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**Nutritional and climatic effects on berry shrivel of grapevines in
Southern Germany**

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Abbreviations

a.s.l. = above sea level

BBCH = **B**iologische Bundesanstalt für Land- und Forstwirtschaft, **B**undessortenamt
und **C**hemische Industrie

BS = berry shrivel

BSN = bunch stem necrosis

Ca = calcium

CaO = Calcium oxide

cvs = cultivars

DAA = days after anthesis

Dw = dry weight

Gibb = Gibberellic acid

HAD = hours after detachment

HPLC = high-performance liquid chromatography

ICPOES = inductively coupled plasma optical emission spectrometry

K = potassium

K₂O = potassium oxide

KCl = potassium chloride

LDL = low density lipoprotein

M&M = Material and methods

Mg = magnesium

MgO = magnesium oxide

MPa = megapascal

N = nitrogen

NOPA = nitrogen available for the yeast

SO₂ = Sulfur dioxide

SPAD = Special Products Analysis Division (Konica Minolta Holdings, Inc.)

UTC = untreated control

UV = ultraviolet

Summary

Berry shrivel (BS) is one of the economically most important physiological disorders in grapevine, but it is also one of the least studied ones. At the beginning of the ripening process, berries stop accumulating sugars and lose their turgescence. The must weight remains low, the berries taste sour and bitter. During the last ten years, several studies were conducted to narrow eliciting factors. As a result, an unbalanced ratio of K and Mg in the soil was considered likely to induce BS. To verify this hypothesis, two fertilization trials, one via the soil and one foliar fertilization trial, were initiated. The soil fertilization trials were situated at two sites in the South of Germany, one with the variety Zweigelt in Weinstadt-Endersbach in the North of Baden-Wurttemberg and one with Pinot Blanc in Ehrenkirchen in the Southwest of Baden-Wurttemberg. In Weinstadt, three fertilization treatments were chosen: (1) no fertilization of K and Mg, (2) fertilization of K and Mg as recommended, (3) 300 % of the recommended amount of K and Mg as recommended. At the Pinot Blanc site, the following treatments were chosen: (1) no fertilization of K and Mg, (2) recommended amount of K and Mg, (3) 300 % of the recommended amount of each K and Mg, (4) no K and 300 % of the recommended amount of Mg (5) no Mg and 300 % of the recommended amount of K. Within two years, no effect on the incidence of BS was evident. In the foliar fertilization trials, Mg, K and Ca fertilizers were tested with their effect on BS and bunch stem necrosis (BSN). The first year the trial was conducted in Zweigelt and Pinot Blanc and the second year it was repeated in Pinot Blanc. For the first year, no significant differences could be observed. In the second year, more berries were affected by BS in the plots treated with Ca fertilizer. The plots with Mg fertilizer showed significantly less berries affected by BS than the untreated control.

Extensive analysis of concentrations of Ca, Mg and K in berries illustrate that berries affected by BS show significantly higher concentrations of Ca compared to healthy berries. The loss of quality of wines made of berries affected by BS was demonstrated by must and wine analysis as well as by a consumer preference analysis.

Since an artificial interruption of the phloem (Erhardt, 2010) caused symptoms similar to BS, callose deposition in the phloem was investigated microscopically in rachis of healthy grapes and grapes affected by BS. In both cases callose deposition was found.

Phenolic compounds (in particular resveratrol) that are often used as indicators of abiotic stress were analysed as well. It was supposed that BS is caused or increased by abiotic stress so that the concentration of resveratrol in berries affected by BS is increased as well. However, in all varieties analysed (Pinot Blanc, Pinot Gris and Zweigelt), the concentration of resveratrol was higher in healthy berries than in affected ones.

A direct connection between weather condition at bloom and especially at ripening was examined. Observations at monitoring sites and reports from other countries seem to show that cool and wet weather at these phenological stages might increase the risk of BS. It was only possible to statistically prove a connection between the precipitation at bloom and the incidence of BS.

Zusammenfassung

Traubenwelke wurde seit Beginn des 21. Jahrhunderts immer öfter in den Weinbergen Süddeutschlands beobachtet. Bei dieser physiologischen Störung der Rebe verlieren die befallenen Beeren zu Beginn der Reife ihre Turgeszenz, und mit dem Stop des Reifeprozesses hört auch die Zuckereinlagerung auf und das Mostgewicht ist verringert. Da diese Beeren auf Grund der niedrigen Qualität nicht zur Weinbereitung gelesen werden dürfen, ist eine Vorlese in befallenen Anlagen unerlässlich. Die Auslöser der Traubenwelke sind nicht bekannt. In früheren Studien wurde vor allem zu wenig K im Boden bzw. ein unausgewogenes K/Mg-Verhältnis als Auslöser diskutiert. In zwei Boden- und Blattdüngungsversuchen wurden verschiedene Kalium- und Magnesiumdüngestufen und deren Auswirkung auf die Befallshäufigkeit der Traubenwelke bonitiert. Die Versuche fanden auf zwei Standorten in Baden-Württemberg statt, auf einer Zweigeltfläche in Weinstadt-Endersbach sowie einer Weißburgunderfläche in Ehrenkirchen bei Freiburg. Für den Bodendüngungsversuch im Zweigelt wurden drei Düngevarianten ausgebracht: 1) ohne K-, Mg- Düngung, 2) empfohlene Menge K, Mg, 3) dreifache Menge der empfohlenen Menge K und die empfohlene Menge Mg. Auf der Weißburgunderfläche wurden folgende Düngemengen gewählt: 1) ohne K-, Mg-Düngung, 2) empfohlene Menge K sowie Mg, 3) dreifache Menge der empfohlenen Menge K und die empfohlene Menge Mg, 4) ohne K-Düngung und dreifache Menge der empfohlenen Menge Mg, 5) dreifache Menge der empfohlenen Menge K und keine Mg-Düngung. Die erhöhte Zufuhr von Kalium in Form von Bodendüngern mit Kalisulfat konnte das Auftreten der Traubenwelke auf den beiden Versuchsstandorten nicht verhindern. Auch die gleichzeitige Reduktion der Magnesiumdüngung zeigte keine signifikante Erhöhung oder Reduzierung des Befalls. Bei den Blattdüngungsversuchen wurden

2010 im Zweigelt sowie im Weißburgunder ein Mg-, K-, sowie Ca-Dünger getestet; 2011 wurde der Versuch im Weißburgunder wiederholt. Hier zeigte wider Erwarten die mit Mg gedüngte Variante im Weißburgunder einen signifikant niedrigeren Befall von Traubenwelke und die mit Ca-Dünger behandelten Parzellen zeigten einen höheren Anteil an befallenen Beeren.

Die umfangreichen Untersuchungen zu den Konzentrationen an K, Mg und Ca in den Beeren zeigen deutlich, dass in traubenwelken Beeren die Ca-Konzentration erhöht ist. Auch der Qualitätsverlust der Weine aus befallenen Trauben wurde im Versuchsweinausbau sowie der Analyse der Moste und Weine und der Verkostung ersichtlich. Da durch eine künstliche Unterbrechung des Phloems durch Ringeln traubenwelke-ähnliche Symptome hervorgerufen werden konnten (Erhardt, 2010), wurden Stielgerüste befallener und gesunder Trauben mikroskopisch untersucht. Kalloseeinlagerungen, die zu einer Unterbrechung des Phloems führen, konnten in Stielgerüsten traubenwelker, aber auch im Stielgerüst gesunder Trauben festgestellt werden.

Phenolische Inhaltsstoffe (v.a. Resveratrol), die oft als Indikatoren für abiotischen und biotischen Stress herangezogen werden, wurden ebenfalls analysiert. Es wurde angenommen, dass Traubenwelke auch durch abiotischen Stress ausgelöst oder verstärkt werden kann, und der Resveratrolgehalt in welken Beeren dementsprechend höher sein sollte. Hier zeigte sich aber in allen Sorten ein höherer Resveratrolgehalt in gesunden Beeren.

Ein Zusammenhang zwischen der Witterung zur Blüte und vor allem zur Reife wurde untersucht. Kühles und feuchtes Wetter zu beiden phänologischen Stadien scheint das Auftreten der Traubenwelke zu fördern, was Beobachtungen auf mehreren Monitoringflächen sowie Berichte aus anderen Ländern bestätigen. Statistisch konnte

nur ein Zusammenhang zwischen dem Niederschlag zur Blüte und dem Traubenwelkebefall belegt werden.

1 Introduction

1.1 *Biology of grapevine*

Grapevines have existed for more than 60 million years. Wild grapevines belong to the oldest dicotyledonous plants and were distributed only in the Northern hemisphere. The ice age about one million years ago forced them to the southern parts of Europe. After the ice age they spread again in middle Europe and nowadays wild grapevines can still be found in some protected parts along the river Rhine between Basle and Mannheim (Vogt and Götz, 1987). Grapevines belong to the order of the *Rhamnales*, family of *Vitaceae* and genus of *Vitis*. All cultivated varieties belong to the group of *Viniferae*, the species *vinifera* and subspecies *sativa*. From the groups of *Cinerascentes*, *Rupestres* and *Ripariae* originate the species *cinerae*, *rupestris*, *riparia* and *berlandieri* which are used to breed today's rootstocks (Huglin and Schneider, 1998).

Grapevines require certain temperatures and water supply to grow and produce mature fruits. Viticulture is mainly performed between 30 °N and 50 °N and 30 °S and 40 °S latitude, which corresponds approximately with the 10 °C and 20 °C isotherms (Mullins et al., 1992). An indicator of the suitability of an area for viticulture might be the sum of degree days above 10 °C according to Winkler (1965). In the Northern hemisphere this sum should lie above 1700 degree days for the vegetation period from the beginning of April until the end of October. At the end of the vegetation period, buds start their dormancy. Temperatures below 10 °C break this inner dormancy and buds are able to pullulate again (vernalization). However, this is inhibited by a so-called forced dormancy by cool outside temperatures. During this

winter dormancy, woody parts of vines start storing starch. The maximum of the content of starch in the shoots occurs with leaf fall in November. Later on, starch is partly converted into soluble sugars as an adaptation to temperatures below 0 °C. Since this process takes time and reaches its maximum between December and January, frost events prior to this period can damage the vines severely. Warmer temperatures at the end of February or beginning of March induce the reconversion of soluble sugars into starch, and therefore the frost resistance is again reduced (Vogt and Götz, 1987).

1.2 Soil, water & nutrition

The type of soil, soil structure, soil moisture, root distribution and the supply of nutrients in the soil are the deciding factors whether a plant is sufficiently supplied by required nutrients (Schachtschabel et al., 1984). In sandy soils, nutrients can be leached out easily and the water-holding capacity is reduced as well. In contrast, clayey soils hold water and nutrients strongly up to a point where they are not available for plants anymore. The structure of clay minerals resembles a sandwich with negative charges between the layers where cations can bind. Cations that bind at the outside of these minerals can be easily exchanged and released. Cations in the inner layers have stronger bonds and are usually easily available for the plants under wet conditions. When the soil runs dry, the cations are locked-in between the layers of the minerals and are not available for the plants anymore (Müller, 2008).

A sufficient supply of water in the soil is necessary for the uptake of nutrients via the roots. As grapevines belong to the group of lianas, they have very efficient tracheae with relatively wide diameters. In the soil, water is bound to particles and the

transpiration flow enables the plant to take up water against gravity. To enlarge the absorbing surface of the roots, the root system is often highly branched. Grapevines are able to grow roots up to 15 m in length (Blaich, 2006), and their root system is often extended to enlarge their ability to absorb water. In our climatic regions, there is usually always enough water available for the grapevine to survive. However, to obtain healthy and ripe berries with the desired size and must weight for wine production, more water than just the amount to survive is necessary. The soil water is mainly taken up by the roots with a continuous flow driven by the transpiration through the stomata and the cuticles of the leaves. This transpiration flow can be regulated by the plant via the stomatal aperture which is controlled amongst others by the availability of water. Drought conditions cause an efflux of K^+ -ions of the guard cells resulting in a closure of the stomata. This also negatively affects the photosynthetic rate of the plant. Under water-stress conditions, the stomata are closed and the whole metabolism of the grapevine is reduced (Huglin and Schneider, 1998), which leads to low yield and low quality of the grapes. Plants being sufficiently supplied with K can also increase the water use efficiency as it helps maintaining optimal performances under water shortage conditions (Lips et al., 1990).

Nutrients solved in the liquid soil phase are moved to the roots by the transpiration flow of the plant and diffuse through the rhizodermis and exodermis. The inner layer containing the Casparian strip blocks the apoplastic transport and requires energy for further uptake of ions. Mg^{2+} and K^+ -ions are highly mobile via the phloem whereas Ca^{2+} -ions are mostly present in the xylem but not very mobile (Marschner, 1986). All three cations act as antagonists, which means that the excess supply and uptake of one of them can inhibit the uptake of the others. Therefore the ratio of these ions should always be balanced to inhibit deficiencies in the grapevine. For K and Mg, a ratio of 3:1 or 2:1 in the soil is considered optimal (Müller, 2008). Mg is important for

the building of chlorophyll as central ion and therefore for conducting photosynthesis. It also acts as an activator for many enzymes. Together with K^+ -ions, Mg acts alkaline in the chloroplasts and cytoplasm, leading to a pH-level that is higher compared to that in the vacuoles, to regulate enzyme activity (Marschner, 1986). The amount of K in grapevines and in wines is higher than the amounts of other mineral nutrients. In wines, it plays an important role to smoothen wines and regulate the pH (Mpelasoka et al., 2003). In the plant, its major role is controlling the opening and closing of the stomata. K^+ -ions are also mobile in the xylem and often act as a carrier in contrast to Mg^{2+} -ions and Ca^{2+} -ions that form strong complexes and are not easily exchanged. Ca is important for the stability of cell walls and mostly found outside the cytoplasm due to its inability to move into the cytoplasm. It also acts as a binding element in biomembranes and can change their transport properties (Demarty et al., 1984). Ca^{2+} -ions are involved in the regulation of the enzyme activities, e.g. can inhibit the PEP-carboxylase or stimulate the α -amylase. These ions also play a role as secondary messenger in signaling pathways that regulate enzymes (Dodd et al., 2010, McAinsh and Pittman, 2009).

1.3 Xylem and phloem in grapevine

The functionality of the xylem in fleshy fruits was discussed contrarily for long. In several studies it was observed that apoplastic dye uptake decreased or ceased in post-veraison grapes (Creasy et al., 1993, Findlay et al., 1987, Rogiers et al., 2001, Düring et al., 1987). The tracheides of the xylem were supposed to collapse with the growth of the fast berry development. Recent research showed that the xylem remains functional even in berries after veraison (Bondada et al., 2005, Keller et al.,

2006). While the phloem flow transports sugars and other solutes into the developing berries, a backflow via the xylem can occur (Lang, 1990) under high potential evapotranspiration which means a change of the mineral composition of the berries. It is known though that an exchange between xylem and phloem might occur (Minchin and Thorpe, 1987) and the sugars are transferred to the phloem that re-exports them again into the berries. As the stomata in ripening berries suberise to lenticels, no transpiration is possible any more. The driving factor for the phloem flow is the big difference in the water potential between berries and vine. The water potential of the berries is about -2.5 MPa (Lang and Thorpe, 1989), whereas the potential of well-watered vines should be around 0 MPa before sunrise and up to -1 - -2 MPa at midday. This indicates the berries as the main sink, followed by the leaves.

1.4 Phenolic compounds in grapes

The most known and the most investigated phenol in grapevine is resveratrol. It belongs to the group of stilbenes as well as viniferin. Stilbenes act as antifungal substances and are found in many plant families. In non-woody parts of grapevine such as leaves, berries or shoots they are mainly induced by fungal infection. In woody parts and the seeds they are constitutively synthesized (Bavaresco et al., 2009).

Flavanoles are mostly found as (+)-catechin and (-)-epicatechin in wines and berries. They often build oligomers in the berries that are called procyanidins. A high concentration of procyanidins contributes to a high colour intensity, as they form compounds with anthocyanins that show low sensitivity to oxidation. In white wines

and berries, flavanols are found in lower concentrations than in red wines, as the flesh of berries contains nearly no flavanols.

