PHYSIOLOGICAL AND HISTOLOGICAL APPROACHES TO STUDY BERRY SHRIVEL IN GRAPES

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Abstract

Aims : The current work aims to study berry shrivel in grapes (a grape-ripening disorder) in relation to vine water status and climatic conditions using physiological and histological approaches.

Methods and results : Measurements of rachis hydraulic conductance on grapevine clusters (*Vitis vinifera* L.) and observations of the vascular tissues (xylem and phloem) using transmission electron microscopy were conducted on rachises from healthy clusters and clusters having berry shrivel (BS) symptoms during the season. BS intensity was largely dependent on the vine water status: BS was greater in vines without water stress than in vines with moderate to high water stress around veraison time. Preliminary results showed that rachis hydraulic conductance declined sharply after veraison but remained slightly higher in healthy clusters in comparison with clusters presenting BS symptoms. An important degradation of the primary phloem was observed in the rachises of BS clusters, with the appearance of hard, non-functional liber (secondary phloem) and a disorganization of the cell content in the phloem tissue. An alteration of the primary xylem was also observed in the middle of the rachis and in the secondary rachis ramifications.

Conclusion : These results suggest that the decrease in sugar and water accumulation in BS berries would primarily be associated with a decline in rachis phloem functionality.

Significance and impact of the study : The management of the vine water status plays a key role in berry shrivel development.

Key words : berry shrivel, rachis hydraulic conductance, berry vascular system, xylem, phloem

Résumé

Objectifs : Le présent travail a pour but d'étudier, au moyen d'observations physiologiques et histologiques, le phénomène de folletage des baies (perturbation de la maturation du raisin) en relation avec l'alimentation en eau de la vigne et les conditions climatiques.

Méthodes et résultats : Des mesures de conductivité hydraulique des grappes de *Vitis vinifera* L. et des observations de tissus vasculaires (xylème et phloème) ont été menées sur des rafles de grappes saines et des grappes présentant des symptômes de folletage des baies (BS) durant la saison. L'intensité du folletage des baies a été largement dépendante du statut hydrique de la vigne : le BS a été plus important dans les vignes n'ayant subi aucune restriction en eau que dans les vignes ayant supporté un stress hydrique modéré à fort autour de la véraison. Les résultats préliminaires ont montré que la conductivité hydraulique des rafles a fortement diminué après la véraison, et qu'elle est demeurée un peu plus élevée chez les grappes saines en comparaison des grappes présentant du BS. Une importante dégradation du phloème primaire a été observée dans les rafles de grappes atteintes de BS, avec un développement de liber dur, non fonctionnel (phloème secondaire), et une grande désorganisation du contenu cellulaire des tissus du phloème. Une altération du xylème primaire a également été notée au milieu de la rafle et dans les ramifications secondaires.

Conclusion : Ces résultats suggèrent que la diminution d'accumulation des sucres et de la perte en eau des baies atteintes de folletage seraient associées principalement à des perturbations de fonctionnement du phloème.

Signification et impact de l'étude : La gestion du statut hydrique de la vigne joue un rôle clé dans le développement du phénomène de folletage des baies.

Mots clés : folletage des baies, conductivité hydraulique des rafles, système vasculaire de la baie, xylème, phloème

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INTRODUCTION

Berry shrivel (BS) is a grape-ripening disorder that has been increasing for more than a decade in the vineyards of the Northern Hemisphere, particularly in Europe. Although it was previously mentioned by Jensen (1970), this disorder was named « Zweigelt disease » in Austria (Reisenzein and Berger, 1997) in reference to the susceptibility of this variety. Since then, BS symptoms have been observed in a large number of *Vitis vinifera* L. varieties, including Pinot noir, Gamay, Cabernet-Sauvignon, Grüner Veltliner and Sauvignon (white and grey). In Switzerland, grape varieties such as Chasselas and locally grown cultivars such as Humagne rouge and Cornalin are highly susceptible to BS as well.

BS is characterized by an interruption of the normal ripening process resulting in low sugar concentration and high acidity in berries (Krasnow *et al.*, 2009; Knoll *et al.*, 2010). The synthesis of aromatic and colored compounds in the berries is also disturbed (Bondada and Keller, 2007; Krasnow *et al.*, 2009). The BS symptoms may appear as early as the veraison stage (the onset of ripening). In the red varieties, berries remain generally pink, especially when the phenomenon occurs early during the ripening phase. BS is often associated with a loss of turgor and visible shriveling or shrinking of grape berries. However, in some varieties, such as Sauvignon, berries can retain their firmness and not shrivel at all. BS may affect all or part of the grape cluster, although it preferentially affects the extremities of clusters. Unlike bunch stem necrosis (BSN), BS is not accompanied by necrotic lesions on the rachis (Redl, 2007), which remains green and visually healthy. When analyzed, the rachises of BS-affected clusters do not present the same imbalances between potassium, magnesium, and calcium as do BSN-affected clusters (Bachteler *et al.*, 2013).

The BS sensitivity of grape cultivars is influenced by environmental factors, such as precipitation and temperature (Reisenzein, 1998; Raifer and Roschatt, 2001; Schumacher *et al.*, 2007), soil water-holding capacity (Spring and Siegfried, 2007) and vine-growing practices (Bachteler *et al.*, 2013; Raifer *et al.*, 2014). According to some authors, BS intensity may be reduced by working the soil to prevent its compaction, by appropriate potassium fertilization, by early yield regulation, and by integrated irrigation according to soil type (Fardossi, 2000; Redl *et al.*, 2009). Nevertheless, the causes of this disorder are not yet known, and to date, no known pathogen has been associated with BS symptoms (Krasnow *et al.*, 2009). The strong decrease in sugar and water accumulation in BS clusters seems to indicate a decline in phloem function, given that the influx of sugar and water in berries during the ripening phase (after veraison) depends primarily on the phloem flux (Greenspan *et al.*, 1994, 1996; McCarthy, 1999; Keller *et al.*, 2006). In general, berry water supply after veraison via the phloem compensates for water loss related to berry transpiration and water efflux called « reverse flow » via the xylem towards the stem (Tyerman *et al.*, 2004; Keller *et al.*, 2006). When the phloem flux is limited, water loss may exceed supply, and berries may start shriveling (Choat *et al.*, 2009).

