% loss of conductivity at a Ψ_x of –1.5 MPa. $K_{petiole}$ described a daily cycle, decreasing during the day as water stress and evapotranspiration increased, then rising again in the early evening up to the previous morning's $K_{petiole}$ levels. In water-stressed vines, PLC increased sharply during the daytime and reached maximum values (70–90%) in the middle of the afternoon. Embolism repair occurred in petioles from the end of the day through the night. Indeed, PLC decreased in darkness in water-stressed vines. PLC variation in irrigated plants showed the same tendency, but with a smaller amplitude. The Chasselas cultivar appears to develop hydraulic segmentation, in which petiole cavitation plays an important role as a 'hydraulic fuse', thereby limiting leaf transpiration and the propagation of embolism and preserving the integrity of other organs (shoots and roots) during water stress. In the present study, progressive stomatal closure responded to a decrease in $K_{petiole}$ and an increase in cavitation events. Almost total closure of stomata (90%) was measured when PLC in petioles reached >90%.

Key words: grapevines (*Vitis vinifera* L.), hydraulic conductivity, stomatal conductance, water relations, xylem embolism.

Introduction

Water deficit, due to environmental limitations of soil available water and high evapotranspiration demand, generally impacts on leaf gas exchanges (carbon assimilation and transpiration) and growth (Hsiao, 1973). In order to avoid water stress, plants have developed long- and short-term acclimation such as root development, leaf surface area reduction, specialized leaf anatomy, and conducting elements (Schultz and Matthews, 1988). In addition, short-term strategies allow the plant to react rapidly in a changing, restrictive environment.

Amongst short-term strategies, stomatal regulation of leaf gas exchange plays a key role in plant response to water

stress. Nevertheless, stomatal behaviour is a complex phenomenon involving feedback controls which interact with a wide range of environmental conditions (Zweifel *et al.*, 2007). A general distinction is made between CO_2 feedbacks, which probably operate either by substomatal CO_2 concentration (C_i) (Mott, 1988) or by assimilation rates (Wong *et al.*, 1985), and hydraulic feedbacks that are dependent on specific aspects of stomatal or whole-plant water status (Monteith, 1995; Jarvis and Davies, 1998) and hydraulic continuity in the soil–plant–atmosphere system (Sperry *et al.*, 2002; Brodribb and Jordan, 2008). Various studies have indeed shown that a relationship exists between

stomatal closure during water restriction and the mainte-nance of xylem integrity (Tyree and Sperry, 1988; Jones and Sutherland, 1991). Under conditions of water restriction, the progressive closure of stomata can be observed when the vapour pressure deficit (VPD) increases and hydraulic conductance along the soil–plant–atmosphere pathway decreases, so that Ψ_{leaf} (leaf water potential) remains above a critical threshold required to prevent xylem cavitation and hydraulic rupture (Tyree and Sperry, 1988; Hubbard *et al.*, 2001; Cochard *et al.*, 2002).

The regulation of xylem pressure by stomatal closure may play a role in limiting the risk of cavitation and, consequently, the loss of hydraulic conductivity in the plant (Bucci *et al.*, 2003; Brodridd and Holbrook, 2003; Brodribb *et al.*, 2003). A positive relationship between hydraulic conductance and stomatal conductance (g_s) has been described in a number of studies (Meinzer and Grantz, 1990; Saliendra *et al.*, 1995; Comstock, 2000), when Ψ_{leaf} remains constant, which would suggest a feedback link between g_s and some form of hydraulic signal (Nardini *et al.*, 2001). Moreover, the vulnerability of vessels to cavitation depends on the balance between embolism formation and its possible repair. Recent works have highlighted diurnal changes in xylem hydraulic conductance and water refilling, in spite of the presence of significant tension inside xylem vessels in roots (McCully *et al.*, 1998; McCully, 1999; Domec *et al.*, 2006; Lovisolo *et al.*, 2008), in shoots (Salleo *et al.*, 1996, 2004; Zwieniecki and Holbrook, 1998; Melcher *et al.*, 2001), in petioles (Zwieniecki *et al.*, 2000; Bucci *et al.*, 2003; Hacke and Sperry, 2003; Loviosolo *et al.*, 2008), and in leaves (Lo Gullo *et al.*, 2003; Brodribb and Holbrook, 2004; Johnson *et al.*, 2009). The first *in vivo* visualization and quantification of the refilling process in grapevines were carried out by Holbrook *et al.* (2001) using magnetic resonance imaging, and were recently improved using high-resolution X-ray computed tomography (CT) (Brodersen *et al.*, 2010). Furthermore, although cavitation appears to be reversible within a rela-tively short time lapse (from tens of minutes to several hours), the diurnal cycles of embolism and leaf-specific hydraulic conductance (K_{plant}), followed by the refilling of leaves and petioles, are considered to play an important role in the regulation of gas exchanges (Lo Gullo *et al.*, 2003). Moreover, other studies (Tyree *et al.*, 1993; Salleo *et al.*, 2001) have been able to show that petioles and leaves from various plant species were more vulnerable to cavitation than stems. In grapevines, the findings of Choat *et al.* (2010) and Alsina *et al.* (2007) demonstrated a relative resistance in stems to xylem embolism as water stress rose, whereas the petioles were found to be more vulnerable (Schultz, 2003). Zimmermann (1983) put forward the hypothesis of hydraulic segmentation, according to which distal organs, such as petioles and leaves, were more vulnerable to embolism. Acting as hydraulic fuses, they would be able to prevent the propagation of embolism in the stems, trunk, or roots, and constitute an effective means of resistance to drought and diminish the risks of hydraulic rupture.