The formation of the aromatic system of phenols in the plant occurs via the Shikimat pathway. It starts with phosphoenolpyruvat, has shikimate that gives the pathway its name as intermediate and finally results in the formation of cinnamic acid and p-cumaric acid. The formation of cinnamic acid from phenylalanine is catalyzed by the phenylalanine ammonia-lyase (PAL) and the source of cumaric acid is tyrosine and the catalyzing enzyme tyrosine ammonia-lyase. Both enzymes are controlled by light, elicitors and ethylene (Richter, 1998b).

Phenols are often used as indicators of biotic and abiotic stress in grapevine (Vezzulli et al., 2007, Gonçalves van Zeller de Macedo Basto, M. et al., 2011, Stein and Hoos, 1984, Favaron et al., 2009, Dixon and Paiva, 1995, Weidner et al., 2011, Ojeda et al., 2002, Langcake, 1977). As BS is likely to result from any form of abiotic stress, an increase of phenolic compounds in affected berries seems possible.

1.5 Physiological disorders in grapevine

Since the beginning of the 21st century, the occurrence of BS has been reported from different regions in Germany (Löhnertz, 2007, Stücklin, 2007, Götz, 2007). Berries affected by BS lose their turgescence around the onset of ripening and shrivel. They stop accumulating sugar (Krasnow et al., 2009, Knoll et al., 2010) and the must weight remains low at around 10-14 °Brix. Moreover, the disturbance of the process of ripening leads to smaller berries, higher acidity and incompletely dyed berries in red varieties. The rachis remains untouched and green (Figure 1.1). As there is no outer damage visible it is necessary to test the grapes manually for limp, affected

berries. This is often difficult and a time-consuming work which makes BS appear not only to be a danger for good quality wines but also an economic problem for winegrowers.



Figure 1.1: Berries affected by BS in the varieties Pinot Noir (A), Chasselas (B) and Zweigelt (C). Photos: Bachteler (A, B), Erhardt (C).

Similar symptoms can be found in reference to another physiological disorder, called bunch stem necrosis (BSN). Berries affected by BSN shrivel as well, they also show lower pH, less sugar and low must weight. However, compared to BS, the main

difference is the condition of the rachis which stays green when affected by BS but gets necrotic when affected by BSN (Figure 1.2). These necrosis interrupt the xylem and phloem flow into the berries. If the whole rachis is affected even the whole grape can fall off.

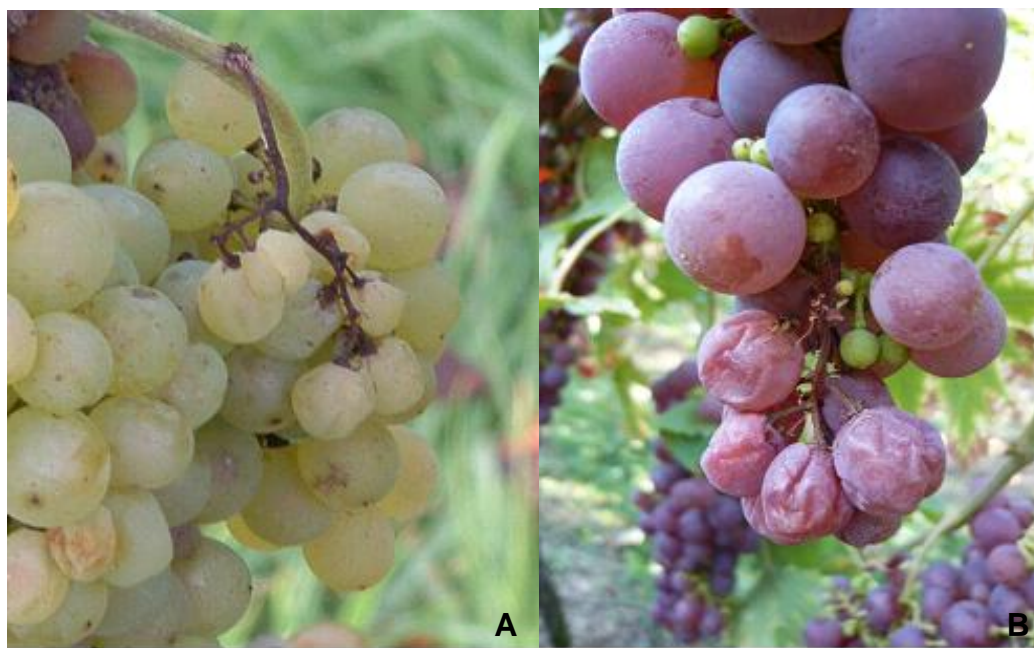


Figure 1.2: Berries affected by BSN in the varieties Pinot Blanc (A) and Chasselas (B). Photos: Bachteler.

Another possible mix-up of BS can happen with sunburn. Sunburn is caused by high solar radiation often after defoliation, when the grapes are uncovered and not adapted to the sun (Bondada and Keller, 2007). This normally happens after the beginning of ripening or just prior to harvest and is limited to berries that are directly exposed to the sun. In white varieties, these berries turn brownish or even golden but the rachis is usually not affected. Only in severe cases of sunburn even the rachis dries and darkens. Sunburned berries shrivel but they still taste sweet and aromatic if they have nearly or even entirely completed the ripening process.

Shrivelled berries occurring at late harvest dates may be caused by natural dehydration. These berries lost water and the sugars accumulated in the berries are concentrated. This late-season dehydration (Krasnow et al., 2010) is mostly known in Shiraz (Rogiers et al., 2006b, Rogiers et al., 2004a, Rogiers et al., 2004b) but can occur in any other variety as well.

Only few publications dealing with BS, which was called a 'new disease' in 2007 (Stücklin, 2007), are related to German vineyards. In 2005, there was the first attempt to distinguish BS from other abiotically caused diseases in grapevine, such as BSN or sunburn (Götz and Petgen, 2005). Since the middle of the 1990s BS has been registered with winegrowers in Austria as a distinctive disease that causes shrivelled berries at a green and normal-looking rachis (Reisenzein and Berger, 1997). However, it is believed that BS has been present before, but it was often mixed up with the above-mentioned or other diseases. In 1997, BS received its first name "Zweigeltkrankheit" (Reisenzein and Berger, 1997) as it has often been observed in the variety Zweigelt. Later on, when it was obvious that it also occurs in other varieties and other countries where Zweigelt is rarely cultivated, it was changed to "Traubenwelke" or "Welkekrankheit" in Germany. In the USA, Australia or other anglophone countries, the name "Berry Shivel" has been established (Bondada and Keller, 2007, Keller and Bondada, 2007), but one tried to change it in "Sugar Accumulation Disorder" to clarify its dissociation from other shrivelling diseases (Krasnow et al., 2009).

Factors causing BS are frequently discussed all over the world. The most popular hypothesis deals with K and Mg in the soil as the main elicitors for this disease. It has been supposed that the reason for BS is a deficit of K especially in the subsoil or a surplus of Mg either leading to an unbalanced ratio of K/Mg in the soil (Fardossi, 2001). Recent studies widened their focus of research on other factors. Weather

conditions at flowering and after veraison seem to influence the occurrence of BS (Schumacher et al., 2007, Raifer and Roschatt, 2001, Spring, 2006) as well as so-called stress-factors such as high yield, well ventilated soil or an unsuitable green cover (Redl et al., 2009).

1.6 Aims & Scopes

Causes for the occurrence of BS should be identified to develop controlling or avoiding strategies that can be applied in practice. Considering the results of previous studies, many factors seem possible to initiate BS. The most cited factors are a deficiency of K or a ratio below 2:1 for K:Mg in the soil (Stücklin, 2007, Raifer and Roschatt, 2001, Redl, 2005). Therefore two multi-annual fertilization trials on two different sites in the South of Germany were established:

- 1) A soil fertilization trial to investigate the effect of different amounts of K- and Mg-fertilizers on the incidence of BS
- 2) A foliar fertilization trial to investigate the effect of K-, Mg- and Ca-fertilizers on the incidence of both BS and BSN

The annual variability of the degree of BS leads to the assumption that climatic conditions might have an influence, which has already been proposed in former studies (Schumacher et al., 2007, Spring, 2006, Keller, 2008). This impact of weather conditions on BS, namely temperature and precipitation at important phenological stages such as flowering or ripening, was also investigated in this study as a second scope.

Besides these two main objectives, physiological studies have been conducted as well. Within these studies, the focus was set on the biosynthesis of phenolic

compounds in healthy berries and berries affected by BS, the transpiration of berries and the phloem flow in rachis of affected grapes.

2 Effect of soil fertilization on the incidence of berry shrivel and the quality of resulting wine

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Effect of soil fertilization on the incidence of berry shrivel and the quality of resulting wine

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Summary

Berry shrivel is becoming an increasing concern for winegrowers all over the world. Until today, no single factor causing this physiological disorder has been determined. Studies concerning berry shrivel conducted in Austria have shown that an unbalanced ratio of K and Mg in the soil is a likely factor contributing to the disorder. The aims of the present study were to establish a better understanding of the causes and consequences of berry shrivel and observe the effects of K and Mg fertilization *via* the soil on the incidence of berry shrivel, the mineral composition of affected berries and the resulting wine quality. A two-year fertilization trial was conducted on two sites located within southern Germany with the varieties 'Zweigelt' and 'Pinot Blanc'. Different amounts of K and Mg were applied each year at both locations in order to generate different ratios of K and Mg in the soil. Before harvest, the incidences of berry shrivel of the different treatments were determined. In addition, macronutrients including K, Mg and Ca that were translocated in healthy berries and berries affected by berry shrivel were determined at harvest. To compare the quality of wine influenced by berry shrivel, different wines were produced consisting of shrivelled berries, berries affected by bunch stem necrosis and healthy berries.

In the soil fertilization trials, no significant differences in the incidences of berry shrivel were observed in relation to the soil fertilization. Major differences were found in the wine qualities of the different wines. Wines produced from healthy berries were always rated as the best wines, whereas wines produced from shrivelled berries were always rated as the lowest quality. The low quality parameters found in the must did not improve in the wine making process. Wines produced from berries affected by bunch stem necrosis were rated better than berry-shrivel-wines, however, rated less than the wine produced from healthy berries. The determinations of macronutrients' level in the berries showed significant differences regarding the concentration of Ca. In the variety 'Zweigelt' in 2009, an average of 36 mg·L⁻¹ of Ca were found in healthy berries and 107 mg·L⁻¹ in berries affected by berry shrivel. In 'Pinot Blanc' in 2010, the average of Ca in healthy berries was 46 mg·L⁻¹ and 70 mg·L⁻¹ in berries affected by berry shrivel. No significant differences were found for K and Mg in the berries.

Key words: berry shrivel, "Traubenwelke", potassium, magnesium, soil fertilization, wine quality.

Introduction

Incidences of berry shrivel (BS) were first described in Austria in 1997 (REISENZEIN and BERGER 1997), however, since 2005, this disease has also been reported in Southern Germany. It is likely that BS has already existed in Germany for a number of years, since the visible symptoms of BS are similar to those identified with bunch stem necrosis (BSN). Berries affected by BS lose their turgescence during the onset of ripening. These berries show a reduced sugar accumulation, low pH, reduced content of anthocyanins as well as an increase of tannins in the skin (KRASNOW *et al.* 2009). The must weight remains low at around 10 °Brix, therefore affected berries cannot be used for wine-making and must be removed before harvest. Since the rachis remains green and often indicates no form of outer damage when affected by BS, the detection of affected grapes is time-consuming as it is essential to examine each individual grape carefully in order to identify affected berries.

Possible causes of BS have been suggested and discussed in numerous publications (STÜCKLIN 2007, BONDADA and KELLER 2007, SCHUMACHER *et al.* 2007, REDL 2005, KELLER 2008, PARDATSCHER 2004, REISENZEIN and BERGER 2001, GRIESSER *et al.* 2012, HALL *et al.* 2011, KÜHRER and GABLER 2011, KÜHRER 2010). Research was conducted to understand and determine the causes and effects of BS. To date, no single factor has been identified as the main cause of the disease. Furthermore, there is a likely genetic pre-determination, as some varieties, rootstocks or clones were considered more susceptible than others (SCHUMACHER *et al.* 2007, STÜCKLIN 2007). Varieties where BS was observed more often are 'Zweigelt', 'Pinot Noir', 'Pinot Gris' and 'Pinot Blanc' or 'Chasselas'. Investigations in Switzerland showed that the rootstocks 5C and SO4 are more susceptible, whereas 5BB, 125AA or 3309 are less susceptible. Comparing the clones of 'Pinot Noir', the clones Mariafeld, 2/45 or 10/5 seem to be more affected by BS (SCHUMACHER *et al.* 2007). In South Tyrol BS was identified in conjunction with extreme weather conditions, such as wet and cold conditions after full bloom and hot and dry weather towards 14.8 °Brix (RAIFER and ROSCHATT 2001). In the USA research associated with BS tended to focus more on the physiological parameters such as phloem/xylem sap flow (BONDADA and KELLER 2012, KELLER 2008) and

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compositional changes (KRASNOW *et al.* 2009) in affected berries compared to healthy berries. Research conducted in Austria focused on investigating the disproportion of K and Mg in the soil as well as high temperatures as possible causes for the BS disorder (REDL 2005).

A well-balanced ratio between K, Mg and Ca is important for the growth of healthy plants and good quality wines. These cations can act as direct competitors during uptake by the roots. If one of these ions is available within the soil in excess, the roots will then essentially take up a greater proportion of this ion and can limit the uptake of the other essential ions, even if they are sufficiently present in the soil. Physiological disorders such as BS or BSN are supposed to be associated with an unbalanced proportion of these cations in the plant.

The objectives of the present study were to find possible effects of the fertilization of K and Mg on the incidence of BS and thereby affordable ways to decrease the risk of the occurrence of BS. The consequences of the disorder BS should be easier understood by the investigation of the shift of K, Mg and Ca into affected berries and the resulting wine quality.

Material and Methods

Site description: The 'Zweigelt' vineyard was located in the South of Germany, in Weinstadt-Endersbach (48°49'05.54" N, 9°22'18.54" E, 246 m a.s.l.), and consisted of an area of 1438 m². The average annual temperature in 2009 was 9.9 °C and the average annual amount of precipitation was 905 mm, in 2010 the average annual temperature was 8.9 °C and the annual amount of precipitation was 772 mm. The soil type was classified as endostagnic Regosol (calcaric).

The vines were all St 9 clones on 5BB rootstocks, planted in 2002 and trained to one cane with 10-11 buds. The distance between vines within the rows was 1.2 m and between rows 2 m. Three treatments were distributed in a randomized block design consisting of 12 plots in 4 rows. Each treatment was present in one of the rows. One plot contained 24 vines in an area of 57.6 m².

The 'Pinot Blanc' site was located in the South of Germany, in Ehrenkirchen (47° 55' 33.22" N, 7°44'59.92" E, 297 m a.s.l.), and consisted of an area of 2070 m².

The average annual values for temperature and the amount of precipitation in 2009 were 11.7 °C and 984 mm, respectively. In 2010, the average annual temperature was 10.31 °C and the annual amount of precipitation was 888 mm. The soil type was classified as haplic Regosol (calcaric, siltic).

The vines were 0212 clones on 5BB rootstocks, planted in 2000 and trained to one cane with 9-12 buds. The spacing of each vine was 2.4 m² and between rows 2 m. The trial design was a completely randomized 5x5 latin square. The 25 plots consisted of five different treatments in five replicates. One plot contained 20 vines in an area of 48 m².

Weather data were collected by the Landwirtschaftliches Technologiezentrum (LTZ) Augustenberg, Germany,

at meteorological stations near the experimental sites and provided by the program Vitimeteo (State Institute of Viticulture and Enology, Freiburg, Germany and the Agroscope Changins-Wädenswil, Switzerland).

Fertilization: In the block design at the "Zweigelt" site, three fertilizing treatments were used: (1) no fertilization of K and Mg, (2) fertilization of K (41 kg/ha K) and Mg (16 kg·ha⁻¹ Mg) as recommended, (3) 300 % of the recommended amount of K and Mg as recommended. At the 'Pinot Blanc' site, five fertilizing treatments were selected. As the trial site was suitable, two more treatments were implemented in order to examine a wider range of fertilization levels. (1) no fertilization of K and Mg, (2) recommended amount of K (28 kg·ha⁻¹ K) and Mg (24 kg·ha⁻¹ Mg), (3) 300 % of the recommended amount of each K and Mg, (4) no K and 300 % of the recommended amount of Mg, (5) no Mg and 300 % of the recommended amount of K. In order to generate an unbalanced ratio of K and Mg in the soil in a short time, the amounts were tripled in the varieties (3), (4) and (5).