The recent works of Bondada and Keller (2012) and Hall *et al.* (2011) show that the loss of rachis viability can be associated with berry-ripening disorders, especially with BS symptoms. These authors emphasized that the cessation of sugar and water accumulation in BS-affected berries was due to phloem death in the rachis or to a loss of phloem functionality. The cessation of xylem water flux to the berries could occur but would have little impact because a xylem connection with the shoot would not be necessary for good berry ripening. Water loss by berry transpiration and xylem efflux can suffice to explain BS symptoms (Hall *et al.*, 2011).

The goal of this study was to assess the physiological causes of BS through hydraulic conductivity measurements of rachises during the season and histological observations of the conducting tissues (xylem and phloem) in healthy and BS clusters. The measurements were made in the field on mature vines of a highly susceptible red variety (cv. Humagne rouge) subjected to different water regimes (controlled irrigation) during the season.

MATERIALS AND METHODS

1. Experimental site and plant materials

The experiments were conducted from 2000 to 2012 at the Agroscope research station in Leytron (Switzerland), located in an Alpine valley. The plant material was *Vitis vinifera* L. cv. Humagne rouge, an old grapevine variety cultivated in the valleys of Aosta (Italy) and Valais (Switzerland), grafted onto *Vitis berlandieri* x *Vitis riparia* cv. Kober 5BB rootstock. The vines were trained in a Guyot system (vertical shoot positioning) with a planting density of 5500 vines ha^{-1} (planting distance : 1.8 x 1.0 m). The experimental site in Leytron (46°11'N ; 7°12'E, altitude 525 m above sea level) lies on very stony (peyrosol with > 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. The average annual rainfall was 550 mm over the last 30 years. Temperature and precipitation data come from the meteorological station in Leytron located on the site of this experiment. Average daily temperature and the coefficient of variation (CV, ratio of the standard deviation to the mean) around the veraison period (10 days before and after veraison) were calculated for each year (table 1).

From 2000 to 2005, two different irrigation treatments were established. In the first treatment, 9 L m^{-2} (16 L per vine) was drip-fed weekly from bloom to veraison. The second treatment was not irrigated during the whole growing season. From 2006 to 2012 in the same experiment, a third irrigation treatment was added encompassing drip-irrigation (9 L m^{-2} or 16 L per vine and per week) from veraison to harvest. The experiment was carried out using 40 plants per treatment, set out in four randomized blocks of 10 vines each with buffer rows left and right.

2. Hydraulic measurements

Predawn water potential (Ψ_{PD}) was measured using a pressure chamber (Scholander *et al.*, 1965) between 8 and 10 times during the growing season from 2000 to 2012. Ψ_{PD} was measured on eight mature, undamaged and non-senescent leaves per treatment (two per replicate), between 04:00 h and 05:00 h in complete darkness. The rachis xylem hydraulic conductance (K_{rachis}) was measured with the XYL'EM device (Xylem embolism meter, Instrutec, Montigny-Les-Cormeilles, France), based on a high-resolution liquid mass flowmeter, according to Cochard *et al.* (2000).

For each field measurement of K_{rachis}, clusters were cut underwater from a shoot bent into a container filled with water. Healthy (n = 5) and BS (n = 5) clusters were collected from irrigated and non-irrigated vines that had suffered no water stress (Ψ_{PD} > -0.3 MPa). Only the main cluster rachis was kept for K_{rachis} measurement (secondary rachis were excised underwater) ; all berries and their pedicels were removed underwater using a razor blade. The rachis length was reduced to 12 cm. The mean berry number per cluster was 120 ± 10. The rachises were attached to the tubes of the XYL'EM device, and their initial hydraulic conductance was determined with a hydrostatic pressure gradient of approximately 3-4 kPa. Degassed distilled water was used as drip fluid for all measurements. To measure the maximum conductance (K_{max}), the rachises were « flushed » twice during a period of 2 minutes with 0.15 MPa of pressurized water. K_{rachis} measurements were performed on healthy clusters (no BS symptoms) and on BS clusters (clusters with all berries showing shrivel).

3. Anatomical and histological observations

Healthy (n = 4) and BS clusters (n = 4) were collected from irrigated vines (no water stress) during the ripening phase (two weeks before harvest) in 2008, and picked in water to avoid any incidences of embolism. Samples were never exposed to air during any stage of their preparation but were always maintained in a liquid medium. Rachis samples, which were prepared according to Roland and Vian (1991), were prefixed with a solution of 3 % glutaraldehyde-2 % paraformaldehyde in 0.07 M pH 7 phosphate buffer and postfixed with a solution of 1 % OsO_4. The samples were then dehydrated in a graded series of ethanol solutions of 30-50-70-95-100 % (v/v) and embedded in LR White resin (14381-UC London Resin Company). After polymerization (24 h at 60 °C), semi-thin (0.8 µm) and thin (0.08 µm) sections were cut and stained with a solution of 1 % methylene blue, sodium tetraborate and azure II or with 1 % toluidine blue for the semi-thin sections and with 2 % uranyl acetate followed by lead citrate (Reynolds, 1963) for the thin sections. Semi-thin sections were observed using a light microscope (Leica DMLB) equipped with a Leica DFC 490 FX camera. Thin sections were observed with a transmission electron microscope (Philips CM10) with a Mega View II camera.

4. Berry shrivel (BS) and agronomical observations

The BS intensity was estimated on all clusters of the 40 vines per treatment by evaluating the percentage of BS berries on each cluster (scale of 0-100 %). Berry weight, sugar content (g/L), titratable acidity (g/L, expressed as tartaric acid) and yeast assimilable nitrogen (YAN, mg/L) in berries were measured every week from veraison until harvest. Two hundred berries (50 per replicate) were collected weekly on healthy clusters as well as clusters with BS symptoms during the 2012 season. The analytical parameters were measured using WinScan (FOSS NIRSystems, USA) at the Agroscope laboratory in Changins, Switzerland.