Even though the precise mechanisms responsible for embolism recovery are still unknown (Tyree *et al.*, 1999),

various hypotheses have been proposed (Clearwater and Goldstein, 2005) and partially confirmed by different studies. Some authors, for example, have suggested that the refilling of vessels possibly occurs by reverse osmosis, induced by rising pressure in petiole or shoot tissues, established by the hydrolysis of starch into osmotically active sugars (Canny, 1997; Bucci *et al.*, 2003). Other theories were advanced (Hacke and Sperry, 2003) including the activity of several tissues (phloem and xylem), the hydraulic compartmentalization of conducting elements during refilling, or even complex properties specific to membranes and perforations. All appear to indicate that a finely balanced equilibrium exists between xylem pressure and the loss of hydraulic conductivity, and that vulnerabil-ity to cavitation would be part of a feedback mechanism linking stomatal regulation to hydraulic conductance and plant water status (Bond and Kavanagh, 1999). Other authors (Zwieniecki and Holbrook, 1998; Salleo *et al.*, 2004) have pointed out that the process of water refilling is energy demanding and, consequently, requires phloem activity and an adequate supply of carbohydrates. Bro-dersen *et al.* (2010) clearly presented embolism repair as a dynamic process using solutes, parenchyma water, pres-sure gradients, and conduit surface wettability. Under these conditions, recovery from an embolism may be facilitated in organs where close contacts are present between phloem, xylem, and living parenchyma cells. Such would be the case in leaves and petioles, where narrow vessels would favour refilling, as opposed to those in stems (Vesala *et al.*, 2003).

The goal of the present study was to determine the vulnerability to cavitation of a drought-susceptible grape-vine cultivar (*Vitis vinifera* L. cv. Chasselas) when subjected to water restriction. The importance of hydraulic conduc-tance in the soil–plant–atmosphere continuum and, more specifically, of hydraulic conductance in petioles in control-ling stomatal behaviour, was evaluated during water stress. Does stomatal regulation of leaf gas exchanges in grape-vines provide a sufficient guarantee against cavitation development and hydraulic rupture? Is the appearance of midday wilting symptoms in Chasselas leaves, observed during the day when climatic and edaphic constraints are very high, and their disappearance at the end of the day linked to diurnal changes in hydraulic conductance in the plant, and, more especially, in the petioles? To answer these questions, different levels of water stress were imposed upon grapevines growing in the field, and the resulting hydraulic behaviours during the season were studied.

Materials and methods

Study site and plant material

The experiments were conducted in 2009 at the experimental station of Agroscope Changins-Wädenswil ACW in Leytron (Switzerland), located in an Alpine valley. The planting material was the cultivar Chasselas, clone 14/33-4, grafted on *Vitis berlandieri*×*Vitis riparia* cv. Kober 5BB rootstock. Vines were planted in the Guyot training system (vertical shoot-positioned) with a planting density of 5500 vines ha^{-1} (planting distance:

1.8×1.0 m). Six shoots per vine were maintained. The experimental site in Leytron lies on very stony (peyrosol >60% large elements, stones, blocks, and gravel) and deep soil (>2.5 m vine root depth), with a water-holding capacity estimated at 150 mm. In 2009, the annual rainfall was 560 mm, corresponding to the average annual rainfall over the last 30 years. August, September, and October were particularly dry (Table 1). Maximum daily temperatures measured locally were ~30–35 °C during the period of physiological study (July–August). Maximum daily VPDs ranged from 2 kPa to 4.5 kPa.

Irrigation treatments

Three different irrigation treatments were established. In the first treatment, 9.0 l m^{-2} soil (16.0 l per vine) were drip-fed weekly from bloom (152 DOY) to fruit ripening (216 DOY). This level of irrigation corresponded to an approximately weekly compensation of 30% evapotranspiration potential (ETP). The second treatment was not irrigated at all throughout the whole growing season. Neither was the third treatment. In addition, a waterproof and non-reflecting plastic was laid on the soil of the third treatment, from bloom (152 DOY), in order to eliminate the infiltration of water from precipitation events. The trial was carried out using 40 plants per treatment, set out in four split-plot randomized blocks of 10 vines each.