On both sites fertilization was applied by hand in 2009 and 2010 at approximately 60 days prior to anthesis, using ESTA® Kieserit (K+S Kali GmbH, Kassel, Germany) and Kaliumsulfat (K+S Kali GmbH, Kassel, Germany).

During the trial, vineyard management regarding plant protection, defoliation, tipping or soil cultivation remained the same for all treatments at each site according to commercial practices in southern Germany.

Leaf samples: In 2009 and 2010, 60 leaves were sampled for each treatment throughout each season. Twenty leaves were collected on three separate occasions: 1) at full bloom, 2) at veraison and 3) at the ripening stage of the berries of 14.8 °Brix. Leaves were collected at mid-shoot position, opposite the first or second cluster. Contaminations (e.g. fertilizer or fungicide) were removed with a soft brush and deionized water. Subsequently, samples were dried to constant weight in a drying oven at 45 °C, and then placed in plastic bags and sent to the LTZ Augustenberg, Germany, for analysis of K, Ca and Mg. The ratio of K and Mg was calculated by K/Mg and the cation ratio was calculated by K/(Mg+Ca).

Soil samples: For each treatment, a mixed sample across all plots was taken. Per plot, two samples were randomly collected within the rows. Soil samples were collected in January 2009 prior to the first fertilization in order to determine the supply status of K, Mg, humus and pH. Samples were collected with a Pürckhauer auger at two depths ranging from 0-30 cm and 30-60 cm. Analysis of the nutritional status of the soil was conducted at the LTZ Augustenberg, Germany. Due to the results received in the analysis, a recommendation of the amounts of fertilizers was calculated according to the "Düngung von Ertragsreben" (MINISTERIUM FÜR ERNÄHRUNG UND LÄNDLICHEN RAUM BADEN-WÜRTTEMBERG, 2006). This recommendation was taken as a basis for the calculation of the different treatments. In order to monitor the nutritional status in each of the different treatments, soil samples were taken in the same way after harvest in 2009 and 2010.

Berry samples: Berry samples consisting of 100 berries each were collected at BS symptom onset.

One sample of healthy berries and berries affected by BS was collected in each treatment. At the 'Pinot Blanc' site in 2010, one sample of berries affected by BS was collected over all the treatments as not enough affected berries could be sampled for each treatment. All berries were pressed, stabilized with 0.2 ml of a 5 % SO₂ solution and concentrations of Ca, Mg and K were analysed with atomic absorption spectroscopy (AAS).

Wine Production: At the "Zweigelt" site in 2010, healthy grapes of the control treatment with the recommended amount of fertilizers were harvested. Berries affected by BS and BSN were detected and harvested separately. In all treatments, all grapes were mashed after harvest, fermented on the skins and the following additives applied: 20 g/l of Siha 8 yeast (E. Begerow GmbH & Co., Langenlonsheim, Germany), 22.7-37.9 g·L⁻¹ saccharose (depending on the treatment) to adjust the amount of alcohol and 50 g·hL⁻¹ Vitamon Combi (Erbslöh, Geisenheim, Germany) as yeast nutrition. After one week, the mash was pressed and 6 days later, 1 g·hL⁻¹ of sK 2 (Erbslöh, Geisenheim, Germany) was added for biological deacidification. This was stopped after 5 ½ weeks, using 50 mg·L⁻¹ SO₂ and another 30-50 mg·L⁻¹ SO₂ 6 weeks later. Before filling, 15-20 mg·L⁻¹ SO₂ was added.

Musts and wines were analysed with GrapeScan™ (FOSS NIRSystems, Inc., Laurel, USA) for the contents of glucose, fructose, total acidity, tartaric acid, malic acid, volatile acid, alcohol, glycerine, gluconic acid, NOPA, ammonium and must weight. The concentrations of Ca, Mg and K were analysed with AAS.

Consumer preference analysis: Three "Zweigelt" wines were evaluated in a sensory seminar with 137 participants. One was made from healthy grapes, one from berries affected by BS and one from berries affected by BSN. Each participant tested the three wines blindly without knowing which one was which. After tasting, the wines were evaluated with rank number 1 for the best and 3 for the worst wine. These results were statistically analysed with Friedman rank sum test at $\alpha = 0.05$.

Scoring: For each treatment, each grape of 20 vines was rated which added up to about 300 grapes per treatment. The grapes were examined by hand and the amount of shrivelled berries was estimated.

Statistical analysis: Scores and samples were statistically analysed by ANOVA with proc mixed statements (SAS® Institute INC, USA) at $\alpha = 0.05$.

Results

Incidence of BS: In 2009, the 'Zweigelt' site showed BS symptoms at an average of 22.3 % compared with the 'Pinot Blanc' site at 5.1 %. (Fig. 1A). In 2010, BS was present at both sites and incidences similar to 2009. BS was at 29.3 % at the 'Zweigelt' site and 5.9 % at the 'Pinot Blanc' site (Fig. 1B). There were no significant differences between each of the treatments at either of the sites.

Nutritional status in the leaves: The ratio of K/Mg and concentrations of K and Mg in the leaves were higher at the 'Pinot Blanc' site compared to

the 'Zweigelt' site throughout the whole seasons of 2009 (Fig. 2A) and 2010 (Fig. 2B). At each site, the ratios of the different fertilization treatments did not show significant differences. At the 'Pinot Blanc' site in 2009, the treatment with 300 % of the recommended amount of K and Mg showed the highest ratio at 14.8 °Brix, whereas all the other treatments did not differ much. At the 'Zweigelt' site, ratios of all treatments lay in the same range between 2 and 4.

A regression of the cation ratio and the logarithmic transformed incidences of BS showed a linear relationship in 2010 with $y = 4.53 - 5.12x$, $R^2 = 0.75$.

Nutritional status in the berries: The amounts of nutrients in the berries and significant differences are shown in Tab. 1. Significant differences were found for concentrations of Ca. Comparing the results within the treatments at the 'Zweigelt' site, the amounts of Ca in berries affected by BS were significantly higher for all the treatments in 2009: 223 % in treatment 1, 261 % in treatment 2 and 129 % in treatment 3. In 2010, only the affected berries of treatment 1 showed significantly higher Ca levels with 104 % more Ca than healthy berries. At the 'Pinot Blanc' site in 2010, the amount of Ca in affected berries was significantly higher in all treatments with 56 % respectively.

Nutritional status in the soil: It was not possible to change the ratio of K and Mg in the soil in the different treatments considerably. At the 'Zweigelt' site prior to trial initiation, the amount of K in 0-30 cm depth was 24 mg/100 g and Mg was at 22 mg/100 g. In 30-60 cm K was at 14 mg/100 g and Mg at 22 mg/100 g. These levels are considered as sufficiently supplied. After harvest in 2010, in treatment 1 with no fertilization of K and Mg, 26 mg/100 g K were analysed in 0-30 cm and 18 mg/100 g in 30-60 cm. Mg was at 22 mg/100 g in 0-30 cm and 21 mg/100 g in 30-60 cm. In treatment 3 with 300 % of the recommended amount of K and Mg as recommended, 30 mg/100 g K and 23 mg/100 g Mg were observed in 0-30 cm and 22 mg/100 g of both K and Mg in 30-60 cm. The level of Mg supply remains in the range that should be pursued. The level of K rises in treatment 3 and is considered as more than sufficient.

At the 'Pinot Blanc' site, the amount of K was at 26 mg/100 g and Mg at 14 mg/100 g in 0-30 cm prior to trial initiation. In 30-60 cm, K was at 24 mg/100 g and Mg at 16 mg/100 g. These levels are considered as sufficiently supplied. After 2 years, the control plots without fertilization of K and Mg showed 31 mg/100 g of K and 15 mg/100 g of Mg in 0-30 cm and 24 mg/100 g K and 15 mg/100 g Mg in 30-60 cm. Amounts of K in the upper soil varied only from 26 mg/100 g in treatment 3 to 32 mg/100 g in treatment 5.

In the subsoil, highest amounts of K were found in treatment 2 with 28 mg/100 g and lowest amounts in treatment 1 and 5 with 24 mg/100 g. Mg varied from 13 mg/100 g in treatment 5 to 16 mg/100 g in treatment 3 in the upper soil. In the subsoil, the same amount of Mg (15 mg/100 g) was found in all treatments except for treatment 5 with 14 mg/100 g. The level of Mg supply remains in the range that should be pursued. Regarding the level

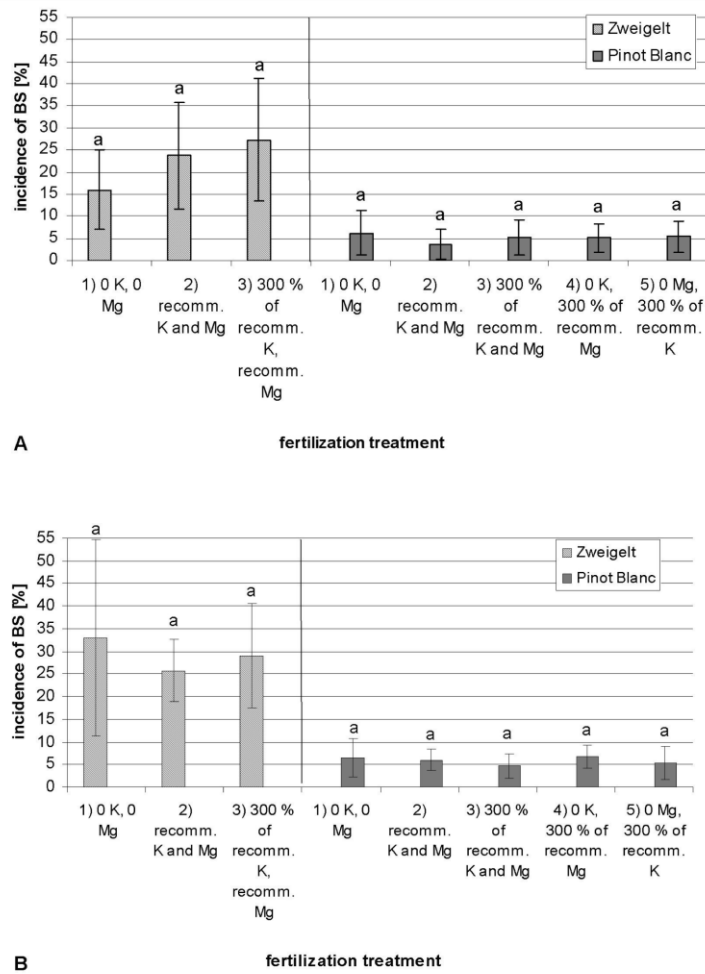


Fig. 1: Incidences of BS at both sites in 2009 (A) and 2010 (B) in the different fertilization treatments. aMeans with the same letter do not differ significantly at $p < 0.05$ within each site. Error bars indicate standard deviation.

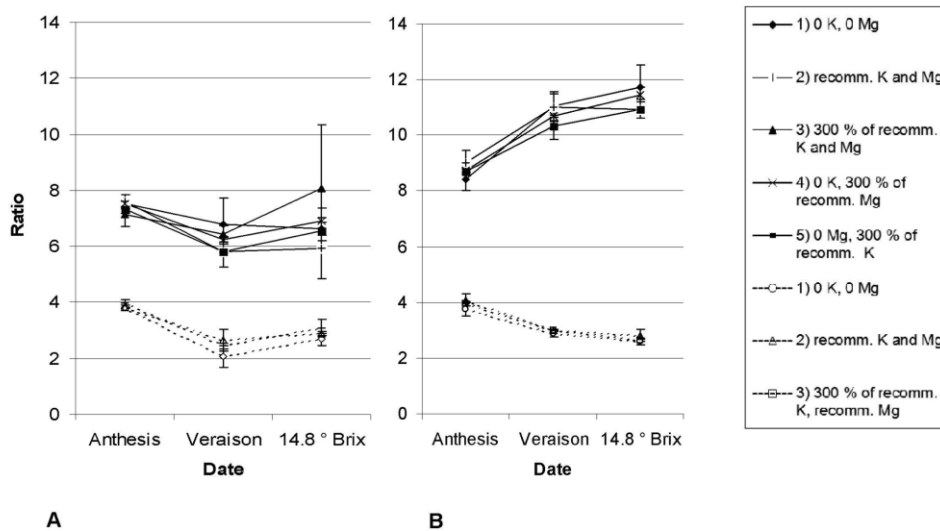


Fig. 2: Ratio of amounts of K and Mg in leaves of treatments 1-5 at the "Pinot Blanc" site (—) and treatments 1-3 at the "Zweigelt" site (---) in 2009 (A) and 2010 (B). Error bars indicate standard deviation.

Table 1

K, Mg and Ca in healthy berries and berries affected by BS in the different treatments at both trial sites in 2009 and 2010.
^{ab}Means followed by the same letter in each column do not differ significantly at $p < 0.05$

Zweigelt 2009		Means		
fertilization	berries	K [mg·L ⁻¹]	Mg [mg·L ⁻¹]	Ca [mg·L ⁻¹]
1	healthy	1253 a	38 a	33 a
	BS	1183 a	34 b	107 b
2	healthy	1336 a	37 a	30 a
	BS	1386 a	36 a	107 b
3	healthy	1288 a	41 b	46 a
	BS	1244 a	39 b	106 b
Zweigelt 2010		Means		
fertilization	berries	K [mg·L ⁻¹]	Mg [mg·L ⁻¹]	Ca [mg·L ⁻¹]
1	healthy	1086 a	58 a	41 a
	BS	833 a	65 a	84 b
2	healthy	1087 a	66 a	48 a
	BS	898 a	59 a	69 a
3	healthy	1072 a	59 a	46 a
	BS	841 a	62 a	72 a
Pinot Blanc 2009		Means		
fertilization	berries	K [mg·L ⁻¹]	Mg [mg·L ⁻¹]	Ca [mg·L ⁻¹]
1	healthy	1302 a	71 a	56 a
	BS	1243 a	65 a	62 a
2	healthy	1384 a	66 a	56 a
	BS	1254 a	69 a	60 a
3	healthy	1365 a	69 a	55 a
	BS	1241 a	69 a	61 a
4	healthy	1191 a	85 a	55 a
	BS	1287 a	76 a	62 a
5	healthy	1372 a	72 a	64 a
	BS	1315 a	68 a	57 a
Pinot Blanc 2010		Means		
fertilization	berries	K [mg·L ⁻¹]	Mg [mg·L ⁻¹]	Ca [mg·L ⁻¹]
1	healthy	1636 a	66 a	45 a
	BS	1335 a	70 a	70 b
2	healthy	1652 a	66 a	45 a
	BS	1335 a	70 a	70 b
3	healthy	1652 a	68 a	45 a
	BS	1335 a	70 a	70 b
4	healthy	1552 a	67 a	45 a
	BS	1335 a	70 a	70 b
5	healthy	1749 a	70 a	46 a
	BS	1335 a	70 a	70 b

of K supply, treatment 4 with 30 mg/100 g in 0-30 cm and treatment 5 with 32 mg/100 g show a supply that is higher than the optimal level.

Ratios of K and Mg in the soil are shown in Fig. 3. After harvest in 2010, all treatments at the 'Zweigelt' site lie under the optimal range of 2:1 and all treatments at the 'Pinot Blanc' site (except for treatment 3) lie above this ratio.

Musts and wines: The results of the analysis of the musts showed differences in all parameters analysed. In the musts, mustweight, NOPA, pH and K showed the

highest values in the control and the lowest in the must of berries affected by BS, whereas acidity and Ca had the highest values in these musts. Furthermore, as expected, in the control wines highest alcohol levels and pH and lowest acidity and concentrations of fermentable sugar were observed (Tab. 2).

The results of the consumer preference analysis indicated significant differences between the wines. Out of 137 tasters, 135 tasters rated the control wine as the best wine, 114 rated the wine made from berries affected by BSN as the second best wine and 115 rated the wine made from berries affected by BS as the worst wine on rank no. 3. Statistical analysis showed rank number 1.0 for the control wine, 2.2 for wines made of berries affected by BSN and 2.8 for wines made of shrivelled berries.