5. Data analysis

Figures 1 to 6 were designed with Sigma Plot v11.0 ; figures 7 to 8 were designed using Photoshop 6.0

RESULTS

1. Sugar content, total acidity and yeast assimilable nitrogen in berries

During the 13 years of observation, the earliest BS symptoms were observed shortly after veraison (about 10 days) at the end of August. By the end of August and until harvest, sugar content was lower in BS clusters than in healthy clusters (fig. 1A). Indeed, sugar accumulation was severely limited during maturation in shriveled berries in comparison to healthy berries. During the ripening phase, titratable acidity was higher in shriveled berries than in healthy ones (figure 1C). YAN values were lower in BS clusters than in healthy ones (figure 1D). The relatively low YAN content is a characteristic of this particular grape variety. The weight of shriveled berries decreased gradually after veraison with a visible wilting to 1.1 g per berry at harvest, which is half of the final weight of healthy berries (figure 1B).

2. Water regime, temperature fluctuations and berry shrivel

Irrigation from flowering to veraison and from veraison to harvest generally favored the development of BS symptoms in comparison to non-irrigation (figure 2). Differences in BS intensity at harvest between irrigated and non-irrigated treatments were significant most of the years. The post-veraison irrigation treatment seemed to increase BS symptoms in 2007 and 2008 in comparison with pre-veraison irrigation (figure 2B). However, during especially dry years (2000, 2003 and 2004), no BS symptoms could be observed regardless of the irrigation level. Moreover, BS intensity was strongly correlated with vine water status during the veraison period (figure 3). The BS symptoms were more severe when the vine did not suffer from water stress (Ψ_{PD} > -0.2 MPa, irrigated vines, wet years). The BS intensity was much lower in a situation of moderate water stress (Ψ_{PD} from -0.3 to -0.8 MPa). Thus, vine water availability during the pre-veraison period and around veraison (during the beginning of the ripening stage (2-3 weeks after veraison)) seemed to play a key role in BS manifestation. Temperature conditions during veraison appeared to be decisive as well. Strong temperature fluctuations during the early ripening period (hot and dry periods alternating with cool and humid periods) seemed to accentuate BS symptoms

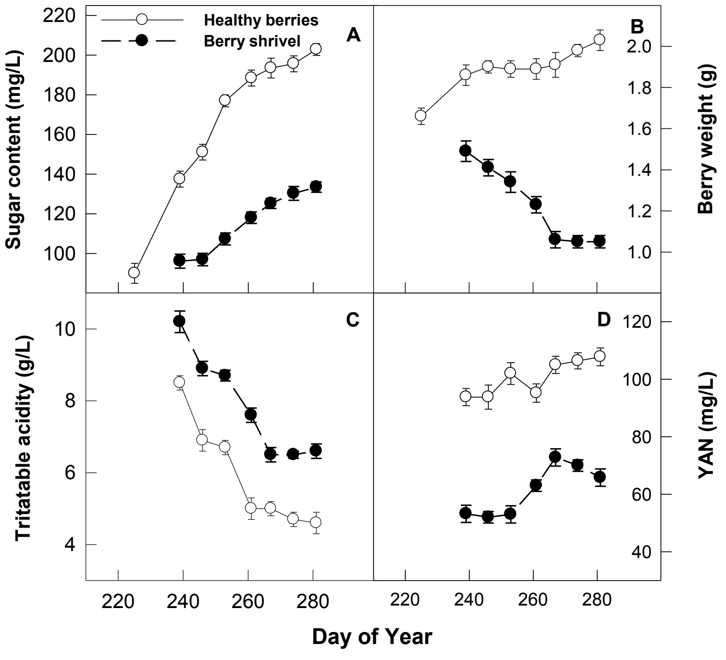

**Figure 1 – Sugar content (A), berry weight (B), titratable acidity (C) and yeast assimilable nitrogen (YAN) (D) in healthy berries and berries with symptoms of berry shrivel.
The beginning of veraison was at DOY 230. Humagne rouge, Leytron (Switzerland), 2012.**

(figure 4). The years 2005 to 2008 presented significant temperature fluctuations (table 1) around mid-August and high precipitations, often coinciding with the veraison period, which resulted in increased BS manifestations, especially in irrigated vines without water restriction. Very hot and dry seasons without important temperature fluctuations around veraison (years 2000, 2003, 2004 and 2009) were less favorable to berry shrivel development (table 1).

3. Rachis xylem hydraulic conductance (K_{rachis})

The 2008 and 2012 seasonal evaluations of K_{rachis} are presented in figure 5. In 2008, K_{rachis} was higher before veraison and then decreased during the grape ripening phase. In 2012, K_{rachis} was measured only during the post-veraison period. In 2008 and 2012, K_{rachis} remained slightly higher in healthy clusters in comparison with clusters presenting BS symptoms after the veraison period. No embolism event was detected in healthy and BS clusters: the initial K_{rachis} measured at very low hydrostatic pressure (3-4 kPa) was equivalent to the maximal K_{rachis} measured at 1.5 MPa in healthy and BS clusters.