Measurements of water relations

Pre-dawn water potential (Ψ_{PD}), Ψ_{leaf}, and stem xylem water potential (Ψ_x) were measured using a pressure chamber (Scholander *et al.*, 1965) according to Turner (1988). Ψ_{PD} was measured between 04:00 h and 05:00 h in complete darkness, on eight mature, undamaged and non-senescent leaves. Ψ_{leaf} and Ψ_x measurements were carried out between 14:00 h and 15:00 h when evapotranspiration was at the greatest. Ψ_{leaf} was determined on eight mature non-senescent leaves exposed at photon flux density (PFD) levels >1200 μmol m^{-2} s^{-1}. Ψ_x was measured on eight leaves bagged with a plastic sheet and covered with aluminium foil to stop transpiration at least 1 h before measurements were taken (Fulton *et al.*, 2001).

The differences in stomatal behaviour and hydraulic conductance in the whole plant were estimated using the K_{plant}. K_{plant} was estimated from the relationship between the rates of single-leaf transpiration and soil–leaf water potential difference. The soil water potential (Ψ_{soil}) was considered to be very close to the Ψ_{PD} in the leaves (Breda *et al.*, 1995). In this way, Ψ_{PD} could be considered to be equivalent to Ψ_{soil} and it was possible to estimate K_{plant} from the rate of single-leaf transpiration (E) and the difference in water potential between the soil and the leaf with the formula $K_{plant}=E/(\Psi_{PD}-\Psi_{leaf})$ (Sperry and Pockman, 1993).

Table 1. Monthly rates of precipitation (mm) at the experimental site of Leytron (CH) during the 2009 season in comparison with the long-term averages (1960–1990)

	2009	Long term
January	55	54
February	32	46
March	17	39
April	35	36
May	22	39
June	40	47
July	58	49
August	2	62
September	16	47
October	11	46
November	60	52
December	105	60
Year	**560**	**570**

Xylem embolism

Xylem embolism, induced by the presence of air bubbles in vessels, was measured using the method introduced by Sperry *et al.* (1988) and established by Cochard *et al.* (2000). The apparatus XYL'EM (Xylem Embolism meter, Instructec, Montigny-les-Cormeilles, France), based on a high-resolution liquid mass flowmeter, was used to determine the percentage loss in conductivity (PLC) in leaf petioles.

For each determination of PLC, the leaf petioles and their leaf blades were cut under water from the shoots bent in a container filled with water in the field: the petioles and their leaf blades were swiftly covered with aluminium foil (kept in darkness), then taken immediately to the laboratory *in situ*. Lengths and diameters of petioles, and the leaf blade surface area were measured. The petioles were attached to the XYL'EM apparatus tubes and their initial hydraulic conductance (K_{init}) was determined with a hydrostatic pressure gradient of ~3–4 kPa. Distilled and degassed water was used as perfusion liquid for all measurements. Since changes in the value of K may be caused by the absence or presence of certain ions in the solution, 10 mmol l^{-1} KCl was added to the solution. To measure the maximum conductance (K_{max}), the petioles were then flushed twice during a period of 2 min with water pressurized at 0.15 MPa. Ψ_x was controlled on shoots at regular intervals during the day as well as just before petiole sampling.

Furthermore, with a view to amplifying water tensions artificially, some PLC measurements were carried out on petioles taken from shoots which were sectioned a few minutes in advance then progressively dehydrated in room conditions (temperatures of 22 °C, relative air humidity 60%).

PLC figures were constructed using the average values obtained from four petioles and as many measurements representing the xylem water potential Ψ_x. The loss of conductivity versus Ψ_x curves were utilized to determine vulnerability to cavitation, expressed as Ψ_{PLC50}, the xylem water potential inducing 50% loss in hydraulic conductivity. The Ψ_{PLC50} was estimated by adjusting a sigmoid PLC curve versus Ψ_x data: PLC=100/{1+exp [a ($\Psi_x-\Psi_{PLC50}$)]}, where the parameter 'a' represents the slope of the curve at the point of inflection. The petiole hydraulic conductance ($K_{petiole}$) was estimated from the following formula: $K_{petiole}=K_h\times L/LA$, K_h being the initial hydraulic conductance described above, L the length of the petiole and LA the surface area of the leaf blade.

Leaf gas exchange measurements

Leaf gas exchange measurements [net photosynthesis (A) and transpiration (E)] and g_s measurements were carried out on adult, non-senescent leaves with a leaf plastochon index (LPI) >15, and well exposed to direct sunlight (PFD >1200 μmol m^{-2} s^{-1}). Gas exchanges were measured using an open gas exchange system (ADC-System, LCA-4, Hoddesdon, UK) equipped with a Parkinson leaf chamber. Maximum photosynthetic rates (A_{max}) and maximum stomatal conductance (g_{max}) to ambient CO_2 concentration were observed between 10:00 h and 11:00 h on different days throughout the season. Supplementary g_s measurements were carried out during the day on adult non-senescent leaves located on the same shoots as those used for PLC and $K_{petiole}$ measurements.