Discussion

The amount of affected berries at the 'Zweigelt' site was higher each year the experiment was conducted compared to the amount of affected berries at the 'Pinot Blanc' site. This might be due to the fact that the variety 'Zweigelt' is more susceptible to BS than the variety 'Pinot Blanc'. The rootstock (5BB) was the same at both sites and is considered to be less susceptible for BS than others. Only in 2008, prior to this trial, the occurrence of BS at the 'Pinot Blanc' site was approximately 40 % - which shows the variation of this disease in a period of one year and indicates that weather conditions are one of the main other factors causing BS, besides the nutritional status in the soil. Cool and wet conditions during blooming and ripening are likely causes for both BSN and BS (SIEGFRIED 2010, pers. comm.). In 2010, such conditions could be found. At the 'Zweigelt' site, 16.7 % of the yearly amount of precipitation were recorded from veraison until harvest. At the 'Pinot Blanc' site it was even 22.5 % of the yearly amount of precipitation in 2010. In 2009, in this period only 3.6 % of the yearly amount of precipitation were recorded at the 'Zweigelt' site and 5.5 % at the 'Pinot Blanc' site. This might explain the higher rates of incidence of BS in 2010.

Comparing the results of the leaf and soil analysis at both field sites, at the 'Pinot Blanc' site, the ratio of K·Mg⁻¹ was within the range considered to be optimal. However, at the 'Zweigelt' site, a very narrow ratio was determined, due to an overfertilization of Mg in the years prior to the trial. For soil, a ratio of K:Mg at 2:1 is considered optimal. Within a two year trial period, it was not possible to alter the proportion of K:Mg in the soil effectively. Former studies have proven that there is little effect on the uptake of K when K fertilizers are applied in excess (MORRIS *et al.* 1980). In tomato plants, the same can be applied to Mg (BRYSON and BARKER 2002). However, 2010 a slight increase of K can be observed at both trial sites in the treatments given 300 % of the recommended amount of K, which might possibly be enlarged by continuing the trials.

The quantity of K and Mg that reached the leaves did not increase in either of the treatments that were given three times the amount of fertilizer considered optimal.

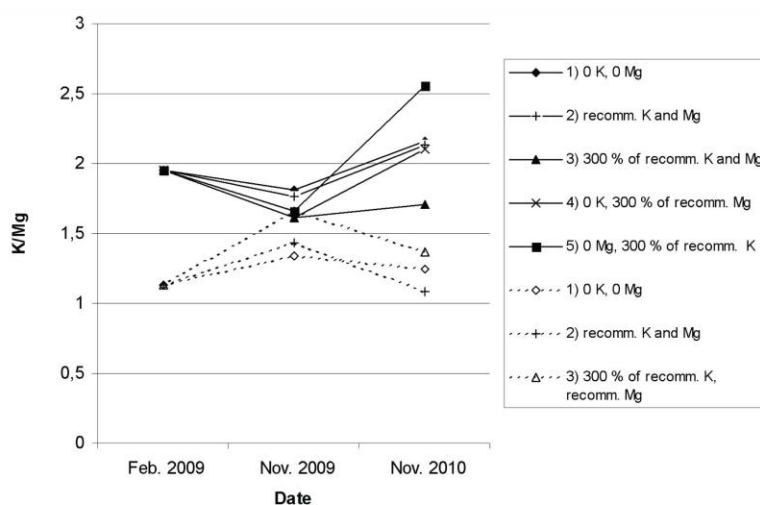


Fig. 3: Ratio of amounts of K and Mg in the soil of treatments 1-5 at the "Pinot Blanc" site (—) and treatments 1-3 at the "Zweigelt" site (---).

Table 2

Analysis of control (healthy), BS and BSN musts and wines harvested in 2010

must	must weight (°Brix)	total acidity (g·L ⁻¹)	Tartaric acid (g·L ⁻¹)	pH	NOPA (mg·L ⁻¹)	K (g·L ⁻¹)	Ca (mg·L ⁻¹)
BSN	17.7	12.5	7.7	3.0	134	1.00	63
control	20.5	8.5	6.0	3.1	197	1.28	37
BS	14.5	14.3	8.7	2.8	81	0.90	79
wine	alcohol (g·L ⁻¹)	total acidity (g·L ⁻¹)	pH	fermentable sugar (g·L ⁻¹)	sugarfree extract (g·L ⁻¹)	volatile acid (g·L ⁻¹)	free SO ₂ (mg·L ⁻¹)
BSN	90.7	7.2	3.1	2.3	24.1	0.47	32
control	101.2	5.3	3.4	1.8	22.2	0.51	20
BS	77.2	8.7	3.0	2.7	23.6	0.39	15

This can be explained with the already sufficient supply of K and Mg in the soil, which can be seen in the treatment without any fertilization. The concentrations of K and Mg in the leaves remained within the normal range even after the second year. Only the ratio of K·Mg⁻¹, which is considered optimal at 3.5.-7.0 (VANEK 1978), does not lie in this range but shows no correlation to the incidences of BS. However, the pathway of nutrients from the roots to the leaves is influenced by a large number of parameters (SCHACHTSCHABEL *et al.* 1984, MARSCHNER 1986, RICHTER 1998a, GISI *et al.* 1990). When the absolute amount of nutrients in the soil can be increased after fertilization for a certain time the uptake can be disturbed by fixation in the soil, pH-value or drought. Nutrients in the shoots and in the trunk were not analysed and therefore storage could be possible.

The ratio of all cations in the leaves showed a significant correlation with the incidence of BS in 2010. The range of all cations in the leaves is considered optimal between 0.30-0.40 (COGA 2009). Almost all of the samples analysed were under or above this range. A ratio higher than 0.4 means that the amount of K rises, which is the case at the 'Pinot Blanc' site. This is also reflected in the K·Mg⁻¹

ratio in the leaves and in the soil. At the 'Zweigelt' site, the ratio is smaller as more Mg is present. Since the petioles were not used for analysis, the amount of nutrients taken up and stored might have been even higher, as the petioles may be seen as a storage for nutrients that can be moved to the blades when needed (GERENDAS 2011, pers. comm.).

The higher amounts of K and Mg given by fertilization were not reflected in the berries. The most obvious and most constant effect is the higher concentration of Ca in berries affected by BS, which was already found in the rachis of affected grapes (KRASNOW *et al.* 2009). Although not significantly, the concentration of K in berries affected by BS is often lower than in healthy berries, which was already found in prior studies (KRASNOW *et al.* 2009, KELLER 2008, MEHOFER and REGNER 2010). As K is mainly transported via the phloem, a cessation of the phloem flow might contribute to the development of BS. Grapes, where the pedicels were girdled and the phloem destroyed, showed similar symptoms to grapes with BS (ERHARDT 2010). In contrast to K, Ca is a xylem mobile element. It reaches its maximum quantity within the berries early compared to other nutrients, at approximately 30 (POSSNER and KLIEWER

1985) - 60 DAA (ROGIERS *et al.* 2006a). If a cessation of xylem and/or phloem flow should occur during ripening when shrivelling becomes apparent, most of the Ca^{2+} -ions are already accumulated within the berries. An increase of oxalic acid which is observed in healthy berries was not identified in shrivelled berries (KELLER and BONDADA 2007). This acid can bind Ca^{2+} -ions and therefore reduce the quantity of free ions. The differences found in the berries were also reflected in the musts. The control contains more sugar, less acid, more K and less Ca. The wines were not cleared or corrected artificially, as the purpose was to show the poor quality wine from affected grapes. The lack of K is reflected in the high acidity, as it is normally involved in controlling the pH in the grape juice (BOULTON 1980).

Factors causing BS still remain unclear. The variation of K and Mg fertilizers did not increase or decrease the incidences of BS. Yet, trials must be continued in order to observe long-time effects. The evaluation of the resulting wine quality illustrates the importance of drawing wine-growers' attention to this disorder and its detection before harvest.

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3 Effects of foliar fertilization on incidence of berry shrivel and bunch stem necrosis in *Vitis vinifera* L. cvs Pinot Blanc and Zweigelt

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Abstract

Berry shrivel and bunch stem necrosis are the economically most important physiological disorders of grapevine. The nutritional status of the plant is considered as one of the main elicitors for the occurrence of these disorders. For foliar fertilization trials, two vineyards that had previously been affected by both diseases were selected in Southwest Germany, one with the variety Zweigelt and one with Pinot Blanc. By the use of potassium, magnesium and calcium foliar fertilizers, a rapid increase of different nutrients in the plants should be caused and the effects on the diseases observed. In 2010, no significant differences in the fertilization treatments were found for both varieties. In 2011, in Pinot Blanc, significantly more berries were affected by berry shrivel in plots treated with calcium fertilizer compared to those treated with potassium and magnesium. Plots with magnesium fertilizer showed significantly less berries affected by berry shrivel than the untreated control.

Introduction

Berry shrivel (BS) was first described in the 1990s in Austria (Reisenzein and Berger, 1997) where it received its German name “Zweigeltkrankheit” after the variety Zweigelt that was affected the most. Since then a number of cases have been reported in other parts of the world including Switzerland (Schumacher et al., 2007, Pardatscher, 2004, Keller, 2008), Italy (Raifer and Roschatt, 2001), the USA (Bondada and Keller, 2007, Krasnow et al., 2009, Krasnow et al., 2010) and Germany (Stücklin, 2007). Over the past years, the occurrence of BS has risen and consequently so has the awareness. BS is a physiological disorder of the berries which start losing their turgescence visibly around the onset of ripening. Shriveled berries have a lower pH-value and accumulate less sugar than healthy berries (Knoll et al., 2010). At first sight these berries resemble berries affected by bunch stem

necrosis (BSN) but the rachis of shriveled berries remains green. In red varieties affected berries remain light red and are more easily detected than in white varieties. One of the main and most expensive problems associated with BS is the detection and removal process which is mostly done by hand. Before harvest affected berries must be completely removed as the must weight of these berries lies around 10.0 °Brix.

In former studies, biotic factors such as fungi, viruses, phytoplasms and bacteria were excluded as possible factors causing BS (Krasnow et al., 2009, Reisenzein and Berger, 2001), however, other theories have been discussed over the last decade. The most widespread theory focuses on a potassium deficiency in the subsoil in connection with a surplus of magnesium (Redl, 2005). Another hypothesis concerning the weather conditions had its origin in South Tyrol and determined an insufficient supply of water for the plants after a period of hot and dry weather (Raifer and Roschatt, 2001), resulting in shrivelling berries. In the USA, a possible interruption of the xylem or phloem was suspected to cause the lack of water in the berries (Keller and Bondada, 2007).

To find effective and financially affordable methods for reducing BS formed the main goal of this research. The aim of the trials was to assess the efficacy of different foliar fertilizers to reduce BS.

Material and Methods

Site description and weather data

The “Zweigelt” vineyard was located in the South of Germany, in Weinstadt-Endersbach (48°49'05.54” N, 9°22'18.54” E, 246 m a.s.l.) and consisted of an area

of 464 m². The average annual temperature in 2010 was 8.9 °C and the annual amount of precipitation was 772 mm, in 2011 the average annual temperature was 10.6 °C and the annual amount of precipitation was 569 mm. The soil type was classified as endostagnic Regosol (calcaric). The vines were all St 9 clones on 5BB rootstocks, planted in 2002 and trained as bended unilateral shoots with 10-11 buds. The distance between vines within the rows was 1.2 m and between rows 2 m. Three fertilization treatments and one control were completely randomly distributed in a trial design consisting of 16 plots in 2 columns. One plot contained 6 vines in an area of 18.24 m².

The “Pinot Blanc” site was located in the South of Germany, in Ehrenkirchen (47° 55' 33.22" N, 7°44'59.92"E, 297 m a.s.l.) and consisted of an area of 475.2 m².

The average annual values for temperature and annual amount of precipitation in 2010 were 10.3 °C and 888 mm, respectively. In 2011, the average annual temperature was 12.6 °C and the annual amount of precipitation was 710 mm. The soil type was classified as haplic Regosol (calcaric, silty). The vines were 0212 clones on 5BB rootstocks, planted in 2000 and trained as unilateral shoots with 9-12 buds. The spacing of each vine within the rows was 2.4 m² and between rows 2 m. The trial design was completely randomized and contained 12 plots in two rows with three different fertilization treatments and one control in three repetitions. One plot contained 12 vines in an area of 28.8 m².

At both experimental sites, weather data was collected by the LTZ Augustenberg at a meteorological station near the experimental site and provided by the program Vitimeteo (State Institute of Viticulture and Enology, Freiburg, Germany and the Agroscope Changins-Wädenswil, Switzerland).

Fertilization

At each site, three different foliar fertilizers were tested: Lebosol[®]-Magnesium⁵⁰⁰ (33% MgO) with 4 l/ha, YaraVita[™] Safe-K (33% K₂O) with 5 l/ha and Folanx[®] Ca29 (40.6% CaO) with 2.5 kg/ha. The fertilizers were dissolved in 0.1 l water/vine and applied in the morning with a SOLO[®]425 knapsack sprayer by hand. In 2010, the sprayings were performed at 7 days after anthesis (DAA), 31 DAA and 43 DAA at the “Zweigelt” site and at the “Pinot blanc” site at 7 DAA, 21 DAA, 28 DAA and 42 DAA. One more spraying was chosen as it started to rain just after the second spraying. The control plot was left untreated. In 2011 the trial was repeated at the “Pinot blanc” site and sprayings were performed at 41 DAA, 48 DAA and 56 DAA. Later dates were chosen in order to find differences compared to the earlier sprayings.

During the trial, the vineyard management remained the same for all treatments.

Leaf samples

Twenty leaves of each treatment were sampled during the ripening stage of the berries of 14.2 °Brix. Leaves were collected at the mid-shoot position, opposite the first or second cluster. Following sampling the chlorophyll content was determined with an SPAD 502 Chlorophyll Meter (Spectrum Technologies, Inc., Plainfield, Illinois, USA) and the fresh weight without petioles was recorded. Subsequently, contaminations were removed with a soft brush and deionized water. Following drying to constant weight at 45 °C in a drying oven, samples were weighed, placed in plastic bags and analyzed for K (extraction with concentrated nitric acid, analysis with ICPOES), N (DUMAS method), Ca (extraction with concentrated nitric acid, analysis with ICPOES), Mg (extraction with concentrated nitric acid, analysis with ICPOES) and B (extraction with ultrapure concentrated nitric acid, analysis with ICPOES) by the Landwirtschaftliches Technologiezentrum, Augustenberg, Germany.

Amounts of leaf nutrients were statistically analyzed by ANOVA with proc mixed statements (SAS[®] Institute INC, USA) at $\alpha=0.05$

Phenological stages

The phenological stages according to the BBCH scale were visually evaluated in the years 2008 - 2011 at the “Pinot Blanc” site.

Scoring

For each plot in the trial design, each grape of two (Zweigelt) or three (Pinot blanc) vines was rated. The grapes were touched by hand and the amount of shrivelled berries estimated. Grapes were divided into five categories: 1) 0 % of the berries shrivelled, 2) 1-10 %, 3) 11-25 %, 4) 26-50 % and 5) 50 % and more of the berries shrivelled after Reisenzein and Berger (2001). The incidence of BSN was scored in the same way as described above.

Scores were statistically analyzed by ANOVA with proc mixed statements (SAS[®] Institute INC, USA) at $\alpha=0.05$.