4. Rachis phloem anatomy

Histological structures of rachises were compared between healthy symptomless and completely shriveled clusters (BS clusters) in two parts of the rachis (the peduncle and the middle of the rachis) using transmission electron microscopy. Semi-thin sections of a peduncle of BS clusters (figure 6) show the existence of a thick cell wall with elongated cells in the phloem section, corresponding to secondary phloem (hard phloem), as well as the occurrence of a secondary layer that splits the xylem part of the vascular bundle in two parts and the shrinkage of parenchyma cells and sclerenchyma. Thin sections of a shriveled peduncle made at the level of the primary phloem (figure 7) show an important disorganization of organelles and cell membranes as well as a thickening of the cell walls in comparison to a

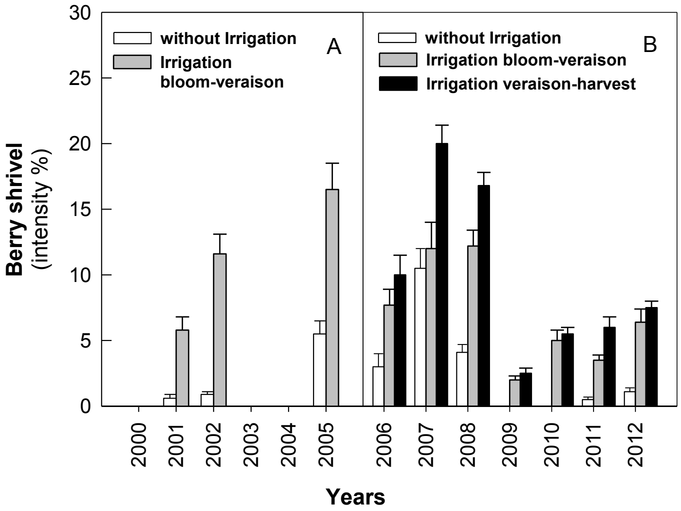

Figure 2 − Influence of irrigation treatment on berry shrivel intensity: (A) with irrigation from bloom to veraison and without irrigation (period 2000 to 2005), and (B) with the added irrigation treatment from veraison to harvest (period 2006 to 2012). Means ± standard error. Humagne rouge, Leytron (Switzerland), 2000-2012.

Table 1 − Daily mean temperature calculated around the veraison period (veraison ± 10 days) and the coefficient of variation (CV) for the daily temperature, and the precipitation during this period. Data from the meteorological station of Leytron (Switzerland), 2000-2012.

Years	Period veraison ±10 days	Daily mean temperature (°C)	Coefficient of variation for temperature (%)	Precipitation (mm)
2000	26.7-15.8	19.5	11.0	45
2001	27.7-16.8	20.4	12.1	44
2002	01.8-20.8	18.0	13.0	50
2003	11.7-31.7	22.8	8.5	16
2004	28.7-17.8	21.6	8.8	35
2005	24.7-13.8	19.5	17.0	53
2006	26.7-15.8	18.6	19.4	40
2007	01.8-20.8	18.7	16.2	64
2008	06.8-26.8	18.8	13.8	52
2009	21.7-10.8	20.9	6.7	27
2010	27.7-16.8	18.3	12.5	50
2011	23.7-12.8	18.8	13.0	18
2012	28.7-17.8	21.0	8.8	47

healthy peduncle. Semi-thin sections made at other levels of the rachis show very important degradation of the vascular bundles, essentially alterations of the primary phloem and xylem in comparison to healthy rachis (figure 8).

DISCUSSION

The earliest appearance of BS symptoms, observed shortly after veraison (end of August) in this study, was often associated with particular climatic conditions, such as when cool and wet periods alternated with sudden hot periods. Significant temperature amplitudes around mid-August (at the beginning of veraison) and high precipitations were observed particularly during the years 2005 to 2008, which resulted in increased BS symptoms. Very hot summers without strong temperature fluctuations around veraison time (years 2000, 2003, 2004, 2009) were less favorable for BS manifestations. Moreover, vine water status appears to play a key role in the manifestation of BS. Indeed, BS symptoms most frequently appeared in vines with no water restriction in comparison to vines with moderate or high water stress. The vine water status, assessed by the predawn leaf water potential, explained well the sensitivity of the cultivar Humagne rouge to BS appearance in this study. BS symptoms were generally less frequent in the Swiss vineyards during years with warm and dry ripening conditions and in vines grown in soils with either low water-holding capacity or relative high water stress during the season.

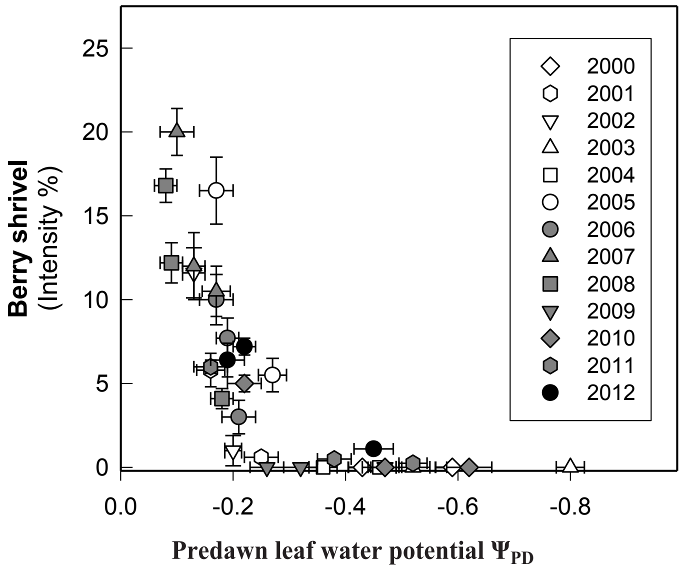

Figure 3 − Relationship between vine water status, measured by the predawn leaf water potential (ΨPD) at veraison (period of 2 weeks before and after veraison), and berry shrivel intensity. Means ± standard error. Humagne rouge, Leytron (Switzerland),2000-2012.