Results

Cavitation vulnerability

The cavitation vulnerability curves (Figs 1, 2) were constructed by plotting the changes in the PLC in relation to Ψ_x. At Ψ_x of around –0.3 MPa, a PLC of 10% was observed (Fig. 1). The 50% PLC threshold was reached when Ψ_x was at –0.95 MPa, according to the parameters of the function employed. Non-irrigated vines with covered
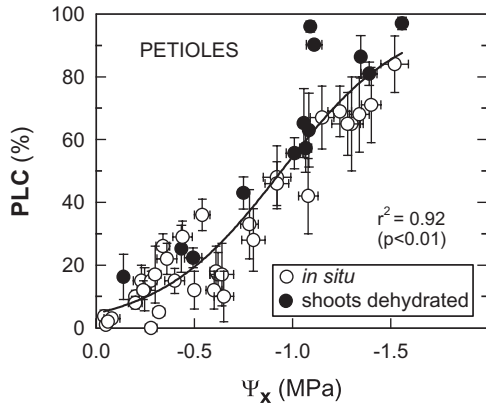
Fig. 1. Vulnerability to cavitation (PLC, percentage loss of conductivity) versus stem xylem water potential (Ψ_x) for grapevine leaf petioles measured *in situ* and of dehydrated shoots. Means ±SE of four petioles. Chasselas, Leytron (CH), 2009.

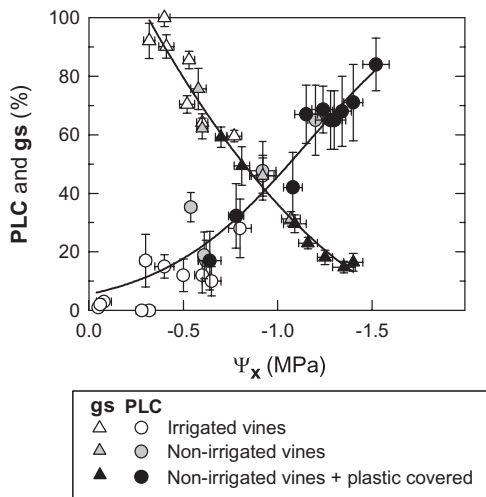**Fig. 2.** Vulnerability to cavitation (PLC, percentage loss of conductivity) and stomatal conductance (g_s) versus stem xylem water potential (Ψ_x) for grapevine leaf petioles. Means ±SE of four petioles (for PLC), and eight leaves (for g_s). Chasselas, Leytron (CH), 2009.

soils presented values of PLC >60% in petioles when Ψ_x fell below –1.1 MPa (Fig. 2). Irrigated vine plants showed a lower vulnerability to cavitation (PLC <30%) with values of Ψ_x greater than –0.8 MPa. An intermediate situation was observed in non-irrigated vines.

The leaf g_s decreased with increasing xylem tension (low xylem water potentials) (Fig. 2). Stomatal closure reached a level of 50% when the values of Ψ_x were of the order of –0.8 MPa, and of 90% with values of Ψ_x at –1.5 MPa.

Diurnal variations of $K_{petiole}$ and PLC

The $K_{petiole}$ (Fig. 3C–D) decreased progressively during the first hours of the day as water stress increased. The lowest values of $K_{petiole}$ were observed in the afternoon when the evapotranspiration was at its highest (high VPD) and water stress at its greatest. At the end of the afternoon and in the
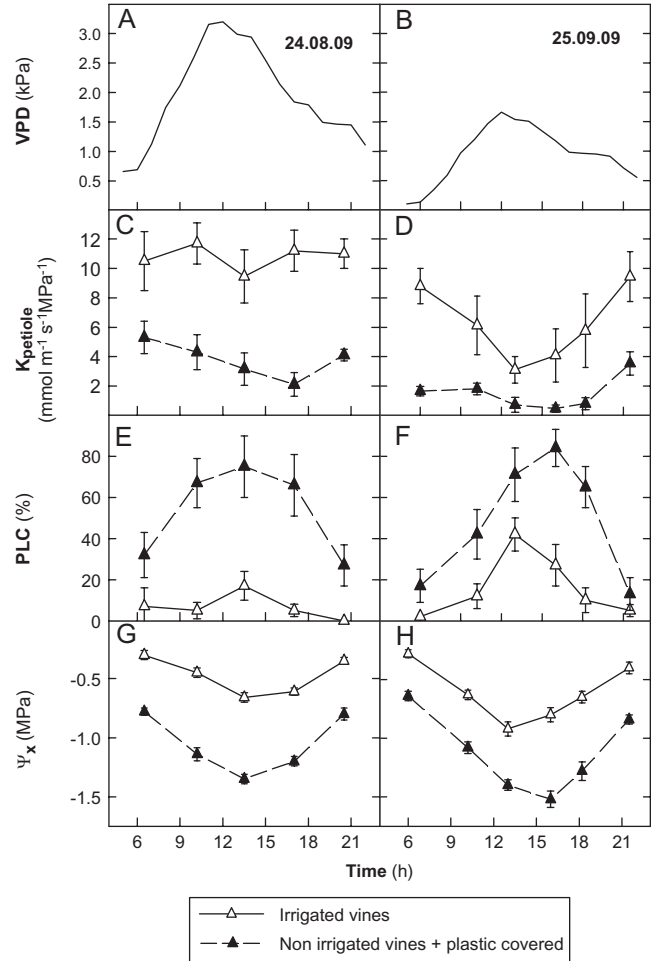

Fig. 3. Diurnal courses of leaf-atmospheric (VPD) (A, B), petiole hydraulic conductance ($K_{petiole}$) (C, D), vulnerability of petioles to cavitation (PLC, percentage loss of conductivity) (E, F), and stem xylem water potential (Ψ_x) (G, H) during two days (24 August and 25 September) for different irrigation treatments. Chasselas, Leytron (CH), 2009.

early evening, $K_{petiole}$ rose again, reaching levels close to those measured during the earliest hours of the day. This type of behaviour in $K_{petiole}$ variation was observed mainly in the irrigated vines, which, incidentally, presented $K_{petiole}$ values 2–3 times greater than those in the non-irrigated and soil-covered vines where the water stress was very high (Ψ_x – 1.5 MPa) (Fig. 3G, H).

