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THE EFFECT OF CLUSTER TIPPING AND LEAF REMOVAL ON THE YIELD OF THE GRAPEVINE CULTIVAR ZWEIGELT, ON BERRY SHRIVEL AND THE CHEMICAL COMPONENTS OF THE MUST

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1. INTRODUCTION

Zweigelt is one of the most important red grapevine variety in Hungary. In the case of Zweigelt, berry shrivel - which is most probably a physiological disease - is occurring more and more frequently, and its causes are not even known. This phenomenon causes significant losses in yield and quality, so it is necessary to have such technologies which can mitigate the damage. As Zweigelt is highly prone to rot, it is worth using technologies that can solve both berry shrivel and botrytis at the same time.

In Hungary, berry shrivel has not been studied at the scientific level before, although it is also an increasingly significant problem in Hungary.As a precaution, the literature recommends cluster tipping (KÜHRER, 2009; LEICHTFRIED et al., 2010), which I supplemented with an additional crop-regulating operation: leaf removal at bloom.As there are very few studies internationally dealing with the relationships between viticultural practices and biogenic amines, my experiment also included measuring the amount of biogenic amines, as these substances are very important from a human physiological point of view.The experiment took place in three production sites (Dunakeszi, Vác, Erdőkertes), where, based on previous practical experiences, the Zweigelt variety showed different degrees of berry shrivel. The characteristics of the plantation and bud load of the three sites were similar.

In my research I was looking for answers to the following questions:

- How do the applied treatments affect the yield and quality?
- How do the applied treatments affect the incidence and severity of berry shrivel?
- Do weather factors play a specific role in the appearance of berry shrivel??
- How do the soils' nutrient conditions affect berry shrivel?
- How do the amount of biogenic amines changes in shrivelled berries compared to healthy ones?
- How do the applied technolgies effect the amount of biogenic amines?
- Is there a difference between sites in respect of the amount of biogenic amines?
- Can phytoplasma play a role in the occurrence of berry shrivel?

2. MATERIALS AND METHODS

2.1. The experimental sites

The experiments were set up in Dunakeszi, Vác and Erdőkertes for three years (2014-2016). The soils are significantly different in composition and watercapacity. In Dunakeszi, the soil is well supplied with humus and is poorly water retention. The soil in Vác has very low humus content but it has high water storage capacity. The soil in Erdőkertes is characterized by low humus content and poor water storage capacity.

2.2. The characteristics of the plantations

In all of the three sites the training system was modified Moser, the variety *Vitis vinifera L. cv* Zweigelt (rootstock: Teleki 5C). In Dunakeszi, the row orientation is N-S, the spacing is 3 x 1.2 m, the planting took place in 1983. The plantation in Vác has the same parameters except for the year of planting (1982) and the row orientation (E-W). In Erdőkertes (planting: 1986) the spacing is 3 x 1 m, and the row orientation is NE-SW.Bud load was 4-5 bud/m^2.

2.3. Method and time of treatments

I selected 6 rows per location. I selected similar conditioned grapevines and randomly distributed the treatments with the help of a randomization program: 4-4-4 control, cluster tipped and defoliated stocks (a total of 72 examined grapevine / site).

Leaf removal at bloom: treatment was carried out shortly after the onset of bloom, at a time close to full bloom (BBCH 65) (SABBATINI, 2011), on the same day at the three sites. I removed the leaves opposite the cluster, as well as the ones below and above it, by tearing them at the base of the leaf blade by hand. Leaving the petiole on the shoot avoids damage to the shoot (FAZEKAS, 2012).

Cluster tipping: this treatment was also carried out on the same day at the three sites. I did cluster tipping when the berry size reached the same size as a pepper, but did not yet exceed the size of the green peas (BBCH 75) (HAFNER, 2001). Depending on the size of the bunch, I cut its lower, apical third, and in some cases, if the size of the cluster was big, half of it. (FOX, 2000; PRIOR, 2005; ZANATHY, 2006).

