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Influence of leaf canopy height on the occurrence of berry shrivel

B. Raifer, F. Haas and A. Cassar

Research Centre for Agriculture and Forestry Laimburg, Auer, Italy

Summary

Berry shrivel (BS) is a physiological disorder of grapevine that causes severe grape quality losses. The origin of BS is still unknown. In a trial with different treatments of the leaf canopy, a higher BS incidence in the plots with reduced leaf area was observed. Therefore it was investigated, whether a strong reduction of the canopy height regularly promotes BS development. Further, berry and leaf analyses should give evidence of possible changes in mineral contents which could be related to BS incidence. In three out of four trials, a strong reduction in leaf canopy height at the beginning of berry ripening, resulted in the appearance of berry shrivel (BS) symptoms. Intensive shoot topping during berry ripening therefore strongly increases the likelihood of BS occurrence, but does not always result in the appearance of BS symptoms. At the end of the berry ripening period, leaves in the reduced canopy height treatment (RCH) showed higher nitrogen content and lower potassium content than those in the normal canopy height treatment (NCH), suggesting a high and maybe also passive absorption of nitrogen, which hinders the absorption of potassium. These findings open new approaches for further studies on the causes of BS.

K e y w o r d s : berry shrivel, canopy height, nitrogen, potassium, viticulture.

Introduction

Berry shrivel (BS), also known as SAD (sugar accumulation disorder) (Krasnow *et al.* 2009) or sour shrivel (Bondada and Keller 2012a), is a physiological disorder of grapevine. In South Tyrol (Italy) it has been detected with increasing incidence and severity since the year 2000. BS has also been recorded in other grapevine growing areas of the Alps, especially in Austria, Switzerland, and Friuli (Italy). The disorder is present also in some grapevine growing areas of the USA (California and Washington state) (Krasnow *et al.* 2009, Bondada and Keller 2012a and b), and in many other grapevine growing areas throughout the world, although to a lower extent.

BS symptoms can be observed from the beginning of berry ripening up to harvest. The major symptoms consist in more or less pronounced shrivelling of berries, stunted berry ripening, and the development of unripe up to very unpleasant green, herbaceous flavours (Krasnow *et al.* 2009, Bondada and Keller, 2012a and b). Unlike in the case of bunch stem necrosis, at least at the first appearance of BS, no apparent changes in the rachis are visible (Bondada and Keller 2012a and b). Later on, shrivelling of portions of the rachis or even withering of the entire rachis may be observed, but frequently the rachis remains green for weeks without showing any evident symptoms.

Affected berries shrivel and may even appear wrinkled. The appearance of the symptoms of BS goes along with mesocarp cell death (Krasnow *et al*. 2008, 2009). The clearly visible symptoms on berries are preceded by a variably long-lasting period of interruption of sugar accumulation in berries (Knoll *et al.* 2010). The first symptoms of stunted berry development may appear a few days after the beginning of berry ripening (in South Tyrol frequently already at the end of July). The disorder is definitely restricted to the ripening stage, and affected berries contain viable seeds (Hall *et al.* 2011). Recent studies showed that cell death is not restricted only to cells inside the berries, but may affect also the phloem cells of the rachis (Hall *et al.* 2011). Remarkable similarities between bunch stem necrosis (also known as waterberry) and BS emerge, and it can thus be assumed that a relation between the two disorders may exist.

Sometimes also healthy bunches on plants affected by BS show a considerably lower content of sugar than bunches on healthy plants. Krasnow *et al.* (2009) therefore suggested that BS may be a disorder of the entire plant and not only of the affected bunches. In addition, Krasnow (2009) found evidence for the fact that BS can be transmitted via grafting of buds from affected vines, but up to now no causal agent has been detected.

Usually yield losses due to BS do not exceed 10 %, but may reach up to 20 % in years of heavy incidence. More consistent losses have been observed only in isolated cases (Krasnow *et al.* 2009). Thus, direct yield losses are usually a limited problem. However, affected bunches must be removed in order to prevent impairment of the quality of the harvested grapes and consequently of the wine.