The PLC increased sharply during the daytime to reach maximum values (70–90%) in the non-irrigated and soil-covered vines in the middle of the afternoon (Fig. 3E, F). At the end of the afternoon, it then decreased, reaching minimum values during the night, identical to those measured at dawn. The same kind of PLC behaviour was monitored over several days, of which only two days (24 August and 25 September) are presented herein. In the irrigated treatment, the pattern of the PLC daily evolution was found to be identical to that of the non-irrigated vines but with smaller variations. Moreover, a good correlation exists between the $K_{petiole}$ and Ψ_x (Fig. 4) measured in vines subjected to different levels of irrigation. In the same way,
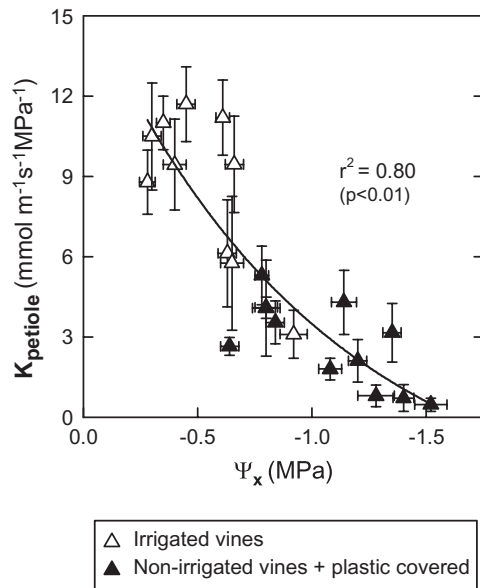
Fig. 4. Relationship between the stem xylem water potential (Ψ_x) and the petiole hydraulic conductance ($K_{petiole}$) for adult leaves and different irrigation treatments. Means ±SE of four petioles/leaves. Chasselas, Leytron (CH), 2009.

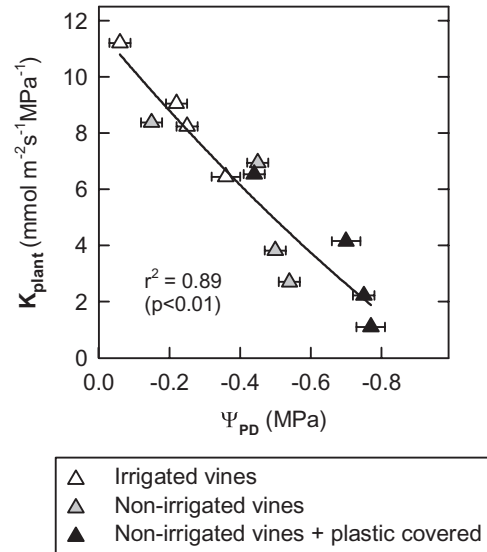**Fig. 5.** Relationship between mid-morning leaf-specific hydraulic conductance [K_{plant}=$E/(\Psi_{PD}-\Psi_{leaf})$, equation from Sperry and Pockman (1993)] and the pre-dawn water potential (Ψ_{PD}) for different irrigation treatments. Means ±SE of eight leaves. Chasselas, Leytron (CH), 2009.

a relationship between K_{plant} (=$E/\Psi_{PD}-\Psi_{leaf}$), estimated according to Sperry and Pockman (1993), and the level of water stress estimated by Ψ_{PD} was established in the present study (Fig. 5). The latter decreases in a linear fashion as water stress rises. Finally, leaf g_s is closely related to K_{plant}, which was measured several times around midday, whether in vines with or without water restriction (Fig. 6).