Low sugar content (Krasnow *et al.*, 2009) and shriveling symptoms (Bondada and Keller, 2012) observed in BS clusters suggest that disruption in sugar and water flows or partial loss in the vascular system functionality (xylem-phloem) happened most likely around veraison and during grape maturation (Hall *et al.*, 2011). The innovative work of Hall *et al.* (2011), using a xylem-mobile dye, showed that BS clusters were mostly unable to transport the dye through the rachis, in contrast to healthy clusters. The authors concluded that the cessation of sugar and water accumulation in BS clusters was associated with a loss of phloem functionality due to a decrease in cell viability in the rachis. Here, anatomical and histological observations seem to corroborate the results of Hall *et al.* (2011). Indeed, microscopic observations have shown an important degradation as well as a loss of functionality of the primary phloem in rachis of BS clusters in comparison to healthy clusters. These two events are both due to the disorganization of the cell contents as well as to the appearance of hard fiber or secondary phloem that is not functional. Even if the xylem tissue is not affected in the peduncle, an alteration of the primary xylem was observed in secondary branching of the rachis and in the middle of the main rachis. The histogenesis of secondary phloem or hard phloem in shoots is normally linked to periderm formation, which occurs slowly after veraison (Fournioux and Adrian, 2011). However, this process has not been studied in grape peduncles. In healthy clusters, this secondary phloem is not present ; all of the conducting bundles in the rachis have only primary phloem. Important questions remain open : which abiotic or biotic stress could initiate the periderm formation process, and what could induce the disorganization and degradation of the primary cell contents in shriveled grape peduncles ? It might be interesting to analyze whether the usual fungal community described on healthy grapevines (Hofstettler *et al.*, 2012) is still the same in BS-affected plants, or if physiological perturbations could change the equilibrium of this fungal community, contributing to the development of unusual microorganisms either secreting cytotoxins or eliciting the biosynthesis of phytotoxic secondary metabolites, such as phytoalexins, as a grapevine defense mechanism. Work is in progress to compare the

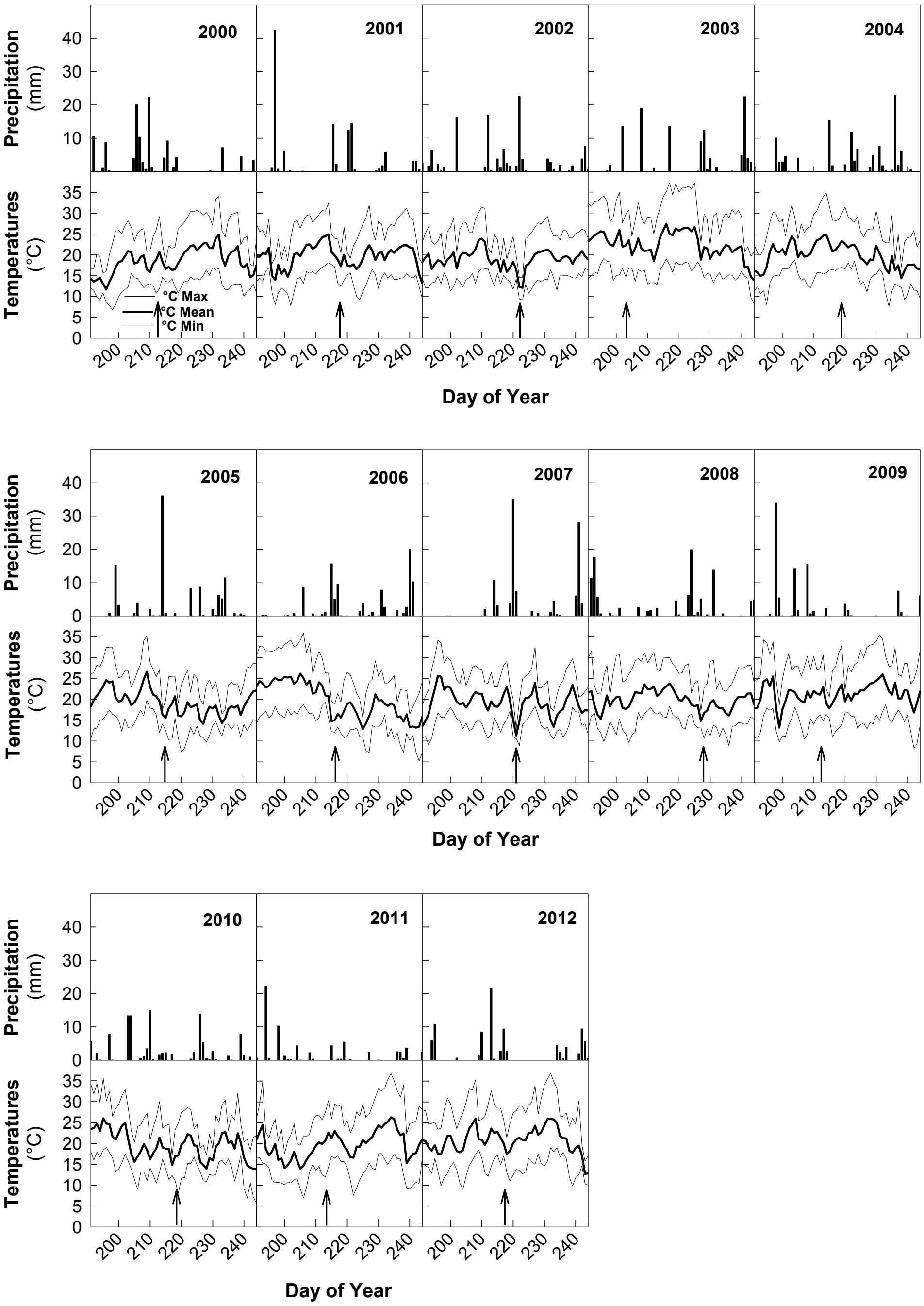

Figure 4 – Seasonal evolution in precipitation and temperatures (mean, maximum and minimum temperatures in °Celsius) in Leytron (Switzerland), 2000-2012. Arrows indicate the onset of ripening (veraison).

fungal communities of symptomatic and asymptomatic plants.