Even though in recent years the knowledge on BS has increased, the actual causes of this disorder are still unknown (Krasnow *et al.* 2010, Bondada and Keller 2012b). Studies on BS are difficult to conduct due to the erratic and unpredictable occurrence of the disorder, and often fail because of the absence of symptoms.

However, striking differences in onset and incidence of BS among adjacent vineyards of the same cultivar, in which different agricultural practices are used, may be observed. It can thus be concluded that management practices strongly influence the occurrence of BS (Smith 2003). We noticed that vineyards affected by BS over several years

Correspondence to: Dr. B. Raifer, Research Centre for Agriculture and Forestry Laimburg, Laimburg 6, 39040 Ora/Auer (BZ), Italy. Fax: +39-0471-969-599. E-mail: Barbara.Raifer@provinz.bz.it

can remit symptom appearance, also in years of heavy disorder incidence, if different agricultural management practices are used. However, it was not yet possible to identify the reasons for this phenomenon, which was also observed by Redl (2008). Differences in BS incidence among years can be observed: in some years the symptoms are widespread throughout the vineyards of a wine-growing area, while in others the disorder appears sporadically or is restricted to parts of the wine-growing area. Within the same grapevine-growing area, the symptoms usually appear almost at the same time within an interval of a few days.

In 2010, at the Research Centre Laimburg (Auer, Italy), different leaf canopy management practices were tested in a vineyard (Stadelhofleiten) of the cultivar 'Gewürztraminer', and a noticeably higher incidence of BS was observed in the treatment which provided for strongest leaf canopy reduction. It can thus be assumed that the unfavourable leaf/fruit ratio may have strongly contributed to the appearance of BS and may have promoted the same mechanisms involved generally in BS development.

The studies conducted in 2011 and 2012 aimed at investigating whether a significant reduction of the leaf canopy at the beginning of ripening can be considered a major trigger for the occurrence of BS. Additionally, first investigations on the impact of the leaf canopy height on mineral accumulation in leafs and berries were carried out.

Material and Methods

Study sites: As mentioned above, in 2010 BS symptoms appeared in a vineyard at the Research Centre Laimburg (46°22'N; 11°17'E), in which different leaf canopy management practices were studied. This vineyard, located on a light slope facing east, was planted with the cultivar 'Gewürztraminer', clone Lb 14 and rootstock SO4, and rows were oriented in slope line. The vertical shoot positioned (VSP) vineyard has been planted in 2006. The vines were pruned during dormancy to one horizontal cane with 9 to 11 buds and one spur with two buds. The base wire that supports the canes was 0.9 m aboveground and 3 pairs of training wires at a distance of 30 cm were used to keep shoots in vertical position. Distance between rows and vines were respectively 2.0 and 0.9 m.

Infertile shoots and shoots in a distance less than 6-7 cm as well as shoots not located on the spur or the cane were removed at ~20 cm shoot length. Crop level was adjusted by cluster thinning at veraison in order to achieve a yield of 8 t·ha^{-1}. The vineyard was not irrigated and the soil management was permanent resident vegetation between rows with herbicide treatments in the row. Climatic conditions (temperature, rainfall and global radiation) recorded during the study period in the three study years 2010-2012 are reported in Tab. 1.

A randomized block design with 3 replicates of 25 plants each per treatment was used. The standard treatment (Standard) consisted in conventional shoot topping as soon as the shoots had grown through the top pair of wires and started to bend downwards. Another treatment (Intensive topping) consisted in similar shoot topping, but shoots were topped more frequently, and a lower amount of leaf mass was removed at each shoot topping. In an additional treatment, all side shoots were removed after fruit set (Lateral shoots eliminated). In one other treatment (NCH = Normal canopy height) no shoot topping was carried out, and the shoot tips were curled around the top pair of wires. Finally, in one additional treatment (RCH = Reduced canopy height) the leaf canopy was kept low by topping shoots just above the second instead of the third (top) pair of training wires.