Water and gas exchange relationships

The different irrigation treatments tested in the present study on grapevines resulted in markedly different conditions of water stress (Fig. 7). Drip-irrigated vines presented no water stress throughout the whole growing period, with values of Ψ_{PD} greater than –0.3 MPa (Fig. 7A) and of Ψ_x greater than –0.8 MPa (Fig. 7B). The absence of irrigation led to a more noticeable water stress from the end of July (210 DOY) with Ψ_{PD} values between –0.3 MPa and –0.6 MPa, and Ψ_x values around –1.0 MPa during August and September. Finally, an elevated water stress was observed (Ψ_{PD} less than –0.6 MPa, and Ψ_x less than –1.1 MPa) from mid-August (DOY 230) until harvesting time in non-irrigated vines where the soil was covered with impermeable plastic. Figure 7 illustrates the impact of the different watering regimes on g_{max} (Fig. 7C) and A_{max} (Fig. 7D) observed mid-morning. The non-irrigated vines presented lower stomatal conductances and photosynthetic rates than the irrigated vines from the end of July (DOY 210), and more especially during September, with a reduction in gas exchange rates of almost 40%. In non-irrigated vines with covered soil, a drop in gas exchanges (g_{max} and A_{max}) was observed from the end of July down to very low levels of g_s and A in the following months, which corresponded more or less to the residual conductance g_{res} (0.03 mol m^{-2} s^1) and a photosynthetic rate of ~2 µmol CO_2 m^{-2} s^{-1}.
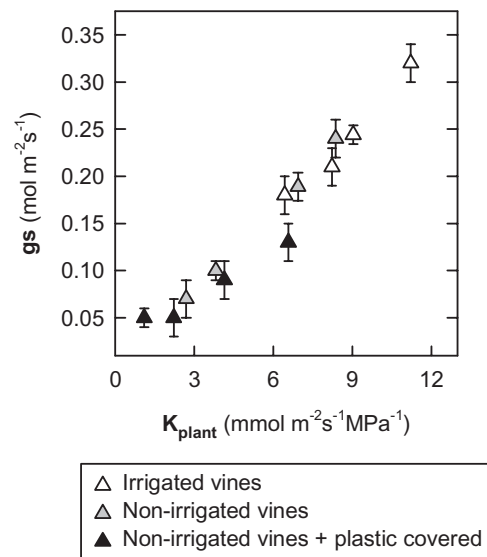

Fig. 6. Stomatal conductance (g_s) as a function of mid-morning leaf-specific hydraulic conductance (K_{plant}) for different irrigation treatments. Means ±SE of eight leaves. Chasselas, Leytron (CH), 2009.

Discussion

Vulnerability to cavitation and diurnal change in PLC and $K_{petiole}$

The different irrigation treatments set up in these trials enabled the development of well-contrasted water regimes in grapevines, which had in turn a strong influence on leaf gas exchanges, hydraulic conductance in petioles, and cavitation vulnerability. The present observations showed
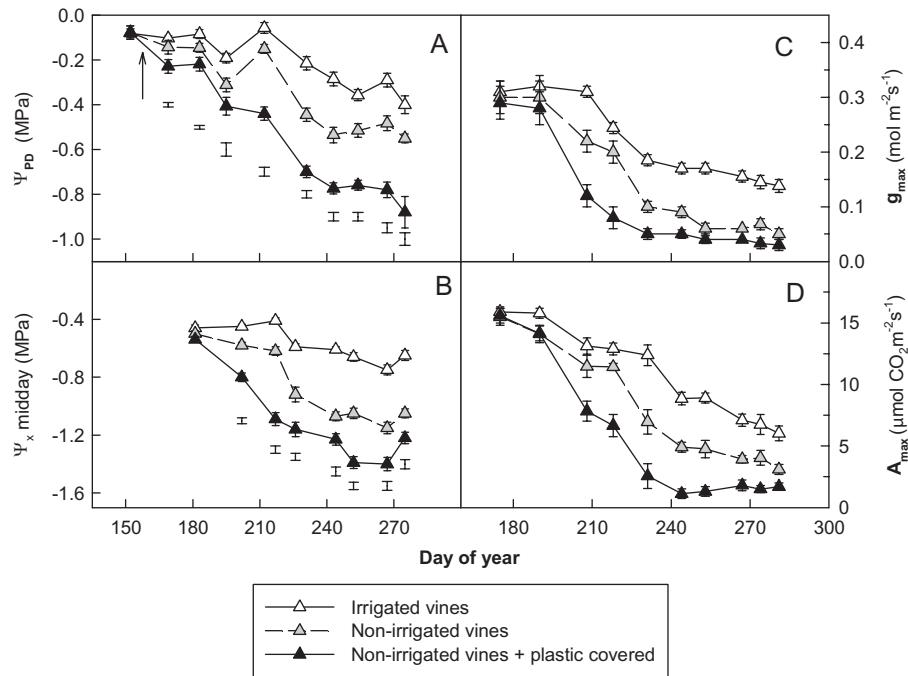
Fig. 7. Changes in (A) pre-dawn water potential (Ψ_{PD}), (B) stem xylem water potential (Ψ_x), (C) maximum stomatal conductance (g_{max}), and (D) maximum photosynthetic rate (A_{max}) during the 2009 season with different irrigation treatments. The arrow indicates irrigation onset. Means ±SE for eight leaves. Bars: statistically significant at the 5% level of probability. Chasselas, Leytron (CH).

that the petioles of the cultivar Chasselas were highly vulnerable to cavitation, with values of Ψ_{PLC50} measured at –0.9 MPa and an almost complete cavitation (90% PLC) with xylem water potential at around –1.5 MPa. Furthermore, a daily depression in $K_{petiole}$ and in Ψ_x became clear over the daily course, coinciding with the highest evapotranspiration (although moderate in the present study). At the end of the afternoon, $K_{petiole}$ and Ψ_x recovered and this trend continued overnight. In parallel, the rate of petiole cavitation increased during the day, regardless of the level of water status (high or low water restriction), followed by repair of embolism during the afternoon and the night.