In our study, the anatomical and histological results obtained from the rachis conducting tissues are observations made only after the visible manifestation of BS symptoms. Further investigations are required to determine when the phloem loses its functionality in relation to cell viability and thus causes a decline in sugar accumulation in berries. Moreover, the very low transfer capacity of the xylem-mobile dye fuchsin through the rachises of BS clusters, highlighted by Hall *et al.* (2011), could indicate an increase in hydraulic resistance present in these rachises. In our experiment, K_{rachis} measurements have shown the rachis hydraulic conductance declining after veraison in comparison with the pre-veraison measurements. These observations confirmed the

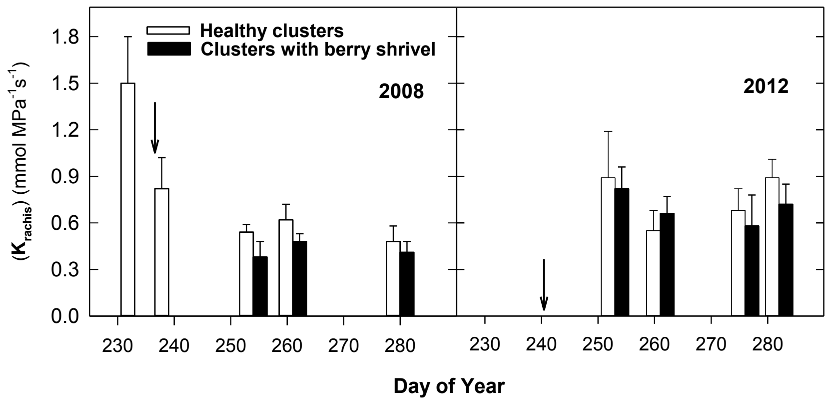

Figure 5 − Seasonal changes (pre- and post-veraison) in rachis hydraulic conductance (Krachis) for healthy clusters and clusters with berry shrivel symptoms. Means ± standard error. Arrows indicate the beginning of veraison. Humagne rouge, Leytron (Switzerland), 2008 and2012.

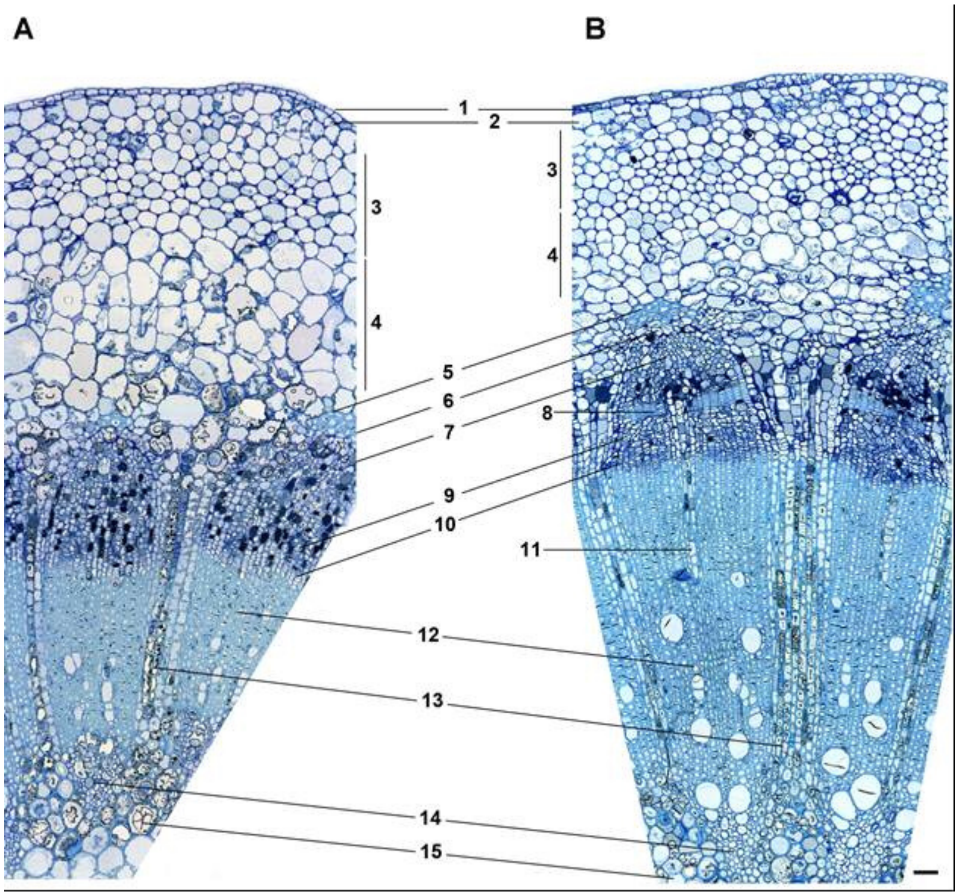

Figure 6 − Semi-thin sections of the peduncle of grape clusters stained with 1 % methylene blue, sodium tetraborate and azure II. A. Cluster without BS symptoms. B. Cluster with berry shrivel. 1. Epidermis, 2. Protoderm, 3. Collenchyma, 4. Parenchyma, 5. Phloem fiber cap, 6. Periderm (sclerenchyma), 7. Primary phloem, 8. Band of hard secondary phloem, 9. Band of soft secondary phloem, 10. Cambium, 11. Secondary shelf, 12. Secondary xylem, 13. Ray parenchyma, 14. Primary xylem and 15. Medulla. Scale bars represent 100 µm.

results of Tyerman *et al.* (2004) and Tilbrook and Tyerman (2009) on berry hydraulic conductivity. K_{rachis} values observed in BS clusters during the ripening phase were slightly lower than those observed in healthy clusters, nevertheless without statistical difference. Formation of new xylem tissue cannot be excluded during the post-veraison period.

Water flow through the xylem to the berry could decrease during the post-veraison period (Düring *et al.*, 1987), with the water requirement for berry transpiration (Dreier *et al.*, 2000) and berry growth being primarily provided through the phloem (Lang and Thorpe, 1989 ; Greenspan *et al.*, 1994, 1996 ; Ollat *et al.*, 2002). Choat *et al.* (2009) clearly highlighted a significant increase in hydraulic resistance in berry xylem only at the end of the ripening phase, associated with the deposition of a solute or gel in the xylem of many berry receptacles. Nevertheless, the work of Chatelet *et al.* (2008) showed that berry xylem remains intact and functional throughout berry development and capable of water transport during ripening. However, no embolism was observed in our study during the pre- or post-veraison periods. Among healthy and BS clusters, it also seems unlikely that tylosis in shoot vessels can lead to BS, but the possibility is not yet totally excluded (Bondada and Keller, 2012).