In 2011 and 2012, the two most extreme leaf canopy management practices, NCH and RCH were tested again, 2011 in the already described 'Gewürztraminer' vineyard and additionally in a nearby 6-year old 'Pinot blanc' vineyard. In 2012, the trials were carried out again in the same 'Pinot blanc' vineyard and in a 12-year old 'Pinot gris' vineyard distant about three kilometres from the Laimburg Research Centre and located in Kaltern/Mazzon (Bozen, Italy). Both the 'Pinot blanc' (clone Lb 16, rootstock SO4) and the 'Pinot gris' (clone unknown, Rootstock SO4) study vineyards were located in a steep slope and were thus terraced (vines were spaced 0.8 x 2 m and 0.8 x 2.1 m, respectively). The row orientation was in both cases north-south, the training system VSP with the base wire that supports the canes at 0.9 m aboveground and 3 pairs of training wires at a distance of 30 cm for keeping shoots in vertical position. The cultural practices in the 'Pinot blanc' and 'Pinot gris' vineyards were consistent with those of the 'Gewürztraminer', with the difference that the target yields were in both cases 10 t·ha^{-1}. The 'Pinot blanc' and the 'Pinot gris' vineyards were provided with an irrigation system and were irrigated as necessary.

Soils of all three vineyards of the study were classified as Dystric Eutrochrepts, coarse loamy, mixed, mesic, with

Table 1

Climatic conditions (temperature, rainfall and global radiation) recorded during the study period in the three study years 2010-2012 (data recorded by the weather station of the Research Centre Laimburg)

	Mean monthly temperature at 2 m (°C)			Monthly rainfall (mm)			Global radiation (J/cm²)		
	2010	2011	2012	2010	2011	2012	2010	2011	2012
June	21.3	20.1	21.9	63.4	96.2	100.2	69,912	57,588	68,069
July	24.7	21.2	22.5	30.6	84.6	96.4	78,522	70,362	70,263
August	21.3	22.8	23.4	173.4	70.2	74.3	59,586	64,170	63,395
September	16.3	19.3	17.6	122.3	141.0	119.0	44,712	45,156	41,160

a depth of 1.5 to 1.7 m (55-65 % sand, 30-35 % silt, 5-15 % clay, organic matter 2.5-2.7 %, pH (H_2O) 6.7-7.0) .

In the 2011 trials, a randomized block design with 3 replicates of 35 plants each per treatment was used. In 2012 twenty-four plants, similar in size and development, were selected, and 12 plants each were randomly assigned to one of the two treatments, NCH and RCH.

Timing of shoot topping: In 2010, the different leaf canopy management practices (*i.e.* treatments) were initiated immediately after flowering. The first shoot topping in the RCH treatment was carried out 8 d after full flowering (BBCH 65), and repeated every time the majority of the shoots had reached the third pair of wires by topping them at a few cm above the second pair of wires.

In the trials conducted in 2011 and 2012, the intensive shoot topping in the RCH treatment (Reduced canopy height, the leaf canopy was kept low by topping shoots just above the second instead of the third pair of training wires) was performed later, shortly after the beginning of ripening stage (BBCH 81). In the 2011 and 2012 trials, no shoot topping was conducted in the NCH treatment after majority of berries touching stage (BBCH 79). The tips of the shoots, instead, were curled around the top pair of wires, when deemed necessary.

In 2011, at the end of July, a strong growth of side shoots occurred due to frequent rainfalls and exceptionally low temperatures considering the season (Tab. 1). To prevent impairment of the effects of reduced leaf canopy height in the RCH treatment by excessive leaf development on side shoots, all side shoots from the mid August were removed in both grapevine cultivars 'Pinot blanc' and 'Gewürztraminer'.

In 2012, in the RCH treatment, no new leaf growth was observed after the first shoot topping. The exact dates on which shoot topping practices were carried out in the RCH treatments in the different study years, are reported in Tab. 2.

Assessments

Effects of NCH and RCH on leaf area: To assess for the effects of the NCH and RCH treatment on leaf area, the number of leaves present on 12 main shoots randomly selected from 12 plants was counted, and the mean number of leaves per main shoot was determined. In addition, the potential exposed leaf area (SFEp) of the two treatments was established according to Carbonneau (1995).