Results

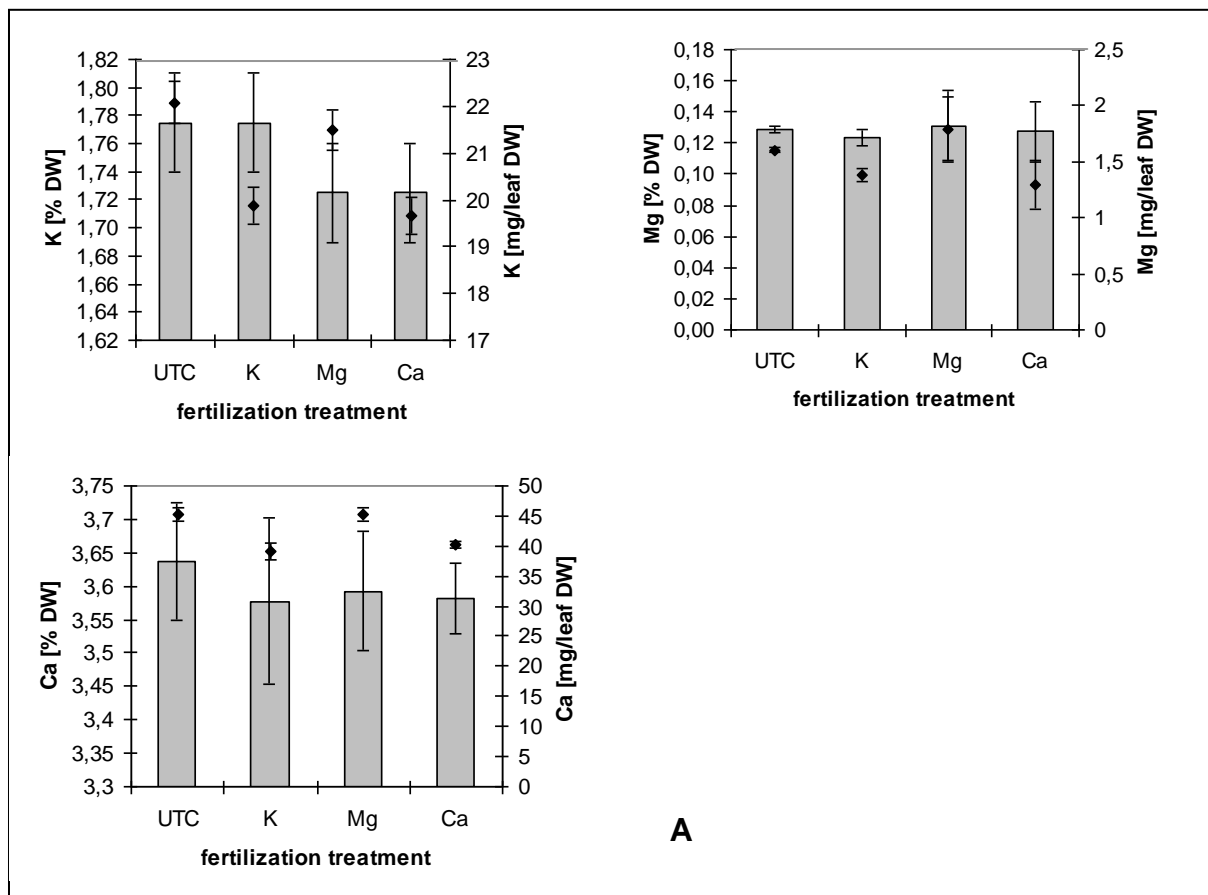
Leaf analysis

In 2010, there were no significant differences between the different fertilizing treatments concerning the amount of nutrients in the leaves at both sites. The leaf fertilizers did not increase the amount of nutrients in the leaves significantly compared to the untreated control (Figure 1A, B). In the variety Pinot Blanc, highest

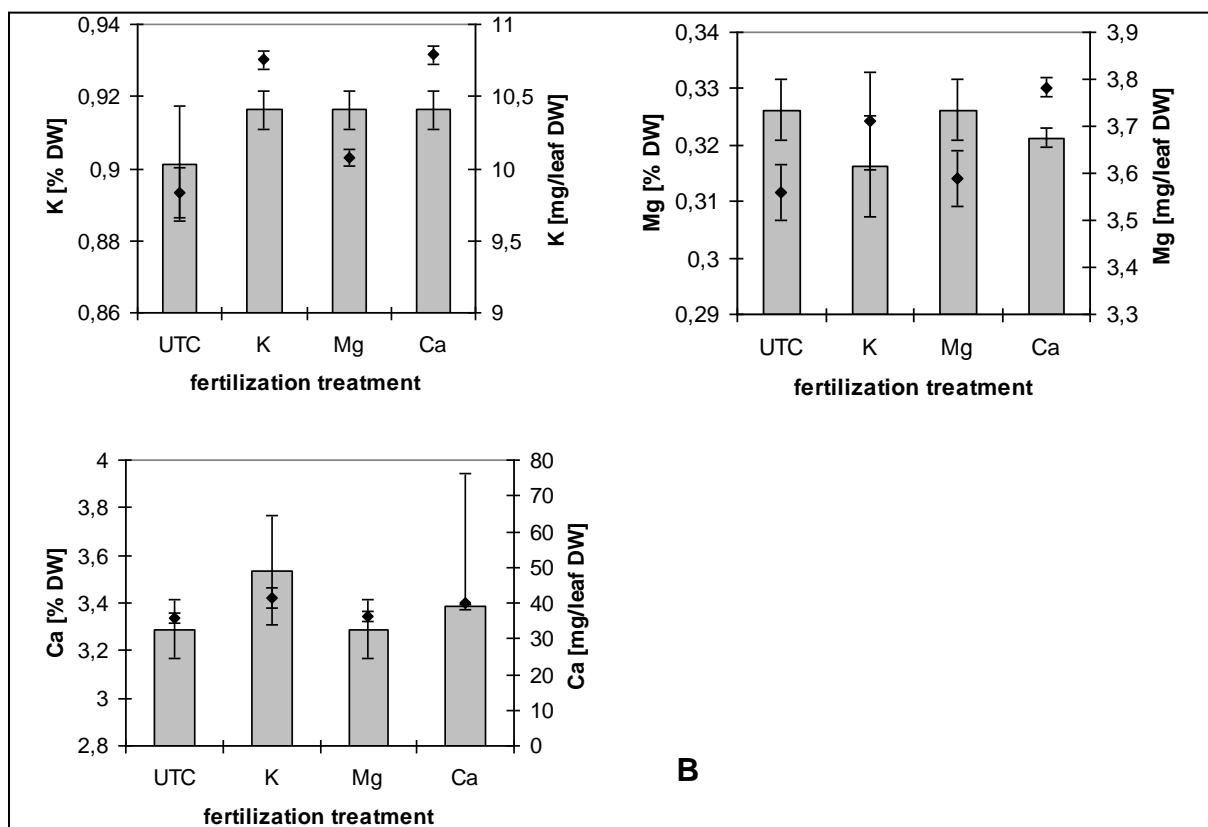
amounts of K were found in the untreated control and lowest in the leaves treated with Ca fertilizer. The leaves treated with Mg fertilizer showed the highest amounts of Mg. The highest amounts of Ca were found in leaves treated with Mg fertilizer and in the control plots. In the variety Zweigelt highest amounts of K were found in the leaves fertilized with Ca fertilizer and lowest amounts in the untreated control. Mg showed highest amounts in the plots treated with Ca fertilizer whereas highest concentrations of Mg were found in the plots treated with Mg fertilizer and in the untreated control. Content of Ca was highest in the leaves treated with K fertilizer and lowest in the untreated control.

In 2011, there were no significant differences in terms of the amount of nutrients in the leaves between the treatments. The amount of nutrients was not increased significantly by the fertilizers used (Figure 1C). Highest amounts of K were found in leaves treated with Ca fertilizer whereas the concentration of K was the same in all treatments. The Mg content showed highest values in the treatment treated with Mg fertilizer and lowest in the untreated control. Ca content was highest in the untreated control and lowest in the plots treated with K fertilizer.

3 Effects of foliar fertilization on incidence of berry shrivel and bunch stem necrosis in *Vitis vinifera* L. cvs Pinot Blanc and Zweigelt



A



B

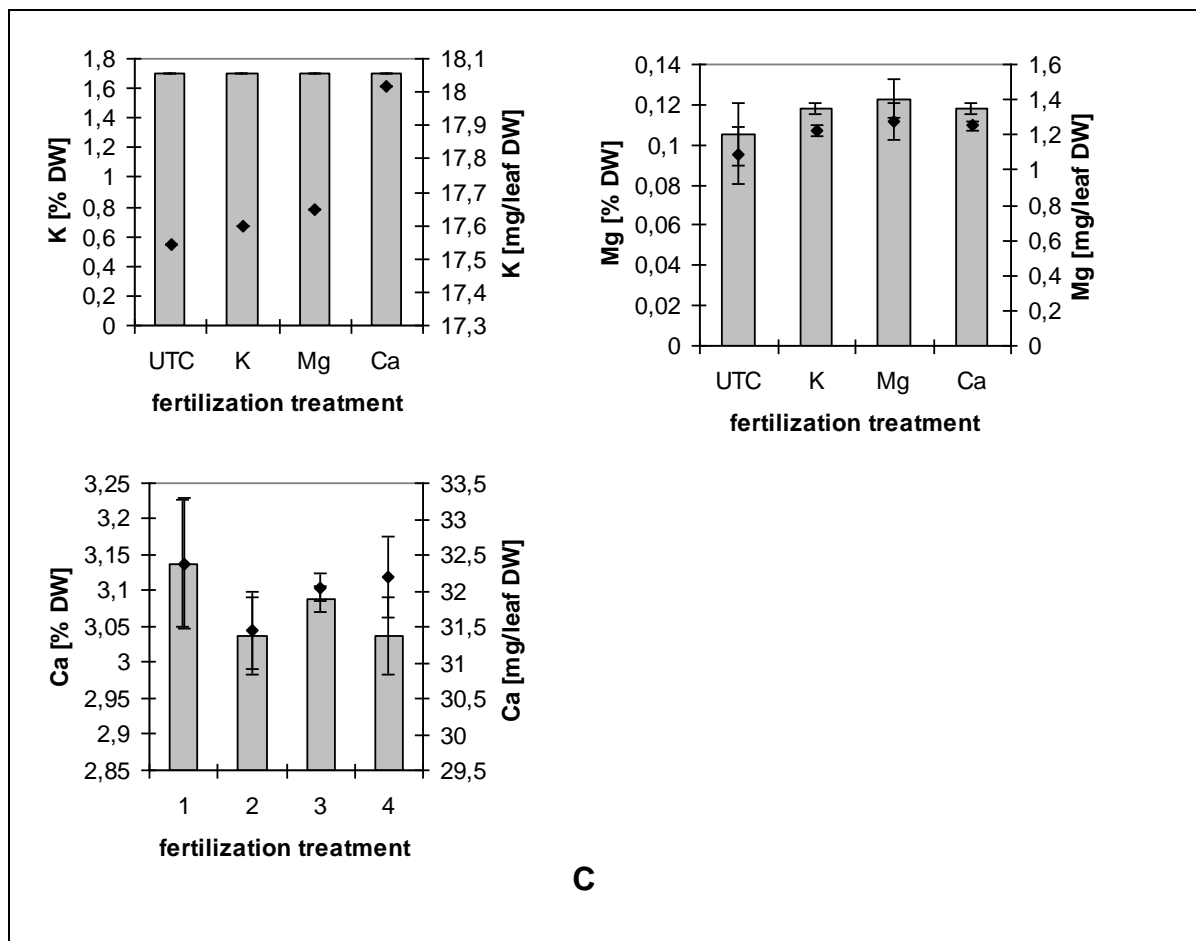


Figure 1: Effect of foliar fertilizer application on leaf nutrients in cv. Pinot Blanc (A) and cv. Zweigelt (B) at 14.8 ° Brix in 2010 and cv. Pinot Blanc in 2011 (C). UTC = untreated control, DW = dry weight. ■ = [% DW], ◆ = [mg/leaf DW].

SPADmeter, fresh and dry weight

In 2010, there were no significant differences between the SPAD-values, fresh and dry weight of the leaves of the different treatments (Table 1). In 2011, SPAD-values of the untreated control were significantly higher than those for leaves treated with calcium fertilizer (Table 1).

Table 1: SPAD-values of both trial sites in 2010 and 2011.

SPAD 502 Chlorophyll	
2010	Meter
Zweigelt	
untreated control	31.3 ± 6.3 a
potassium	33.2 ± 4.4 a
magnesium	34.8 ± 3.8 a
calcium	33.5 ± 4.7 a
Pinot Blanc	
untreated control	38.6 ± 5.2 a
potassium	40.7 ± 3.0 a
magnesium	41.2 ± 3.3 a
calcium	37.4 ± 9.0 a
2011	
Pinot Blanc	
untreated control	34.4 ± 5.8 a
potassium	33.2 ± 7.9 ab
magnesium	33.4 ± 5.9 ab
calcium	28.9 ± 8.5 b

^{ab}Means followed by the same letter do not differ significantly at $p < 0.05$.

Ratings of incidences of BS and BSN

There were no significant differences between incidences of BS and BSN in the fertilization treatments at either of the sites in 2010 (Figure 2A, B). In the variety Pinot Blanc, the highest incidences of BS were found in the plots treated with Mg fertilizer with 7 % and lowest incidences in the untreated control with 4 %. BSN showed highest incidences (32 %) in the plots treated with Ca fertilizer and lowest (16 %) in plots treated with K fertilizer. In the variety Zweigelt, the highest incidence of BS with 33 % was observed in the plots treated with Ca fertilizer and lowest with 23 % in the untreated control. BSN did not occur at all in the untreated control and Mg fertilizer treated plots whereas highest incidence with 4 % was found in the plots treated with K fertilizer.

In 2011 in the variety Pinot Blanc, significant differences were found for BS. The vines of the plots treated with Ca fertilizer were affected most by BS with 24 %. The treatments with Mg (6 %) and K fertilizer (14 %) showed significantly less incidences of BS compared to the Ca treatment. Moreover, significantly less BS was observed in the plots treated with Mg fertilizer compared to the untreated control with 20 % (Figure 2C). However, there were no significant differences between the fertilization treatments for BSN. Highest incidences of BSN were found in the untreated control with 22 % and lowest in the plots treated with K fertilizer (10 %).

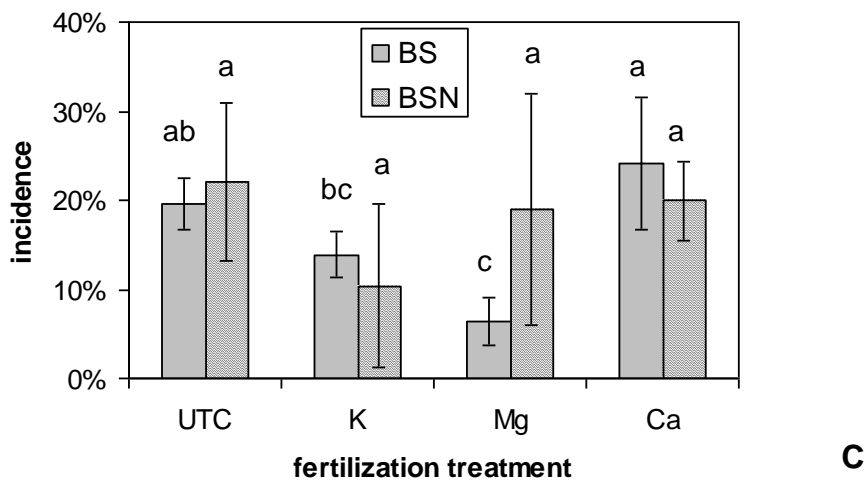
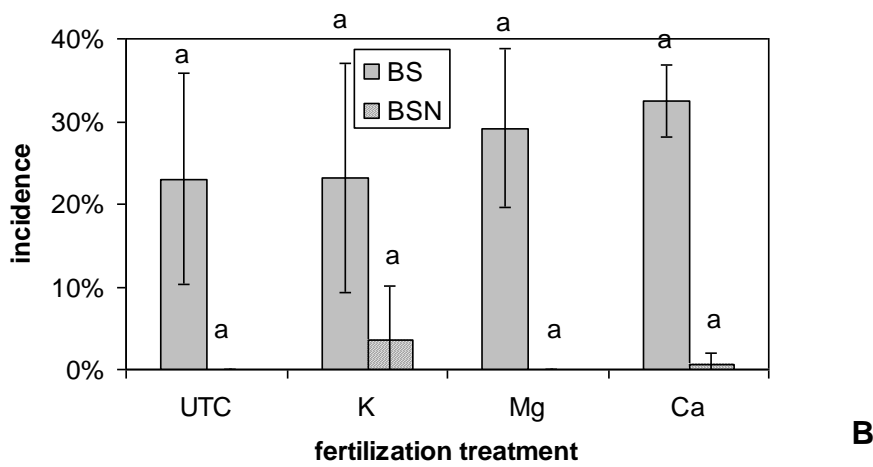
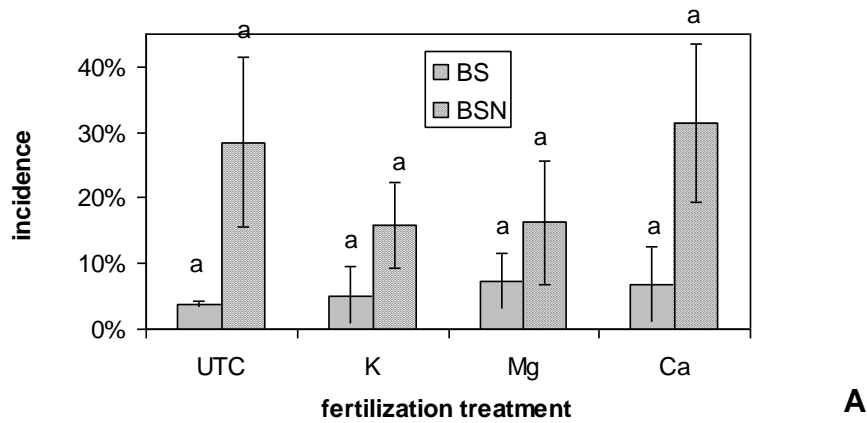


Figure 2: Incidences of BS and BSN at the Pinot Blanc site in 2010 (A), at the Zweigelt site in 2010 (B) and at the Pinot Blanc site in 2011 (C). UTC = untreated control. ^{abc}Means followed by the same letter do not differ significantly at $p < 0.05$ within BS or BSN.