The microscopic examination of the xylem tissues in the peduncle revealed no particular abnormality in BS clusters, but alterations of the primary xylem was rather observed in the middle of the rachis and in the secondary rachis ramifications. Hall *et al.* (2011) noted that a functional xylem connection between the shoot and the peduncle was not necessary for berry ripening. According to these authors, impeded xylem flux would not be the cause of BS. The xylem flux could slow or even stop in the case of the death of the phloem and parenchyma cells surrounding the rachis xylem, leading to a reduction or a complete cessation of water transfer to the phloem on one hand and of the evaporation from the rachis surface on the other hand (Hall *et al.*, 2011). Furthermore, according to Greenspan *et al.* (1996), the berry water status is apparently unrelated to the plant water status after veraison. In the present study, berries started shriveling shortly (approximately 7 to 10 days) after a disruption in sugar accumulation was evident in BS clusters.

The water flux from the berries to the plant via the xylem, called « backflow », could constitute an important element in the berry weight loss (Greenspan *et al.*, 1996 ; Tyerman *et al.*, 2004), particularly if the phloem stops being functional. Phloem influx can indeed cause « back pressure » leading to xylem water backflow due to the limited extensibility of the berry skin (Matthews *et al.*, 1987, 2009 ; Keller *et al.*, 2015), particularly when the phloem influx exceeds berry growth and transpiration. In theory, xylem water backflow can only occur when the xylem is functionally connected to the berry apoplast and when a suitable gradient exists to drive water flow from the berry to the vine (Tyerman *et al.*, 2004). According to Choat *et al.* (2009), fruits or berries are not hydraulically isolated from the rest of the vine but are rather « hydraulically buffered » by phloem water influx. Given the current state of knowledge, we do not know if the xylem water backflow plays an important role in the decline in sugar accumulation and berry shriveling in BS clusters. The loss of phloem functionality causing the decrease of water influx in the berry, in combination with berry transpiration, may suffice to explain the shriveling observed in BS clusters.

Furthermore, Bondada and Keller (2012) showed that berries of BS clusters have high osmotic potential (Ψ_S), mainly related to an inability of the parenchyma cells to accumulate sugars in comparison with healthy clusters. This phenomenon is reflected by poor cluster sink strength for photoassimilates and/or a sharp decline in the phloem's ability to transport sugars into the berries (death of rachis phloem cells). In addition, besides sugars, the role of cations such as potassium (K), magnesium (Mg) or calcium (Ca) in Ψ_S decrease is widely documented (Mpelasoka *et al.*, 2003). Experiments carried out on soil fertilization and its impact on BS manifestation (Bachteler *et al.*, 2013) showed a higher Ca concentration in BS-affected clusters that was also found in the BS rachis (Krasnow *et al.*, 2009). On the other hand, the K content in shriveled berries tended to be lower than in healthy berries (Mehofer and Regner, 2010 ; Bondada and Keller, 2012 ; Bachteler *et al.*, 2013). Because K is primarily imported via the phloem, the disruption of the phloem stream or even its cessation could contribute to BS triggering. Conversely, Ca is mainly carried via the xylem and its maximum concentration is reached 30 to 60 days after flowering (Rogiers *et al.*, 2006). Disruption in the xylem after appearance of BS symptoms (post-veraison) would not have much effect on Ca accumulation in berries.