BS onset and incidence: In 2010, very first symptoms of BS were observed on August, 12. Thus, the first assessment was conducted on that date by inspecting all bunches within each plot for BS, and by counting the number of healthy and affected bunches, and marking affected bunches. Additional assessments were carried out twice a week during the entire study period to document BS development. BS incidence (= % bunches affected by BS) at the final assessment (at harvest) was determined.

In 2011, from the onset of berry ripening, the entire study area was checked for BS onset and development three times a week, by walking slowly along the rows and checking each single bunch. Bunches with symptoms of BS were touched and checked for their turgescence. Clearly affected bunches were marked and counted in order to determine BS incidence (= % affected bunches) as in 2010. In addition, in 2011 in both study vineyards, we distinguished between bunches with light and heavy BS symptoms as follows: bunches with small berries, grayish-green in colour, with lack of turgescence and showing considerable loss of water once removed from the rachis and lightly pressed, were considered bunches with light symptoms, while bunches with wrinkled berries were classified as bunches with heavy symptoms. The percentage of bunches with light and heavy BS symptoms was then calculated.

In 2012, BS onset was determined and BS development was followed as in 2011. Since each plot consisted of a single plant, the BS incidence at harvest was established by counting the number of healthy and affected bunches per plant, and by calculating BS incidence per plant.

Analysis on berries and leaves: In all study years several analyses on asymptomatic leaves and berries, were conducted.

Within each plot, 150 randomly selected berries were collected and then brought to the laboratory to measure: sugar content (°Babo) in must by using a digital refractometer (RFM 840 from Bellingham & Stanley Ltd., United Kingdom), total acidity and pH value by using a tritator with pH electrode (titrator Titromatic 1S from Crison Instruments SA, Spain) and yeast assimilable nitrogen (YAN) *via* both enzymatic and photometric (at 340 nm) reading using the equipment Crony Saturno 300 from Crony Instruments, Italy. Analyses of mineral elements in leaf and berry samples were conducted in the laboratory of the Research Centre Laimburg. 30 leaves and 75 berries per plot were dried and grinded (0.45 µm). Then 4 mL of

Table 2

Beginning of ripening and timing of shoot topping in the RCH-treated plots in 2010, 2011 and 2012

Trial (cultivar, year)	Beginning of ripening	Timing of shoot topping
Gewürztraminer 2010	27.07.2010	08.06.2010, 21.06.2010 and 03.07.2010
Gewürztraminer 2011	17.07.2011	03.08.2011 and 16.08.2011
Pinot blanc 2011	20.07.2011	03.08.2011 and 16.08.2011
Pinot blanc 2012	21.07.2012	25.07.2012
Pinot gris 2012	23.07.2012	26.07.2012

nitric acid (63 % Merck) were added to 0.3 g of grinded leaves/berries. The microwave assisted acid digestion of the organic matrices according to EPA method 3052 was conducted with the microwave digestion laboratory system Ultrawave from Milestone S.r.l, Italy. The concentration of the elements phosphor, potassium, calcium, magnesium, boron, iron, manganese, copper and zinc within the samples was determined by using ICP-OES (Inductively Coupled Plasma Optical Emission Spectroscopy). To determine the content in nitrogen, a nitrogen analyzer (TruSpec N from LECO Corporation (USA)) was used.

Statistical analysis: The assessed parameters were compared across treatments using analysis of variance (ANOVA), followed by Tukey's-b test for post-hoc comparison of means. All analyses were performed with PASW SPSS statistics software version 17.02 (IBM Corporation, USA).

Results

Effects of NCH and RCH on leaf area: The potential exposed leaf area (SFEp) according to CARBONNEAU (1995) and the mean number of leaves on the main shoots in the different treatments are reported in Tab. 3. SFEp in the NCH treatment amounted to approximately half the value recorded in the RCH treatment.