Weather data and phenological stages at the “Pinot Blanc” site

The phenological stages differ from year to year (Table 2). In 2011, the vegetation period started early compared to the other years when data was recorded and also compared to the longtime average. In 2010, the vegetation period started and ended later compared to the other years. The precipitation showed differences in the distribution over the vegetation period (Figure 7). In 2008 and 2010, 34 % and 29 % of the precipitation was recorded during the BBCH stages 5-61 and 19 % and 20 % during the stages 68-81 whereas in 2009 and 2011, 20 % and 17 % of the precipitation was recorded between the stages 5-61 and 63 % and 49 % during the stages 68-81.

Table 2: Dates of phenological stages at the Pinot Blanc site and incidences of berry shrivel and bunch stem necrosis from 2008-2011.

	dates of phenological development stages (BBCH) in the region of Markgräflerland						incidence in Pinot Blanc [%]	
	5	61	65	68	81	89	berry shrivel	bunch stem necrosis
year	furry stage	opening of the caps	full blossoming	end of flowering	bunches closed, ripening	ripeness		
2008	21/04	11/06	15/06	20/06	10/08	30/09	40.0	21.0
2009	11/04	29/05	03/06	06/06	30/07	25/09	5.1	0
2010	22/04	11/06	17/06	28/06	10/08	06/10	5.9	3.6
2011	04/04	23/05	24/05	26/05	27/07	15/09	9.2	4.6

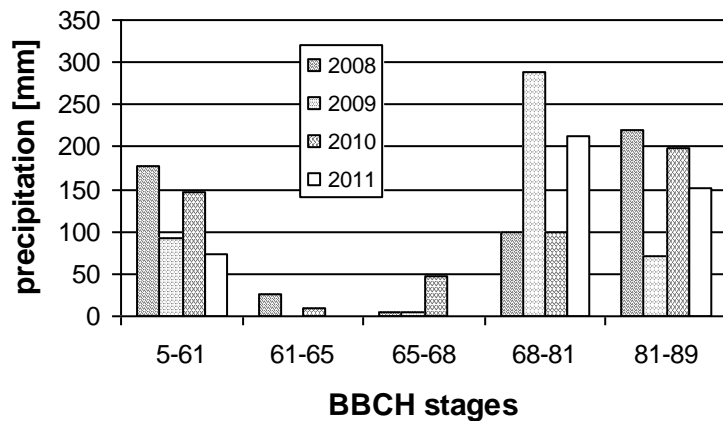


Figure 3: Amounts of precipitation at the Pinot Blanc site at different phenological stages (BBCH) from 2008-2011.

Discussion

Although the Mg fertilizer has been reported to assist in the prevention help against BSN (Riedel, M., Fröhlin, J. and Schies, W. Unpublished.), in 2010 there was no effect. In 2011, differences were observed for the incidences of BS at the “Pinot Blanc” site. The treatment using Mg fertilizer was the least affected by BS. As all the treatments showed Mg deficiencies in the leaves prior to harvest, the use of Mg fertilizer may have an effect on BS as the deficiency stress - although not visibly - was diminished and the health status of the plants generally improved. Despite former studies (Siegfried and Jüstrich, 2006, Mohr, 2007), the use of Mg foliar fertilizer did not decrease the incidence of BSN, however, although not significant, the use of K fertilizer showed the least incidence of BSN. As there were no significant results in 2010, sprayings in 2011 were performed later in order to prevent late Mg deficiencies (Fox et al., 2001) what might have been a reason for the differences. In

2011, the vines treated with Ca fertilizer did not only show the highest affection by BS but also the lowest SPAD values. As in berries affected by BS the amount of Ca is significantly higher than in healthy berries, Ca fertilization might rise the risk of BS. If higher Ca concentration is a reason for the occurrence of BS or if it is the result of this disease remains unclear. Although there are no significant differences between the SPAD measurements of the different treatments in 2010, the SPAD values show an increased chlorophyll content at both sites with the use of Mg fertilizer. As Mg is the central ion in chlorophyll, a higher amount of Mg-ions also increases the amount of chlorophyll built in the leaves (Bybordi and Shabanov, 2010). The use of Ca-fertilizer might inhibit the uptake of Mg^{2+} ions as Ca^{2+} ions act as antagonists (Zharare et al., 2010, Csikasz-Krizsics and Dioófási, 2007). This explains the low SPAD levels of the leaves treated with Ca-fertilizer in 2011 where Mg deficiencies had occurred.

The weather conditions during and after spraying are a factor which has the most influence on the effect of foliar fertilization (Marschner, 1986, Taiz and Zeiger, 2000). Ideally, sprayings are performed in the late evening or early morning to ensure cool and wet conditions which are optimal for the uptake of nutrients via the leaves (Marschner, 1986). For practical reasons, these optimal conditions were not found on all spraying dates which might explain the lack of nutrient increase in the leaves. Another reason may be the late date of collecting the leaf samples as the mobility of K and Mg within the plant is very high (Marschner, 1986). About 20 % of the Mg taken up is assumed to circulate in the plant (Bybordi and Shabanov, 2010) therefore elements move rapidly from the source to the sink which can be the younger leaves, grapes or the shoots in order to store nutrients. Former studies have also shown that foliar application of K did not show an increase in the leaf contents of K (Knoll et al., 2006).

Berry growth follows a double sigmoid curve (Coombe, 1992). The first phase of cell division begins after flowering. Initially growth is very rapid, however prior to veraison it slows down. After veraison, the second sigmoid curve starts and ends with the ripening of the berries (Mpelasoka et al., 2003). Previous research has shown that certain weather conditions in the phases of rapid growth and accumulation of acids (first phase) and sugars (second phase) can cause BSN (Mohr, 2005) and our own observations in the years 2008 until 2011 (Bachteler, K. and Riedel, M. Unpublished.) support the presumption of a relationship to BS. In 2010 like in 2008, BS and BSN occurred in many parts of Germany. In these years, cold and wet conditions during flowering led to a long and unsteady blossom which was then followed by a period of wet and cool weather after veraison in August. In 2009, BSN and BS were observed rarely in Southern Germany. Considering the amount of precipitation during the vegetation period exemplarily at the "Pinot Blanc" in 2009, compared to 2008 and 2010 it is striking that 68 % of the precipitation in 2009 occurred between the stages 68-81 which means after flowering and before ripening. This is the period when the water demand of vines is the highest (Füri and Kozma, 1977). In 2008 and 2011, around 40 % of the precipitation of the whole vegetation period were recorded at the beginning of ripening until harvest, during BBCH 81-89 (Figure 7). In connection with cool and inconstant temperatures this seems to increase the occurrence of BS (Siegfried, 2010). In 2011, the incidence of BS was higher than 2010 which seems inappropriate to this hypothesis. However, 2011 takes a special role concerning the distribution of the precipitation throughout the vegetation period. Although more than 200 mm of precipitation were recorded between the stages 68-81, the period before was the driest within the last four years with only 73 mm of precipitation from furry stage until opening of the caps and no precipitation at all from opening of the caps until end of flowering. This changed towards ripening until harvest, resulting in

150 mm of precipitation which probably was a factor to rise the incidence of BS in this year.

Finally, the use of foliar fertilizers for vines must be assessed critically. An existing deficiency of nutrients can only be corrected for the current vegetation period (Ochßner, 2007, Hamayun et al., 2011). The majority of these elements are absorbed by the roots and therefore must be present in the soil (Taiz and Zeiger, 2000, Wocior et al., 2011).

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4 Influence of berry shrivel on concentrations of resveratrol, catechin, epicatechin and ϵ -viniferin in berries of *Vitis vinifera* L. cvs Zweigelt, Pinot Blanc and Pinot Gris

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Influence of Berry Shrivel on Concentrations of Resveratrol, Catechin, Epicatechin and ϵ -Viniferin in Berries of *Vitis vinifera* L. Cultivars ‘Zweigelt’, ‘Pinot Blanc’ and ‘Pinot Gris’

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Summary

The effect of berry shrivel, a physiological disorder in grapevine, on the concentration of phenolic compounds in the cultivars ‘Zweigelt’, ‘Pinot Gris’ and ‘Pinot Blanc’ berries was studied. Specific phenolic compounds were analysed by HPLC and compared to concentrations in healthy berries of the same varieties and between the varieties. As resveratrol is often used as a marker for infection or stress induced defence mechanisms in grapevine, it was postulated that resveratrol concentration in the grape berries is positively correlated with berry shrivel. Other phenolic substances such as catechin, epicatechin and viniferin were also analysed to evaluate their potential effect on berry shrivel. Healthy

berries from the variety ‘Zweigelt’ contained significantly more trans-resveratrol, trans-resveratrol-glucoside and ϵ -viniferin, whereas shriveled berries contained significantly higher concentrations of catechin and epicatechin. The phenolic concentrations were not significantly different between healthy and affected ‘Pinot Gris’ and ‘Pinot Blanc’ grape berries, respectively, except for ‘Pinot Gris’ with significantly higher catechin concentrations in healthy berries compared to shriveled berries. Cis-resveratrol could not be detected in any of the samples and cis-resveratrol-glucoside could not be definitely identified.

Key words. Phenolic compounds – physiological disorder – berry shrivel – grapevine

Introduction

Resveratrol is a phytoalexin and belongs to the group of the stilbenes. It is generated via the Shikimat biosynthesis pathway (RICHTER 1998). Different monomeric forms can be found in grapevines: trans- and cis-resveratrol and the trans- and cis-resveratrol glucosides (SOLEAS et al. 1995). In plants resveratrol is produced under abiotic stress conditions such as high UV-radiation or biotic stress induced for example by the pathogen *Botrytis cinerea* (STEIN and HOOS 1984; ADRIAN et al. 1997; BAVARESCO et al. 1997, 2007, 2009; SCHOUTEN et al. 2002; FAVARON et al. 2009; GONÇALVES VAN ZELLER DE MACEDO BASTO et al. 2011). It was found to be synthesized in different organs of grapevine such as leaves, shoots and berries (LANGCAKE and PRYCE 1977; VEZZULLI et al. 2007; BAVARESCO et al. 2007). In the berry, the production of resveratrol increases at the beginning of berry development and then ceases around veraison when the production of anthocyanins rises (JEANDET et al. 1991). Both anthocyanins and resveratrol share a similar synthesis pathway so that the constitutive chalcone synthase is competing against the inducible

stilbene synthase (JEANDET et al. 1995). ϵ -Viniferin also belongs to the group of stilbenes and is a dimer of resveratrol (BEZHUASHVILI et al. 2010). It is biosynthesized by an oxidative process of resveratrol (LANGCAKE 1977). Catechin and epicatechin are the flavonoids with the highest concentrations in red wines (GOLDBERG et al. 1998). They act as antioxidants and inhibit the formation of free radicals. It is also known that these flavonoids can inhibit the oxidation of human LDL (low density lipoproteins) and therefore help to prevent coronary artery disease (NAGAO et al. 2005; RIBEIRO DE LIMA et al. 2006). Usually only the diastereomers (+)-catechin and (-)-epicatechin can be found in noteworthy amounts. BUTKHUP et al. (2010) found 52.30 ± 1.13 mg 100 g⁻¹ dry weight base for (+)-catechin content of whole grape extract and 27.92 ± 1.40 mg 100 g⁻¹ dry weight base for (-)-epicatechin respectively. Catechin and epicatechin often form oligomers. Those procyanidins play an important role for the structure and astringency of wines (OJEDA et al. 2002).

Berry shrivel (BS) is a physiological disorder of grapevines. Grapes affected by BS lose their turgescence at the onset of ripening. In red varieties the berries often remain

green and dull. The accumulation of sugars ceases and the must weight remains low around 10–15 °Brix. BS does not cause damage on the outside of the rachis and berries. This distinguishes BS from another common physiological disorder, the bunch stem necrosis. The rachis of grapes affected by bunch stem necrosis become necrotic and sometimes the whole bunch abscises. The factors causing both diseases may be related to nutrient scarcity in the soil or an unbalanced ratio of potassium and magnesium (FARDOSSI 2003). As no plant diseases caused by pathogens such as bacteria, phytoplasma or fungi could be associated with these symptoms (REISENZEIN and BERGER 2001; KELLER 2008), abiotic stress was in the center of many researchers' attention investigating the causal factors for both non-pathological disorders. Temperature during ripening and flowering (W. Siegfried, personal comm. 2010), shortage or excess of water (STÜCKLIN 2007) and yield control (KÜHRER and GABLER 2011) have been mainly suggested. The aim of this study was based on the hypothesis, that resveratrol might be a useful marker for presymptomatic detection of berries affected by BS. Possible differences in the concentration and composition of phenolic compounds between healthy and affected berries would offer some evidence as to which physiological mechanisms are likely associated with the occurrence of BS.

Material and Methods

Site description

Berry samples of 'Zweigelt' grapes were taken at a commercial vineyard in Weinstadt-Endersbach, Germany (48° 49' 05.54" N, 9° 22' 18.54" E, 246 m a.s.l.). The vineyard is orientated north-south and has an area of 1438.0 m². The soil type was classified as endostagnic Regosol (calcaric). The vines were all St 9 clones on 5BB rootstocks, planted in 2002 and trained as bended unilateral shoots with 10–11 buds.

The 'Pinot Blanc' site was located in Ehrenkirchen, Germany (47° 55' 33.22" N, 7° 44' 59.92" E, 297 m a.s.l.), orientated east-west and had a total area of 403.2 m². The soil type was classified as haplic Regosol (calcaric, silty). The vines were 0212 clones on 5BB rootstocks, planted in 2000 and trained as unilateral shoots with 9–12 buds.

The 'Pinot Gris' site was located in Oberrotweil, Germany (48° 05' 30.54" N, 7° 36' 54.81" E, 198 m a. s. l.). The vines are unknown clones on 125AA rootstocks. The vineyard is orientated north-south and had an area of 1134.4 m². The vines are trained as unilateral shoots with 9–11 buds. The soil type was not classified but the parent rock material is volcanic tephrite.

Berry samples

At commercial harvest in 2010 berry samples of 'Zweigelt' were taken on 6 October and those of 'Pinot Blanc' on

7 October. In 2011 healthy and affected 'Pinot Gris' were collected 10 days after commercial harvest on 13 October, a delay that was due to miscommunication. The winegrowers' commercial harvest in both years took place at must weights of healthy berries of around 20.5 °Brix for 'Zweigelt' and 22.0 Brix for 'Pinot Blanc' and 'Pinot Gris'. Berries affected by BS showed must weights around 15.0 °Brix in all three varieties. Following harvest, healthy and shrivelled berries were frozen and stored at -25 °C until extraction. At harvest 12 samples of healthy and affected berries were taken for the variety 'Zweigelt', respectively, as well as 6 samples each of healthy and affected berries of both 'Pinot Gris' and 'Pinot Blanc'. Each sample had a total weight of about 20 g. Healthy individual berry weight was 3.2 ± 0.44 g, whereas one shrivelled berry weighed 1.1 ± 0.23 g.