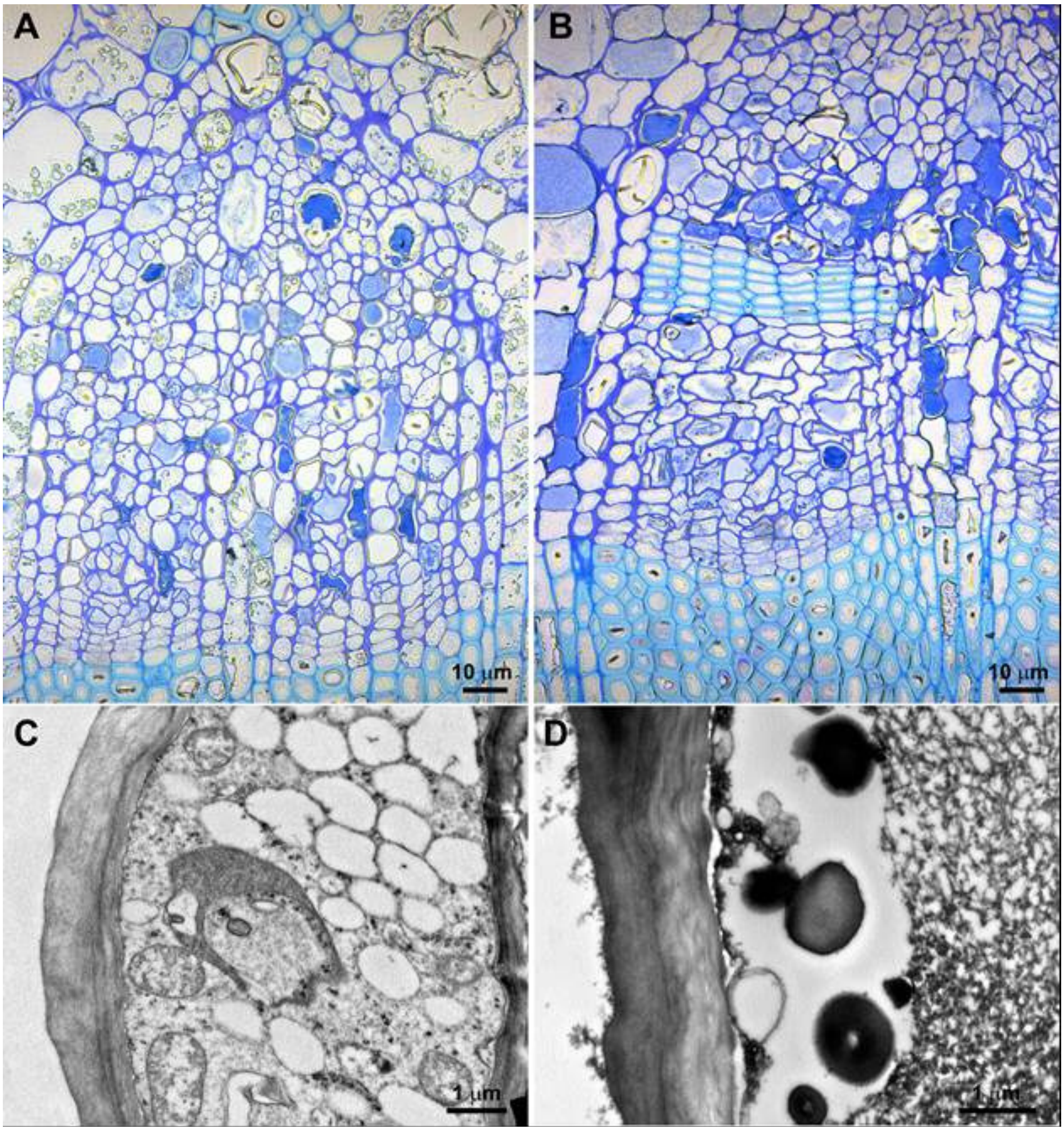

Figure 7 – Semi-thin sections of the peduncles of grape clusters stained with 1 % methylene blue, sodium tetraborate and azure II and thin sections made at the level of the primary phloem. A. Cluster without symptoms. B. Cluster with berry shrivel. C. Primary phloem cells of asymptomatic cluster. D. Primary phloem cells of berry shrivel cluster.

Further investigations on berry vascular functionality (xylem-phloem) in relation with the plant and environmental factors are required to better understand the causes of this grape-ripening disorder and to develop preventive and appropriate management strategies.

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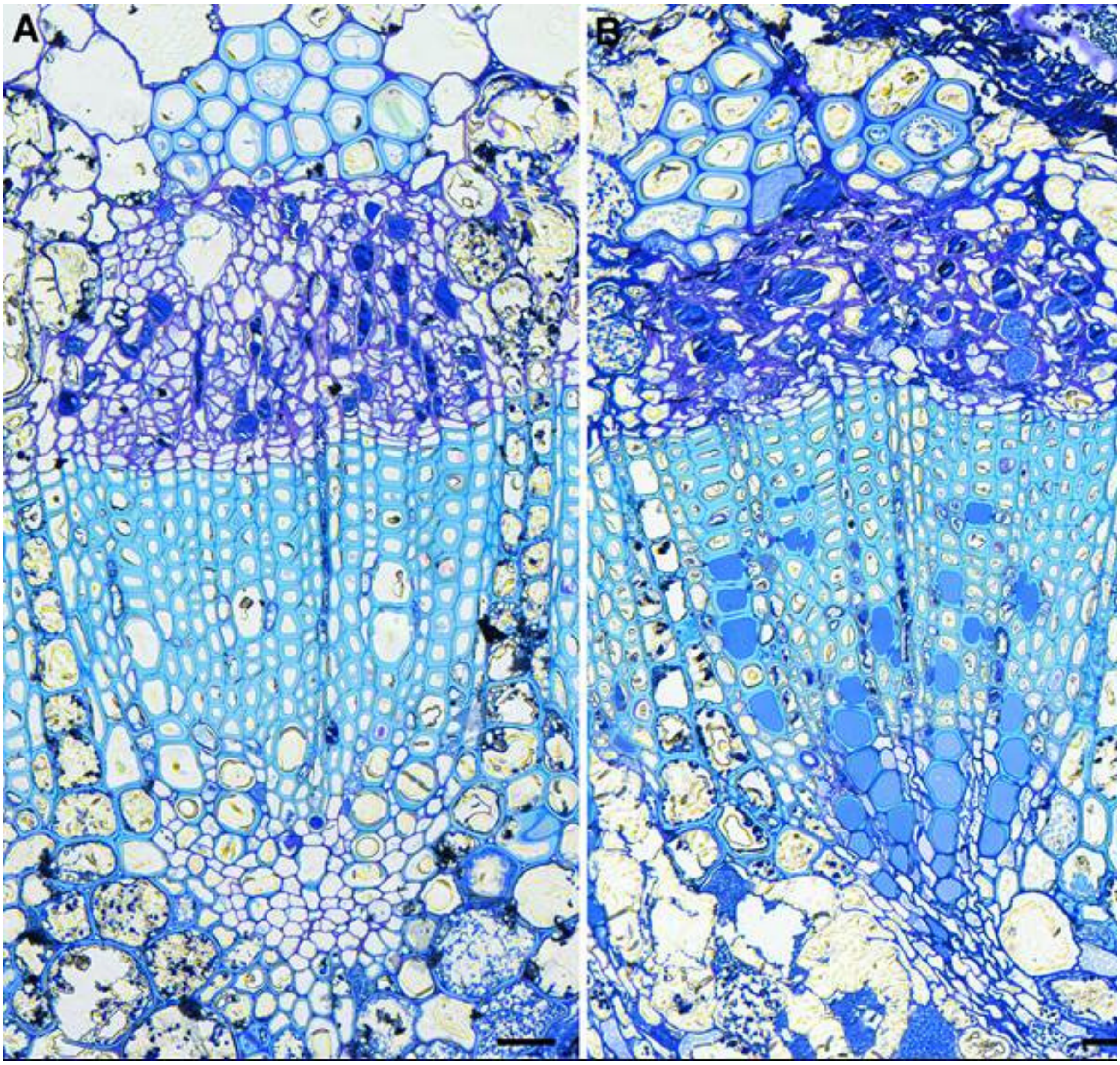

Figure 8 – Semi-thin sections made from the rachis of grape clusters stained with 1 % methylene blue, sodium tetraborate and azure II. A. Healthy rachis. B. Rachis of berry shrivel cluster showing degradation of primary phloem and xylem. Scale bars represent 10 µm.

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