In 2011, evident symptoms of physiological disorder appeared also on leaves in the RCH treatment in the 'Gewürztraminer' trial (Fig. 1). Leaves showed necrosis on edges and between the veins, and abnormal purple to brownish coloration. Abnormal coloration was observed in the RCH treatment also in 2012 on both 'Pinot blanc' and 'Pinot gris', but it was not as evident as in 2011. At the end of the ripening period, nitrogen content was higher and potassium content was lower in leaves of the RCH treatment than in those of the NCH treatment (Fig. 2a and b).

BS onset and incidence: In 2010, the first clear symptoms of BS were observed on 12 August, which was 16 d after beginning of berry ripening. Affected bunches showed evident symptoms. However, no additional increase in BS incidence was detected during the successive assessments. At harvest, BS incidence was significantly higher in the RCH treatment than in the other tested treatments (Fig. 3).

Fig. 1: Symptoms of physiological disorder on leaves of the cultivar 'Gewürztraminer' in the RCH treatment in 2011. The leaves shows purple to brownish coloration, partly also yellowing and necrotic spots. This symptoms were not commonly observed in vines affected with BS, at least not to this extent.

In 2011, on the cultivar 'Pinot blanc' BS symptoms became evident late in the season, a few days before harvest, but the rachis of the bunches was never affected. On the cultivar 'Gewürztraminer', instead, first BS symptoms were observed already on August, 10. At harvest, portions of the rachis appeared withered and berries on bunches affected by BS early in the season appeared heavily wrinkled. Thus, at the end of the ripening period symptoms were similar to those of bunch stem necrosis. As on the cultivar 'Pinot blanc', also on the cultivar 'Gewürztraminer' an additional increase in BS incidence was recorded just before harvest in the RCH treatment, while no symptoms were observed in the NCH treatment (Fig. 4 a and b).

In 2012, on the cultivar 'Pinot gris' BS symptoms were observed neither in the NCH treatment nor in the RCH treatment. Also on the cultivar 'Pinot blanc' no BS symptoms appeared in the NCH treatment, while on the cultivar 'Pinot blanc' only four from total twelve plants in the RCH treatment were free of BS symptoms. Five plants showed BS on one to three grapes per vine, and three plants were heavily affected by BS (9 grapes from on the whole 18 grapes of the vine, respectively 9 from 12 and

Table 3

Mean number of leaves/main shoot and potential exposed leaf area (SFEp) in the NCH and RCH treatment

Trial(cultivar year)	Mean n. leaves/main shoot		Potential exposed leaf area (SFEp)	
	NCH	RCH	NCH	RCH
Gewürztraminer 2010	-	-	1.54b	0.80a
Gewürztraminer 2011	17.9b	9.3a	1.42b	0.73a
Pinot blanc 2011	15.6b	7.2a	1.45b	0.78a
Pinot blanc 2012	17.3b	8.6a	1.38b	0.69a
Pinot gris 2012	19.0b	8.5a	1.23b	0.75a

Different letters within the same column and for the same cultivar and year indicate statistically significant differences ($P = 0.05$).