Weather data

Weather data were collected by the Landwirtschaftliches Technologiezentrum Augustenberg, Germany, at meteorological stations near the experimental sites and provided by the program Vitimeteo (State Institute of Viticulture and Enology, Freiburg, Germany, and the Agroscope Changins-Wädenswil, Switzerland).

Extraction

Phenolic compounds were extracted following the procedure of BAVARESCO et al. (1997). Frozen berries were cut in half and seeds were removed to avoid extraction of constitutive resveratrol. Subsequently the berries were crushed in a mortar with 20 ml of 95 % methanol. The homogenate was transferred in an Erlenmeyer flask with another 10 ml 95 % methanol and then agitated on a shaker (Innova[®] 40, New Brunswick Scientific, USA) at room temperature for 20 min. After filtration through a filter paper (Macherey-Nagel GmbH & Co. KG, Germany), the methanol was vaporized in a rotary evaporator (BÜCHI Rotavapor-RE, Essen, Germany) at 80 mbar and 35–40 °C. The aqueous extract was mixed with 7 ml ethyl acetate and 7 ml sodium bicarbonate and vortexed (IKA[®] MS1 Minishaker, Germany) until phase separation was observed. The upper phase was collected, another 5 ml ethyl acetate was added and the sample was again vortexed. After phase separation, the upper phase was collected again and vaporized until complete dryness. The dry material was resolved with 100 % methanol and stored in a freezer at -25 °C until analysis.

HPLC-Analysis

Phenolic compounds were analysed using high performance liquid chromatography (HPLC; Agilent 1260 Infinity, USA) with Diode Array Detector according to the method of VRHOVSEK et al. (1995). The following standards were used: (+)-catechin hydrate (Fluka[®] Analytical,

Switzerland), (-)-epicatechin (Lancaster Synthesis, USA), trans-resveratrol (Sigma-Aldrich®, Germany), trans-resveratrol-glucoside (specs, Netherlands) and ϵ -viniferin (AK Scientific Inc., USA). Calibration of cis-resveratrol and cis-resveratrol glucoside: Aqueous solutions of the pure trans compounds were partially transformed to cis/trans mixtures by exposition to daylight for several days. Afterwards the chromatograms contained two peaks, peak 1 = remaining trans compound and peak 2 = cis compound formed by isomerization. The amount of remaining trans compound was determined at 306 nm with the existing calibration curve. The difference to the concentration before isomerization gave the amount of formed cis compound. The ratio of peak area to concentration for the cis compound at 285 nm was calculated to create a calibration curve.

The injected volume of each sample was 5 μ l. The samples were separated with a LiCrospher® 100 RP18e column (250 \times 4 mm; 5 μ m) with a LiCrospher 100 RP18e guard-column (4 \times 4 mm; 5 μ m). 0.1 % phosphoric acid (A) and a mixture of 0.1 % phosphoric acid plus 80 % acetonitrile (B) were used as mobile phase. The flow rate was 0.8 ml min⁻¹ and UV-absorption was measured from 200 to 400 nm. Catechin and epicatechin were quantified at 210 nm, trans-resveratrol and trans-resveratrol-glucoside at 306 nm, cis-resveratrol and cis-resveratrol-glucoside at 285 nm. Between two cycles the column was washed for 15 min with solvent A. See Fig. 1 for exemplary chromatograms.

Detection of *Botrytis cinerea*

To ensure that the analysis of resveratrol was not affected by *Botrytis cinerea*, the samples were tested with the LOEWE®FAST Kit *Botrytis cinerea* (Loewe® Biochemica GmbH, Sauerlach, Germany). Samples were mashed, the grape juice mixed with the buffer and then applied onto the test cassettes containing specific antibodies. After a few minutes, besides a control band, a red band can be observed when an infection with *Botrytis cinerea* exists.

Statistical analysis

12 affected and 12 healthy samples of Zweigelt berries as well as 6 affected and 6 healthy samples each of Pinot Gris and Pinot Blanc berries employed to evaluate treatment differences (variety and BS) using a bi-factorial analysis of variance (ANOVA, SAS® Institute INC, USA). Least significant differences (LSD) were computed and significance was indicated at a probability level of $P > 0.05$.

Results

Phenol analysis

The results indicate a great variability of phenol concentration in berries of the different varieties (Table 1).

Table 1. Average phenol concentration [μ g kg⁻¹ fresh weight] in healthy berries and berries affected by berry shrivel of 'Zweigelt', 'Pinot Gris' and 'Pinot Blanc' grapevines.

Variety	Healthy berries			Berries affected by BS		
<u>Catechin</u>						
'Zweigelt'	1392	a	A	5390	b	A
'Pinot Gris'	4790	a	B	2953	b	B
'Pinot Blanc'	2767	a	A	3408	a	B
<u>Epicatechin</u>						
'Zweigelt'	963	a	A	2425	b	A
'Pinot Gris'	1048	a	A	771	a	B
'Pinot Blanc'	656	a	A	350	a	B
<u>Trans-resveratrol</u>						
'Zweigelt'	443	a	A	17	b	A
'Pinot Gris'	62	a	B	31	a	A
'Pinot Blanc'	67	a	B	0	a	A
<u>Trans-resveratrol-glucoside</u>						
'Zweigelt'	469	a	A	219	b	A
'Pinot Gris'	102	a	B	56	a	B
'Pinot Blanc'	33	a	B	50	a	B
<u>Viniferin</u>						
'Zweigelt'	2035	a	A	621	b	A
'Pinot Gris'	162	a	B	183	a	B
'Pinot Blanc'	94	a	B	42	a	B

Means of healthy and affected berries of one variety followed by the same small letter in each line do not differ significantly at $P < 0.05$.

Comparing the three varieties, means of healthy berries or affected berries followed by the same capital letter in each column do not differ significantly at $P < 0.05$.

Comparing healthy and affected berries of the same variety, significant differences in phenol concentration were found in 'Zweigelt'. In this variety berries affected by BS had nearly four times higher catechin concentration and 2.5-fold higher epicatechin concentration than healthy berries. In contrast, affected 'Zweigelt' berries had concentrations of 96 % less trans-resveratrol, 53 % less trans-resveratrol-glucoside and 69 % less ϵ -viniferin when compared to healthy berries. For 'Pinot Gris' berries affected by BS had 38 % less catechin concentration than healthy berries. 'Pinot Blanc' berries did not show any significant differences between healthy berries and berries affected by BS. Cis-resveratrol could not be detected in any of the samples and cis-resveratrol-glucoside could not be definitely identified due to overlaying signals.

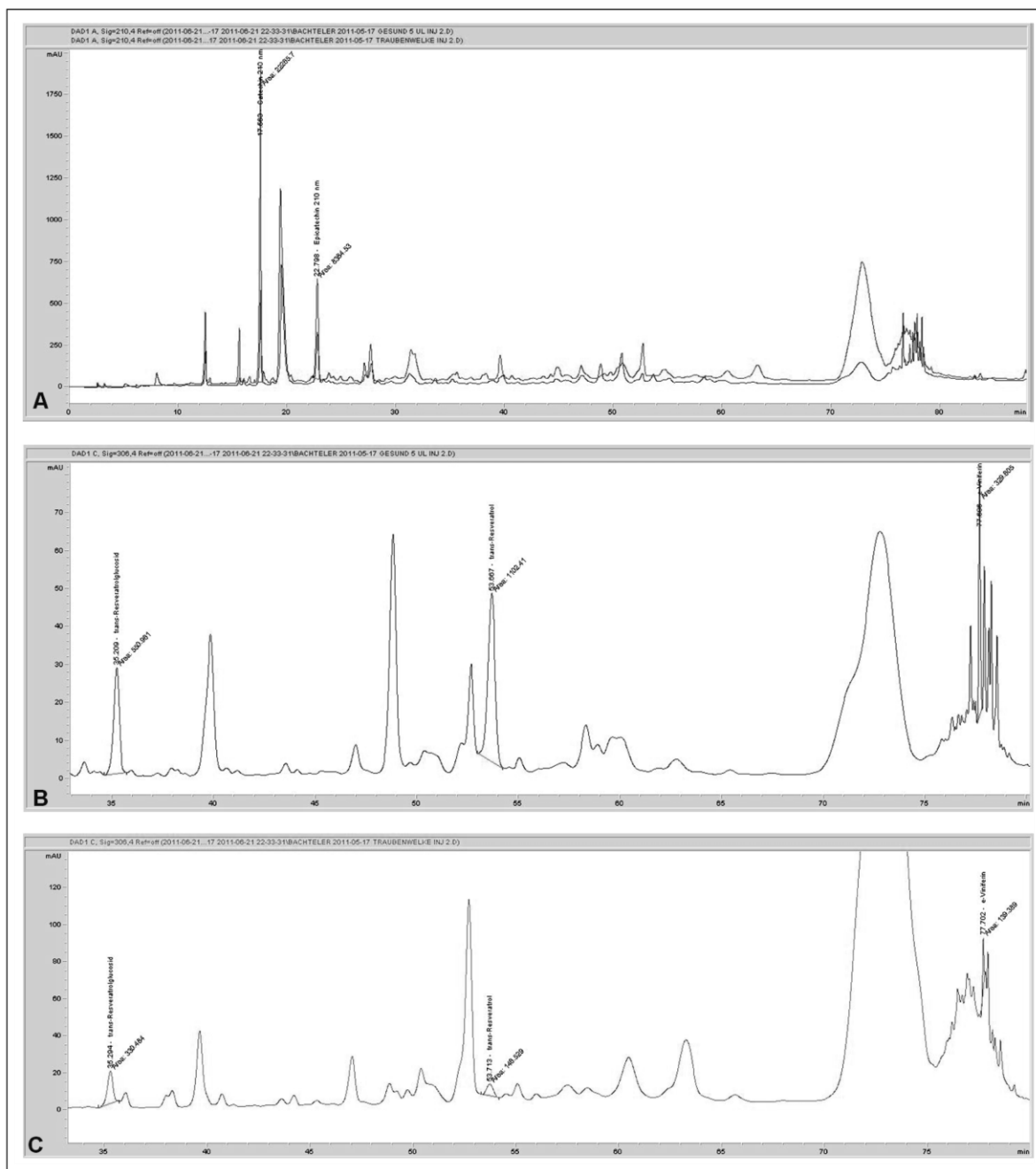


Fig. 1. Exemplary chromatograms of two 'Zweigelt' samples (healthy and affected by BS). A: Detection at 210 nm of catechin (17.5 min) and epicatechin (22.6 min) in healthy (lower line) 'Zweigelt' berries and berries affected by BS (upper line); B: Detection at 306 nm of trans-resveratrol-glucoside (35.2 min), trans-resveratrol (53.6 min) and viniferin (77.6 min) in healthy 'Zweigelt' berries; C: Detection at 306 nm of trans-resveratrol-glucoside (35.2 min), trans-resveratrol (53.6 min) and viniferin (77.6 min) in 'Zweigelt' berries affected by BS.

Weather data

In 2010, the average daily temperature was 8.9 °C and 10.3 °C and the amount of precipitation was 772 mm and

888 mm at the 'Zweigelt' and 'Pinot Blanc' sites, respectively. At the 'Pinot Gris' site, the average daily temperature was 11.1 °C and the annual rainfall was 495 mm in 2011.

Detection of *Botrytis cinerea*

None of the samples tested with the Lateral Flow Test showed an infection with *Botrytis cinerea*.

Discussion

In this study, the concentration of trans-resveratrol was higher in healthy berries of all varieties compared to berries affected by BS of the same variety. The role of phenolic compounds such as catechin and epicatechin under abiotic stress conditions is not clear and with contradictory results in the literature. Some studies showed an inhibition of these substances e. g. in seeds of *Vitis californica* (WEIDNER et al. 2011) under osmotic stress. Others reported an increased production of phenols under different stress conditions such as pathogen attack, wounding, high light or low temperature (DIXON and PAIVA 1995; WEIDNER et al. 2009). It has also been shown that vines under water deficit before and after veraison contain more flavan-3-ols than plants without or with less stress (OJEDA et al. 2002). If BS indicates another form of abiotic stress for the plant or if stress leads into BS symptoms, this may explain the increased production of catechin and epicatechin in 'Zweigelt' berries affected by BS. Another reason for the differences might be that healthy berries contain more anthocyanins than affected ones (KRASNOW et al. 2009) so there is more conversion from catechins to procyanidins (GOLDBERG et al. 1998). The non-red varieties 'Pinot Blanc' and 'Pinot Gris' berries do not show an increased concentration of catechin and epicatechin when affected by BS. The fact that 'Pinot Gris' even shows significantly higher concentrations of catechin in healthy berries indicates that our results are not consistent with other findings (BALIK et al. 2008).

It is difficult to compare different varieties especially red and white ones. White varieties naturally contain less resveratrol and viniferin (ECTOR et al. 1996; BAVARESCO et al. 2007) which becomes apparent when comparing 'Zweigelt' with 'Pinot Blanc' or 'Pinot Gris' berries. However, exceptions can be found too, as SOLEAS et al. (1995) and OKUDA and YOKOTSUKA (1996) obtained results for resveratrol in white varieties that were in the range of red skinned varieties or even above. Differences between 'Zweigelt' and 'Pinot Gris' might be explained by the different times of sampling and the intermediate role of 'Pinot Gris' between white and red varieties. 'Zweigelt' berries were sampled at commercial harvest, whereas Pinot Gris berries were sampled after harvest. The later sampling date may have caused lower concentrations of resveratrol as the concentration decreases constantly after veraison (JEANDET et al. 1991; EDER et al. 2001). The effect of variety, e.g. 'Zweigelt', 'Blaufränkisch', 'Pinot Noir' and 'Cabernet Sauvignon' and harvest time on the concentration of resveratrol was shown by EDER et al. (2001).

Comparing these results with previous studies is difficult as concentrations of phenolic compounds differ with varieties, years, dates of sampling and trial site. The concentrations found in the present study for catechin and epicatechin are 20 times lower than those obtained by ZHAO et al. (2006) in 'Cabernet Sauvignon' or those obtained by BUTKHUP et al. (2010) in dry berries of 'Shiraz'. The concentrations of trans-resveratrol found in earlier studies differ also substantially with those obtained in the present study. JEANDET et al. (1991) reported concentrations below $1000 \mu\text{g kg}^{-1}$ in 'Pinot Noir' at harvest and OKUDA and YOKOTSUKA (1996) describe a range from $59\text{--}1795 \mu\text{g kg}^{-1}$ in white varieties and $92\text{--}1604 \mu\text{g kg}^{-1}$ in red or pink varieties. Others stated much higher concentrations such as ECTOR et al. (1996) with $6390\text{--}14000 \mu\text{g kg}^{-1}$ in 'Muscadine' berries or BAVARESCO et al. (1997) obtaining $5000 \mu\text{g kg}^{-1}$ in 'Castor' berries and $2110 \mu\text{g kg}^{-1}$ in 'Huxelrebe'. For ϵ -viniferin little data can be found. GONÇALVES VAN ZELLER DE MACEDO BASTO et al. (2011) indicated a minimum of $37 \mu\text{g kg}^{-1}$ just prior to an infection with *Plasmopara viticola* as elicitor and a maximum concentration of $3327 \mu\text{g kg}^{-1}$ after infection. The present results are within the range of these values.

If abiotic stress causes or increases symptoms of BS, it does not seem to increase the concentration of resveratrol in affected berries. Therefore, the use of resveratrol as a marker for an early detection of berries affected by BS is not suitable. Nevertheless, well ventilated soils, healthy roots, a suitable green cover and adapted yield targets can help to minimize abiotic stress for the vines in general and may also help to minimize the incidence of BS (REDL et al. 2009).

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5 Physiological aspects and monitoring of berry shrivel in grapevine

5.1 Introduction

Berry shrivel (BS) is one of the most important physiological disorders in grapevine. Physiological aspects of BS were mainly studied in the USA and Austria (Keller and Bondada, 2007, Hall et al., 2011, Griesser and Forneck, 2010). Girdling experiments in cv. Zweigelt were conducted in Germany connected to this present study (Erhardt, 2010). Girdled grapes showed symptoms similar to those of grapes affected by BS. Blocked phloem vessels would also explain the low sugar content of affected grapes. As callose is present in sieve tubes in grapevines during winter dormancy (Aloni and Peterson, 1991), rachis of berries affected by BS were examined under the microscope to find possible callose deposition. Transpiration of berries was investigated as the loss of turgescence might be caused by an increased transpiration by the berries

Many factors were discussed for causing BS; some of them can be influenced directly by the winegrowers such as potassium deficiency in the soil (Redl, 2005), unbalanced yield and leaf-fruit ratio (Kührer and Gabler, 2011), choice of clones, rootstocks or varieties (Schumacher et al., 2007) or the use of bioregulators (Kührer, 2010). To get an idea of possible correlations between incidences of BS and any of these factors, 26 monitoring sites in the Southwest of Germany were selected and data concerning the vineyard management were collected.