Several studies have also mentioned this cavitation daily cycle in petioles (Zwieniecki *et al.*, 2000; Bucci *et al.*, 2003) and in leaf blades (Brodribb and Holbrook, 2003, 2004), which shows a recovery in hydraulic conductance at the end of the day. Brodersen *et al.* (2010) recently succeeded in *in vivo* visualization of the xylem embolism refilling phenomenon in grapevines, using high-resolution X-ray CT. This unequivocal method of visualization demonstrates that water refilling can occur in embolized vessels within a few hours and depends on the water flow from surrounding parenchyma. Brodersen *et al.* (2010) mentioned that water refilling can happen under water tensions between –0.6 MPa and –0.8 MPa, which correspond to the present results (Ψ_x measured in the evening at around –0.8 MPa with PLC values of ~20–30%). Accordingly, the grapevine does appear to recuperate from embolism and very rapidly re-establishes water flow in the petioles during mid-afternoon and the night. In addition, Choat *et al.* (2010) showed that stems of grapevines, in contrast to petioles, were relatively resistant to

cavitation, with embolism rates remaining under 30% even with high hydraulic tensions measured in stem vessels (Ψ_x less than –1.5 MPa), confirming Alsina's results on different cultivars (Alsina *et al.*, 2007). These authors have not, however, excluded the possibility of cavitation phenomena occurring on a wider scale and to a greater extent in the roots, petioles, or leaves, when the grapevine is subjected to water stress, which would concord with the present results and those of Lovisolo *et al.* (2008). Indeed, it seems that the grapevine develops a hydraulic segmentation in which petiole cavitation would play an important part as 'hydraulic fuses', thus limiting leaf transpiration and the spread of embolism, and preserving the hydraulic integrity of shoots and grapes during periods of high water restriction and/or evapotranspiration. This hypothesis of hydraulic segmentation, originally put forward by Zimmermann (1983), would account for the maintenance of a water flux in basal organs, such as grapevine stems, which represent a greater investment in carbon and in which embolism repair would be more difficult due to higher metabolic cost. Furthermore, in some tree species, the distal organs (petioles and leaves) have been observed to be more vulnerable to cavitation than the stems (Tyree *et al.*, 1993; Salleo *et al.*, 2001), the trunk, and the roots (Choat *et al.*, 2005). The findings of Choat *et al.* (2005) have shown, in addition, that the primary xylem/secondary xylem ratio of the organs is of greater importance in the vulnerability to cavitation than the diameter of vessels. The petioles, effectively possessing a sparse secondary thickening of primary xylem conduits, would thus be more likely to be subjected to hydraulic rupture (lower air seeding threshold; Choat *et al.*, 2005). The hypothesis according to which the

petioles may act as hydraulic fuses is partly proved by the studies carried out on the xylem structure in grapevines by Chatelet *et al.* (2006). The latter have shown that the continuity of open and continuous conduits is guaranteed for three internodes only through the primary xylem of grapevine shoots. Pit connections towards the secondary xylem, on the other hand, restrict the displacement of air from the primary xylem towards the secondary xylem, and thus represent an effective segmentation for the propagation of embolism throughout the stems.

In different plants, the combination of a very rapid stomatal response to high evaporation, coupled with a very large maximum hydraulic conductance, may help to minimize the risks of cavitation development and xylem failure, after a decrease in K_{plant} over the daily course (Brodribb and Holbrook, 2003). In grapevines, K_{plant} and $K_{petiole}$ present maximum values which are relatively modest in comparison with other species. Moreover, decreases in $K_{petiole}$ might not be directly related to cavitation and refilling, but could well also be influenced by environmental factors, such as the temperature, VPD, or light intensity. Brodribb and Holbrook (2004) have suggested that the reversible loss of leaf hydraulic conductivity could be a means of amplifying the signal of evaporative demand to the stomata in order to trigger a stomatal response. The major benefit would be protection of upstream xylem. The results of the present study clearly illustrate that the phenomena of daily variations of cavitation and embolism are linked to rising xylem water tension in the conducting elements and high evaporative demand (VPD). In the presence of low xylem water potentials, between –1.3 MPa and –1.5 MPa, the observed cavitation rates were at the highest, in the order of 70–90% PLC. The relationship established between K_{plant} and $K_{petiole}$ (r^2=0.75, P <0.01) demonstrates that changes in hydraulic conductance in the leaves themselves were mainly influenced by the efficiency of water transport in sites of evaporation. Furthermore, g_s decreased progressively from the mid-morning throughout the afternoon, in both experimental treatments (with and without water restriction), to reach its lowest level when $K_{petiole}$ was also at its lowest (unpublished results). At the end of the afternoon, a slight increase in g_s was observed, in parallel with $K_{petiole}$ rising as well, even though no variation in VPD was recorded. With present knowledge, it was not possible to determine whether shoots and roots have the same faculty for re-establishing hydraulic conductivity as quickly as petioles.