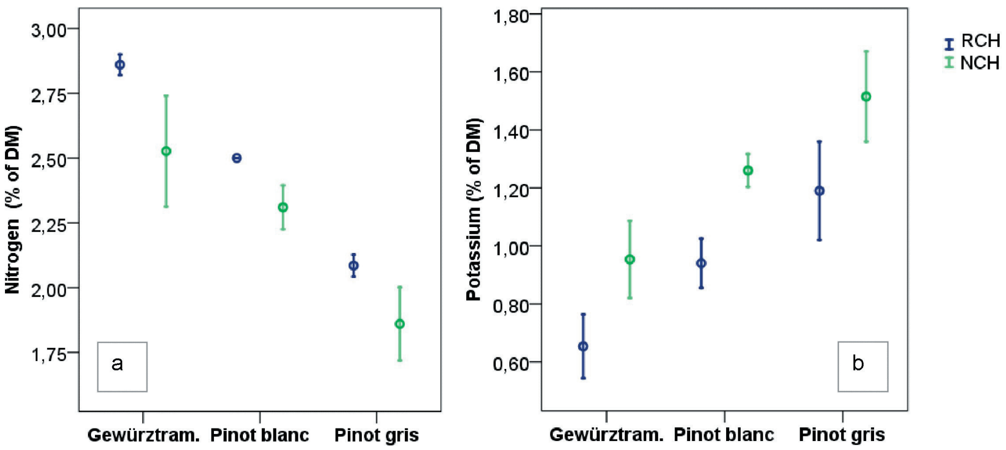

Fig. 2: Nitrogen (a) and potassium (b) content (mean ± 2 s.d.) in leaves of the cultivars 'Gewürztraminer' (2011), 'Pinot blanc' (2012), and 'Pinot gris' (2012) at the end of the berry ripening period (NCH = Normal canopy height, RCH = Reduced canopy height).

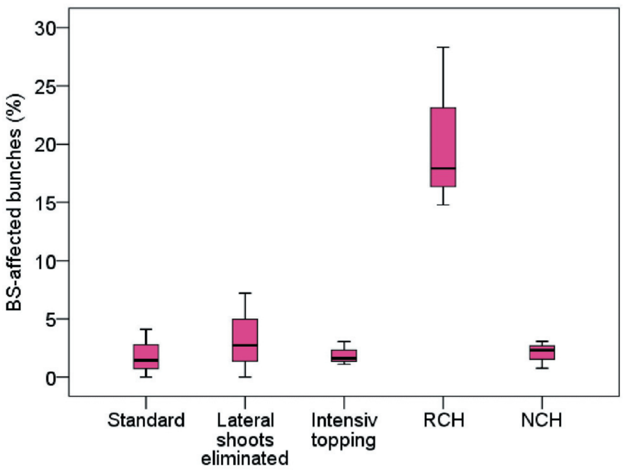

Fig. 3: Berry shrivel incidence (% affected bunches) in the different treatments in 2010.

10 from 13). On this cultivar, in the RCH treatment, also bunches without any clear BS symptom and thus apparently healthy, showed some sort of physiological disorder. In fact, the sugar content of these asymptomatic bunches in the RCH treatment was significantly lower than in the NCH treatment (Tab. 4).

Analysis of berries and leaves: Nitrogen and potassium content of leaves and berries, yeast assimilable nitrogen (YAN), and sugar content recorded just before harvest in the RCH and NCH treatment for the different cultivars in the three study years are reported in Tab. 4. Except for 'Pinot blanc' in 2012, where all bunches appeared to be slightly affected by some sort of physiological disorder, assessments were always carried out on asymptomatic and thus apparently healthy leaves and berries. Nitrogen content in leaves, when assessed (Tab. 4), was always significantly higher in RCH than in NCH. Except for 'Pinot blanc' in 2012, yeast assimilable nitrogen in must was higher in RCH than in NCH, with differences being significant on 'Gewürztraminer' in 2011 and on 'Pinot gris' in 2012. For the nitrogen content in berries, no similar trend was observed: nitrogen content in berries was either comparable among treatments or lower in the RCH than in the NCH treatment. Potassium content in leaves, when assessed, was always significantly lower in RCH than in NCH. Also in berries the potassium content was always lower in the RCH treatment than in the NCH treatment (slightly though not significantly lower in the 2010 and 2011 trials, and significantly lower in the 2012 trials).

For all the other assessed parameters, no statistically significant differences between the RCH and NCH treatment emerged, and results are therefore not shown.

Discussion

Considerable leaf canopy height reduction (RCH) resulted in remarkable BS incidence in comparison to no leaf canopy height reduction (NCH) in three out of four trials in 2011 and 2012. No BS symptoms were observed in the NCH treatment in the four studies. Therefore, strong leaf canopy height reduction definitely favours the occurrence of BS, but does not necessarily result in the appearance of BS symptoms. Kührer and Gabler (2012) obtained similar results by showing that a low ratio leaf area/yield is related to increased BS incidence.

Common agricultural practices in South Tyrol involve intensive shoot topping at the beginning of berry ripening, so that possibly no additional shoot topping must be performed up to harvest. Given the results of our studies, intensive shoot topping at berry ripening should definitely be avoided. However, in order to provide generally applicable indications on how and when shoot topping should be performed, the underlying mechanisms causing BS must be determined.