5.2 Material and Methods

Transpiration of berries

Healthy berries and berries affected by BS of the variety Pinot Blanc were sampled at harvest in 2011. 10 berries of each group that did not show any outer injury were selected and detached from the stem including the receptacle. The berries were sealed with silicone rubber (Dow Corning[®] 3140 RTV Coating) and weighed at 5 different times (1 ½ hours after detachment (HAD), 4 ½ HAD, 24 HAD and 48 HAD). The weight loss was calculated in percent compared to the starting time. Statistical analysis was conducted using a Kruskal-Wallis-Test (SAS[®] Institute INC, USA) at $\alpha=0.05$.

Callose deposition in the phloem

Healthy grapes and grapes affected by BS were sampled at harvest in 2011 at different monitoring sites in Southern Germany. Samples included the varieties Pinot Gris, Muscaris, Zweigelt and Pinot Blanc. Berries were detached and the rachis stored in a mixture containing 90 % of 70 % ethanol, 5 % of 40 % formaldehyde and 5 % of acetic acid (Gerlach, 1977). Rachis were cut and cross sections microscopically analysed for callose deposition. Sections were stained with aniline blue (Merck KGaA, Darmstadt, Germany) solution (Gerlach, 1977, Mouzeyar et al., 1993, Smith and McCully, 1978, Hood and Shew, 1996) and examined under the microscope with fluorescence light (365 nm). Callose deposition was then visually evaluated for each section.

Monitoring sites

26 monitoring sites in the South of Germany were observed for three years. The sites had been reported by wine-growers to be affected by BS in the last years. Soil

samples and leaf samples were collected (s. M&M in 2 *Soil fertilization to alleviate berry shrivel, evaluation of the resulting wine quality and determination of cations in affected berries*) to determine the nutritional status of the sites. Questionnaires about the vineyard management were completed by the wine-growers. The questionnaires included 1) general questions about variety, rootstock, scion, age and site 2) questions about soil management, 3) questions about training and yield, 4) questions about maintenance such as blowing out after anthesis, use of growth regulators and defoliation and 5) questions about the incidence of BS in the last year and the years before. Questionnaires were evaluated and attempts were made to correlate the incidence of BS on the sites with the vineyard management.

5.3 Results

Transpiration

No significant differences between the percentaged weight loss of healthy berries and shrivelled berries were found for the different times (Figure 5.1).

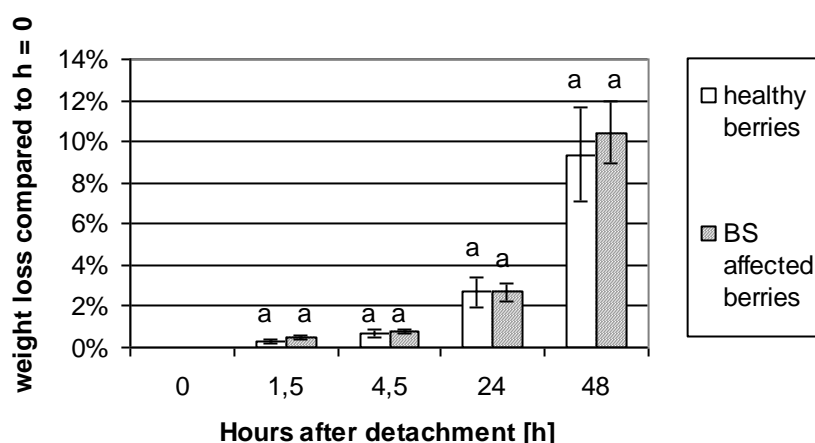


Figure 5.1: Weight loss of detached healthy berries and berries affected by BS compared to start h=0. ^aMeans followed by the same letter do not differ significantly at $p < 0.05$.

Callose deposition

Callose deposition was observed in all varieties examined, that is Pinot Gris, Muscaris, Zweigelt and Pinot Blanc. In stems of healthy grapes as well as in stems of grapes affected by BS callose formation was observed. No connection with the affection by BS could be assessed.

Monitoring sites

The main focus lay on the nutritional status of K and Mg in the soil, use of growth regulators, year of planting and target yield (Table 5.1). As not all information needed could be provided by the wine-growers, blanks occur in the table. No connection with the incidence of BS could be found for any of the factors given above. However, it seems likely that a high yield and any stress in form of a surplus or deficiency of nutrients in the soil might raise the risk of affection by BS.

Table 5.1: Overview of selected recorded data of the monitoring sites in Southern Germany from 2008 - 2011. "Site" gives the name of the winegrowing sector (in italics) and the name of the place.

Site	incidence of BS [%]			mean	year of planting	variety	rootstock	K:Mg (soil)		Gibb
	2008	2009	2010					2011	upper soil	
<i>Markgräflerland</i>										
Ehrenkirchen 1	40%	5%	6%	9%	2000	Pinot Blanc	5 BB	2,3	1,88	no
Ehrenkirchen 2	46%	3%			2000	Pinot Meunier	5 BB	2,9	1,84	no
Zunzingen	52%	39%	2%		2003	Red Chasselas				
Dottingen	25%				2000	Pinot Blanc		2,76	1,76	no
Leutersberg	15%				2004	Pinot Blanc	5 BB	2,7		no
Müllheim 1	59%	3%	2%	21%	2002	Pinot Blanc	5 BB	1,12		yes
Müllheim 2		0%	5%	2%	1997+2000	Pinot Blanc	5 BB	3,3		
Badenweiler	25%		1%	13%		Red Chasselas				
Bollschweil		3%		3%	2003	Pinot Blanc	125 AA	1,5		no
<i>Kaiserstuhl</i>										
Jechtingen	69%	7%	0%	25%	2004	Pinot Noir	5 BB	4,1		no
Bischoffingen		63%	11%	37%	2003	Muscaris	5 BB	2,9		no
Bötzingen WB		3%	1%	2%	2000	Pinot Blanc	125 AA	1,5		no
Bötzingen		11%		11%	2000	Müller- Thurgau	5 BB	1,5		no
Oberrotweil			3%	3%	1982	Pinot Gris	125 AA	2,2		no
Irringen	0%	12%	4%	5%	2000	Pinot Blanc	125 AA	1,6		
Merdingen	0%			0%	1996	Pinot Blanc		2,2		yes
<i>Fernstal</i>										
Weinstadt		22%	29%	27%	2001	Zweigelt	5 BB	1,36	0,8	no
Weinstadt		1%		1%		Pinot Meunier		0,83		yes
<i>Lauffen/Neckar</i>										
Rembold	26%			26%	1985	Pinot Meunier	5 BB	2,15	1,5	yes
Schiefer	21%			21%	1985	Pinot Meunier	5 BB	2,3	1,6	yes
<i>Ortenau</i>										
Nesselried 1	40%	23%	5%	23%	2003	Pinot Gris	SO 4			
Bottenau			3%	3%	2003	Pinot Noir	SO4	1,4		no
Nesselried 2	0%		3%	2%	2003	Pinot Gris	1103 Paulsen	1,5		
Kippenheim		5%	3%	4%	2000	Pinot Blanc	SO4			no
Zell		4%	6%	5%	1994	Pinot Blanc	125 AA	0,72		no
Lahr			5%	5%	2005	Pinot Noir	125 AA			no

5.4 Discussion

Becker and Knoche (2011) showed that more transpiration occurs via the rachis or the stem than the berry itself. As berries affected by BS did not show altered transpiration, it may be possible that water loss happened via the stem or backflow via the xylem (Tilbrook and Tyerman, 2008). As an interruption of the phloem by callose could be detected in both affected and healthy berries, the deposition seems to have no effect on the occurrence of BS. Molecular investigations on expression of callose synthase genes in cv. Zweigelt were conducted in Austria (Griesser and Forneck, 2010) and showed the same results. None of the genes were up- or down-regulated in the rachis of grapes affected by BS compared to healthy grapes.

On the monitoring sites, the data analysis of the factors observed did not confirm any of them as the main elicitor for the incidence of BS. Personal observations of the winegrowers often show the importance of reacting individually on the special needs and requirements every site has. For example, on a deep and nutrient-rich soil, a combination of vigorous rootstocks and varieties is increasing the risk of an affection by BS. The same seems to apply for young fields with too high a target yield. Therefore it is essential to assess every site individually to find suitable ways to reduce the incidence of BS.

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6 General Discussion

The following overall discussion of the results presented above follows the outline given in *1.6 Aims & Scopes* and focuses on the factors that have been investigated regarding their effect on the occurrence of BS.

6.1 *Effect of K and Mg fertilization via the soil and the leaves*

The use of soil fertilization did not effect the incidence of BS. In the fertilization trials the amounts of K and Mg in the soil could only be slightly increased after two years. It is possible that a certain amount of K was fixated in the clay minerals and therefore was not available for the plant. The Zweigelt site was classified as clayey loam. As K needs water to diffuse to the roots for uptake (Albert et al., 2008), periods of dry weather could also be a reason for the fact that the higher amounts of K were not observed in the leaves. Mg is known to be highly mobile in the soil which leads to leaching and dislocation (Vogt and Götz, 1987) and reduces the availability for the plant. Yet, the soil fertilization of K and Mg as well as foliar fertilization obviously could not reduce the occurrence of BS - at least not in the years the trials were carried out. Research that reports K deficiency on sites affected by BS probably only considered one factor as elicitor. The weather conditions might play another important role as well as the ratio of K/Mg - and not the amount of K alone. By evaluating the nutritional status on the monitoring sites, it was obvious that there were sites affected by BS that showed low K concentrations in the soil, but there were also sites with the optimal amount of K in the soil. Vice versa, the same applies for sites that had not been affected by BS. There were sites with a K deficiency in the

soil but also sites that were optimally supplied with K. Yet it has to be considered that 26 sites is probably not a sufficient number of samples.

Although not significantly in this study, it is a fact that berries affected by BS often contain less K than healthy berries, which has been shown in former studies (Krasnow et al., 2009). As the blocking of K import into the plant is not the result of too little K in the soil but of other physiological disturbances, this led to the investigations on callose deposition in the phloem (s. 7.3 Physiological processes in grapes affected by BS, namely transpiration, phloem flow and synthesis of phenolic compounds).

6.2 Effect of temperature and distribution of precipitation at different phenological stages

The annual variation of the incidence of BS at the same sites suggests an impact of the weather conditions on BS. Studies conducted in Switzerland showed that cool conditions with 2-3 °C at night and 10 °C cooler than the normal ambient air temperature during the day starting at BBCH 81 could induce BS (Siegfried, 2010).

The impact of weather conditions on this disease may even have started in the winter the year before. In 2008, temperatures in the month after harvest stayed above 0 °C, in 2009 and 2010, however, harvesting was followed by a period of cold weather in October with temperatures below zero. If temperatures decline fast shortly after harvest, the shoots of the vines do not lignify properly and therefore important storage compounds such as starch and sugars are missing the next year during foliation. This may cause physiological disorders such as BS (Keller, 2008). Therefore, the vines might have had a predestination for physiological disorders in

the following years 2010 and 2011 but not in 2009. This assumption turned out to be true in 2009 with little incidences of BS in the Southwest of Germany as well as in 2010 when BS and particularly BSN occurred on many fields. In 2011, however, incidences of BS were comparably low, which might be due to the effect of precipitation on BS. At the start of the ripening process of the grapes until harvest, 150 mm of precipitation were recorded. As it is known that extensive precipitation during the ripening process can push the vegetative growth of the vines and increase the risk of BS as well as BSN (Raifer, 2012), incidences were expected to be higher. Since the weather conditions during flowering were extraordinarily warm and dry as well as the last two weeks before harvest, this might have balanced the high amounts of precipitation during ripening.

6.3 Physiological processes in grapes affected by BS, namely transpiration, phloem flow and synthesis of phenolic compounds

As berries lose their turgescence when affected by BS, the idea of water loss of the berry itself is obvious. Normally, stomata in the berry skin are no longer functional after veraison and are closed by epicuticular waxes (Mullins et al., 1992, Ollat et al., 2002). As the weight loss of berries affected by BS after detachment did not differ significantly from the weight loss of healthy berries, an altered transpiration or other ways of losing water via the berry surface such as microcracks can be excluded. The water loss must occur via the xylem or the phloem back to the shoot. As the girdling experiments carried out during a diploma thesis (Erhardt, 2010) connected to this study resulted in similar symptoms as BS, phloem disruption was investigated. Blocking sieve tubes in plants usually occurs via callose deposition (Marschner,

1986). Ca^{2+} -ions activate the callose-synthase (Musetti, 2010, Schmele and Kaus, 1990) and phloem specific proteins (Taiz and Zeiger, 2000, Knoblauch, 2001), which could explain the increased amount of Ca^{2+} -ions in affected berries or show an effect of this surplus. Cold temperatures can induce the building of callose as well (Chen and Kim, 2009). However, cross-sections of rachis of affected and healthy grapes did not show any differences in callose formation. So the loss of turgescence might not be explained by an interruption of the phloem but by an interruption of the xylem. Experimentally interrupting the xylem has not been successful, as by interrupting the xylem with a needle, bits of phloem are interrupted as well. Hall et al. (2011) showed that destroying the xylem in the peduncle by drilling a hole did not result in symptoms similar to BS. However, phloem girdling can affect the xylem flow as well. Experiments carried out by Zwieniecki et al. (2004) with *Acer rubrum* L. and *Acer saccharum* Marsh showed that girdling the phloem reduced the hydraulic conductance of the xylem. The decrease in the conductance might be explained by a reduction of ion exchange between phloem and xylem, as adding KCl solution annihilated the effects on xylem conductance. This may be a reason why girdling resulted in symptoms similar to BS but no callose could be found.

Recent studies show that the xylem remains functional in post-veraison grapes (Bondada et al., 2005, Keller et al., 2006), so a possible backflow via the xylem might be investigated in further studies.

7 Conclusion

Factors causing BS still remain unclear. Potassium deficiency could not be identified as the only factor, but a balanced ratio of K and Mg in the soil should nonetheless be pursued. Fertilization trials should be continued to verify longtime effects of K and Mg on BS. To ensure optimal conditions for nutrition uptake via the roots, site-specific soil management must be conducted which includes not only fertilization and monitoring of the nutritional status in the soil but also green cover, tillage and foliage treatment.

The annual variability of the incidence of BS suggests that climatic conditions during the vegetation period have an effect on the occurrence of BS. Although not statistically approved during this study, wet and cool weather conditions during flowering and the ripening process of the berries seem to promote BS.

One physiological effect of BS definitely is the increased concentration of Ca in the berries and the blocked conversion from catechin and epicatechin to anthocyanidins, which explains the incomplete colouring of affected berries in red varieties. Further investigations should focus on this physiological aspect of BS. Xylem flow measurements, canopy height (leaf/fruit-ratio) or greenhouse trials under controlled climatic conditions may help to find new approaches to BS.

Although specific factors causing BS could not be identified during this study, it was shown that this disease is one of the most complex diseases in grapevine and results from a combination of various factors. Therefore, basic studies in the vascular system of grapes and mineral composition of affected berries have to be pursued.

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Declaration of Originality

Hereby I declare that this doctoral thesis was independently written by myself. In addition, I confirm that no other sources than those specified in the thesis have been used. I assure that this thesis, in the current or similar format, has not been submitted to any other institution in order to obtain a Ph.D. or any other academic degree.

Ich erkläre hiermit, dass ich diese Dissertation selbständig angefertigt habe. Es wurden nur die im Literaturverzeichnis aufgeführten Hilfsmittel benutzt und fremdes Gedankengut als solches kenntlich gemacht. Ich versichere, dass ich diese Arbeit in gleicher oder ähnlicher Form noch keiner anderen Institution zur Prüfung vorgelegt habe.

Hohenheim, August 2012

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