Water xylem refilling under tension

It has been difficult to explain xylem water refilling in the presence of adjacent conduits under high tensions, requiring large positive pressures to clear the air bubbles or to cause their re-absorption in the solution (Tyree, 1997). Although several hypotheses have been formulated on the capacity of various plant organs to refill xylem vessels rapidly (Clearwater and Goldstein, 2005), it appears that a pre-existing condition is necessary. This condition requires the hydraulic compartmentalization of vessels so that local pressurization is possible (Zwieniecki and Holbrook, 2000) in order to enable embolism repair in transpiring plants. In other words, without an effective compartmentalization, water and solute flows, which have been transferred temporarily to the embolized vessel, would rapidly be lost and would be redirected towards neighbouring vessels with more negative hydraulic potentials. Brodersen *et al.* (2010), using high-resolution X-ray CT, pointed out the requirement of vessels for hydraulic compartmentalization towards surrounding vessels with higher water tensions. Thus, embolism repair would be a dynamic process. From this perspective, Brodersen *et al.* (2010) put forward a model for vessel refilling, based on the phloem load of water and solutes, as well as pressure gradients and vessel surface wettability. Various authors (Canny, 1997; Bucci *et al.*, 2003) have been able to demonstrate that the hydrolysis of starch into osmotically active sugars in the daytime may well participate in the water refilling of xylem by means of a reverse osmotic mechanism. Recent research projects on grapevines undergoing rehydration after a drought (Lovisolo *et al.*, 2008) confirm that the phenomenon of embolism repair is quite frequent in *V. vinifera* L. These authors have further suggested that the control of transpiration, induced by abscisic acid, is likely to be favourable to the gradual repair of embolisms in rehydrated grapevines.

Cavitation and stomatal behaviour

The cavitation phenomena may act as a signal for progressive stomatal closure (Nardini *et al.*, 2001) or stomatal closure could occur to prevent cavitation (Tyree and Sperry, 1988). Under conditions of high climatic demand (high VPD) and dehydrated soils (restricted water), the stomata move towards closure by limiting evapotranspiration of the guard cells, while maintaining the plant water status above a threshold value of Ψ in order to avoid the development of xylem cavitation (Jones and Sutherland, 1991). In walnut (Cochard *et al.*, 2002), a high restriction in g_s (~90% stomatal closure) precedes the onset of xylem cavitation. In this case, the effective closure of stomata in response to the water stress allows the leaf rachis xylem pressure to be maintained above the value of –1.4 MPa and the leaf water potential (Ψ_{leaf}) above the value of –1.6 MPa. These values represent thresholds of water status in the walnut, below which phenomena of embolism begin to develop. With regards to avoiding the risks of a rapidly developing embolism, the strategy of efficient stomatal closure performed well in this case. However, the major disadvantage of such a strategy resides in the concomitant limitation of CO_2 fixation and a reduction in plant growth. For the Chasselas cultivar, on the other hand, stomatal control of xylem cavitation would appear to be less effective since almost complete closure of stomata (90%) intervenes only when the loss in hydraulic conductivity has already reached 90%, with a xylem potential recorded at –1.5 MPa. In grapevines, stomatal closure alone does not appear capable of regulating the increasing risk of cavitation as water stress rises. The cultivated grapevine (*V. vinifera* L.) is generally

considered to be a 'drought-avoiding' plant (Smart and Combe, 1983) or, according to a more eco-physiological classification, an isohydric plant (Tardieu and Simmoneau, 1998), in accordance with its stomatal behaviour. Some *V. vinifera* L. cultivars of different geographical origin may display very diverse Ψ_{leaf} responses under water stress (Schultz, 2003), indicating that both iso- and anisohydric behaviours can co-exist within a single species. Nevertheless, Chouzouri and Schultz (2005) showed that there was a good correlation between drought tolerance and vulnerability to embolism in four grapevine cultivars of different geographic origin, and that the decrease in g_s occurred concomitantly with an increase in cavitation events for all varieties, as soil water availability became restricted.

In the present study, the Ψ_x and Ψ_{leaf} (non-presented results) of Chasselas dropped steeply in the daytime and were lower in water-stressed plants than in well-watered plants, indicating a more anisohydric behaviour. The most negative values of Ψ_{PD} and Ψ_x were stabilized during the growing season at around –0.8 MPa and –1.5 MPa, respectively, in the most stressed situation (soil covered over, no irrigation). The significant defoliation (50% of leaf surface per vine, non-presented results) observed under highly restricted water conditions meant that the available water reserves in the soil were not used up too rapidly, by adaptation of water flux along the soil–plant–atmosphere continuum, while more or less stable values of Ψ_x and Ψ_{PD} were able to be maintained.

The stomatal closure in the present study probably responded to a decrease in petiole hydraulic conductance and an increase in cavitation events, but other factors cannot be totally excluded. Feed-forward behaviour of stomata with respect to regulation of Ψ_{leaf} has been attributed to the presence of chemical signals which modify stomatal opening and which are brought to the leaf in the transpiration flow (Davies *et al.*, 1994).

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