Given the results of our 2011 and 2012 trials, it can be supposed that increased BS incidence may rather be associated with a high and perhaps passive nitrogen absorption during berry ripening. Currently several indications exist that BS may be associated with excessive nitrogen supply, especially if one assumes that there might be parallels between BS and bunch stem necrosis (Hall *et al.* 2011). Krasnow *et al.* (2010) stated that BS symptoms could be triggered by toxic ammonium accumulation. These authors

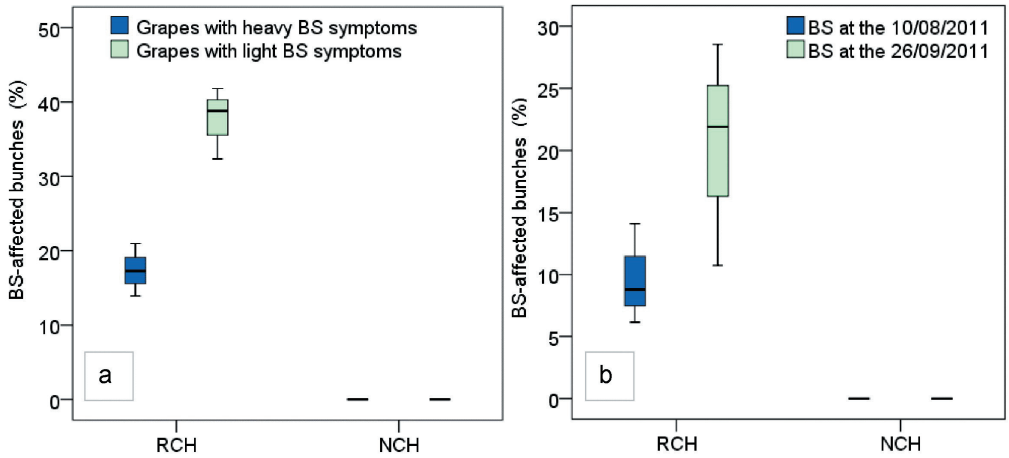

Fig. 4: Percentage of bunches with berry shrivel incidence in the NCH and RCH treatment in 2011 on 'Pinot blanc' (a) and Gewürztraminer (b) at harvest.

Table 4

Mean nitrogen and potassium content in leaves (% dry weight) and berries (mg/berry), mean yeast assimilable nitrogen (YAN) in must (mg·L^{-1}), and mean sugar content (°Babo) in the NCH and RCH treatment for the different cultivars in the 3 study years

Cultivar/year	Leaf-N (% dw)		Must-YAN (mg/l)		Berry-N (mg/berry)		Leaf-K (% dw)		Berry-K (mg/berry)		Sugar (°Babo)	
	NCH	RCH	NCH	RCH	NCH	RCH	NCH	RCH	NCH	RCH	NCH	RCH
Gewürztraminer 2010	*	*	160a	211a	2.06a	2.15a	*	*	3.11a	2.94a	20.2a	19.8a
Gewürztraminer2011	2.53a	2.86b	311a	490b	2.76a	2.87a	0.95b	0.65a	5.83a	5.54a	20.3b	17.7a
Pinot blanc 2011	*	*	206a	251a	2.81a	2.68a	*	*	5.48a	4.61a	19.4b	16.7a
Pinot blanc 2012	2.31a	2.50b	194a	180a	2.35b	2.06a	1.26b	0.94a	4.51b	4.04a	19.5b	15.5a
Pinot gris 2012	1.86a	2.09b	111a	137b	1.84b	1.42a	1.51b	1.19a	4.07b	3.03a	21.5b	19.1a

* Data not assessed.
Different letters within the same column and for the same cultivar and year indicate statistically significant differences ($P = 0.05$).

found significant changes in the spectrum of amino acids of BS-affected berries compared to healthy berries, and considerably higher ammonium content in BS-affected than in healthy berries, but the total nitrogen content was lower in BS-affected than in healthy berries. However, KRASNOW *et al.* (2010) initiated their analysis only after BS symptoms were already present. It is therefore not clear whether the observed changes in ammonium content are causes or consequences of BS. GYSI (1983, 1984) carried out open-field trials and experiments on potted plants, and showed that bunch stem necrosis definitely increased with increased nitrogen supply. CHRISTENSEN and BOGGERO (1985) found considerably increased nitrogen and ammonium content values in the rachis of bunches affected by bunch stem necrosis. They showed that the incidence of the disorder was always higher in the areas of the vineyard with higher nitrogen supply. However, in ad hoc plant nutrition trials, bunch stem necrosis occurred only in some, but not in all trials, maybe because the influence of climatic conditions concealed the effects of plant nutrition on bunch stem necrosis. In addition, no scientific evidence has yet been found for a direct relation between increased nitrogen absorption and increased ammonium content in rachises of bunches affected by bunch stem necrosis. On the contrary, studies conducted by KELLER (1995) showed that the increased ammonium content may be due to degradation of tissues and proteins as a result of carbohydrate depletion.

The higher nitrogen content in leaves of the RCH treatment compared to the NCH treatment found in our study provides further evidence that nitrogen may be a factor involved in BS incidence. According to KELLER (2010), among the metabolic processes in grapevine, nitrate reduction and assimilation are reactions of high energetic cost. In case of extremely limited availability of carbohydrates, grapevine plants are not able to gain sufficient energy for the active regulation of nitrogen absorption, and this may lead to passive absorption (KELLER 2010). If under unfavourable conditions for carbohydrate assimilation also shoot topping is performed, the availability of carbohydrates may be further limited, because the leaf area available for assimilation is suddenly reduced, while the root system responsible for nitrogen absorption remains of the same size. It furthermore may be assumed that not only reduced leaf area, but also other factors limiting assimilation, especially in periods of intense nitrogen absorption, may result in similar effects. To summarize, the preconditions for such a scenario are: a high readiness of plants to absorb nitrogen, a high nitrogen availability in the soil solution and this along with a reduced carbohydrate availability due to limited assimilation in consequence of persisting conditions of high temperatures, low relative humidity, reduced leaf area and others.

Whether these preconditions alone can lead to permanent insufficiency in sugar accumulation, and finally to death of mesocarp cells in berries needs to be further examined.

The lower potassium content observed in the RCH treatment compared to the NCH treatment indicates a high ammonium content of the soil. According to Keller (2010) and Christensen and Boggero (1985), potassium absorption may be impaired when ammonium is the dominant form of nitrogen present in the soil. This occurs especially in acidic soils, in case of nitrogen degradation from organic matter, due to the use of ammonium fertilizers, and in case of ammonium input through the air.

Fardossi (2000) assumed that reduced potassium absorption may contribute directly to BS occurrence, and not be just a side effect. However, additional studies are needed to verify whether this is actually the case.

Finally, in our study, the nitrogen content in berries was not higher in RCH-treated than in NCH-treated berries. On the contrary, nitrogen content in berries tended to be higher in the NCH than in the RCH treatment. However, except when almost all bunches were slightly damaged as on 'Pinot blanc' in 2011, yeast assimilable nitrogen (YAN) in must was in some cases significantly higher in the RCH treatment than in the NCH treatment. These results confirm the observations made by Spring *et al.* (2012). The higher nitrogen concentration (YAN) in RCH may be partially explained by lower sugar content in RCH berries. However, the higher YAN values recorded in the RCH-treated plots meanwhile the nitrogen content in berries was similar in RCH and NCH or tended to be higher in NCH, could also indicate a reduced availability of carbohydrates in the RCH treatment. There may thus have been a shift of the nitrogen fractions to yeast assimilable components, and potentially also to increased ammonium content.

In conclusion, the results of this work give evidence that strong leaf canopy height reduction at veraison or during ripening, favours the occurrence of BS. Further, in the leafs of the reduced leaf canopy height treatment, at the end of the ripening period, the nitrogen contents were higher meanwhile the potassium values were lower compared to the leafs of the normal leaf canopy height plants. Whether the occurrence of BS is triggered by the change in the mineral balance remains to confirm.

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