2.4. Evaluation of the incindence and severity of botrytis and berry shrivel

Botrytis: it was made prior to harvest, based on eye estimation. The healthy bunches (asymptomatic) received a value of 0 %, the completely rotten were valued as 100 %. Intermediate values were determined in 10% breakdown. Incidence was determined by the ratio of healthy (0 %)

to diseased clusters (10-100 %). The severity of botrytis was obtained by averaging the condition of all clusters recorded on each grapevine.

Berry shrivel: it was done by visual evaluation before harvest. The cluster that did not contain any shrivelled berries received a value of 0 % and the cluster consisting only of shrivelled berries received a value of 100 %. The intermediate states were evaluated with a 10% breakdown in this case as well. The method of evaluation was the same as I used for botrytis.

2.5. Methods

Yield analysis and basic analytics: during the harvest I measured the yield (kg/m2).During the pre-harvest evaluation, I got the number of the clusters and the yield of the given grapvine, based on these values I calculated the average weight of the cluster.The average weight of the berries was determined on the basis of 30 berries/bunch.For the analysis of the must samples, I took a cluster (representing the given grapevine) from each examined grapevine.In 2016 in Vác, I also sampled 1 healthy and 1 shrivelled cluster from each examined grapevine.The sugar content was measured with a Brix meter.After that, I converted the value to Hungarian sugar degree (MM°)(TÖRÖK; 2009). The pH value was examined with a pH measuring instrument, and the titratable acid content was examined by acid-base titration (NaOH, 0,1 M).

Leaf and soil analysis: the leaf and soil analysis was performed at the Badacsony Research Station of the NAIK Viticulture and Enology Research Institute. Sampling was also performed for leaves and soil at the time of the harvest according to the protocol.

Determination of biogenic amine content: to examine the ripening process, I sampled each site three times from the onset and at the time of harvest from the examined grapevines (control and two treatments). The biogenic amine content was determined by HPLC at the Department of Oenology of Hungarian University of Agriculture and Life Sciences (BAUZA et.al., 1995; LETHONEN, 1996; KÁLLAY és NYITRAINÉ SÁRDY, 2003).

Method of phytoplasma examination: I considered it necessary to investigate the relationship between phytoplasma infection and berry shrivel. For molecular analysis, samples were taken in 2016 (the only vintage when berry shrivel occurred) in August and October. For this, I collected 10-10 leaves from 50 grapevines. The detection was done by PCR-RFLP. PCR was performed in a nested system with P1 / P7 and R16F2nR2 universal phytoplasmic (DNA encoding 16S rRNA) primers (DENG and HIRUKI, 1991; GUNDERSEN and LEE, 1996; DAIRE et al., 1997; EMBER et al., 2011).

Statistical methods: statistical analyzes were performed using IBM SPSS v25 software. The figures were created with Excel 2016.

3. RESULTS AND DISCUSSION

3.1. The incidence and severity of botrytis

In 2014, due to the rainy weather, this disease severely damaged the examined plantations. With regard to botrytis, there was a significant difference between the areas, however, due to the unfavorable weather, the effect of the treatments did not affect the incidence and severity of botrytis. The lower values observed in Erdőkertes confirm previous observations that inter-row cover crop cultivation may help reduce botrytis infestation due to increased nitrogen uptake. In 2015, due to the drier weather, the incidence and severity of rot also declined. In this season, I did not find any significant differences between the sites, nor the effect of the treatments, but this can also be explained by the weak infestation of the weather. In 2016, favorable conditions for the occurrence of this disease again arose, the incidence of botrytis was significantly different in each location. From the two treatments, cluster tipping proved to be more effective; in 2015 and 2016, in Dunakeszi significantly reduced the clusters' infestation.

3.2. The incidence and severity of berry shrivel and the effect of the vintages and soils on this phenomenon

I can explain the lack of berry shrivel in 2014, only by the effect of the rainy weather (especially from the beginning of the ripening period), because according to the literature, dry periods can cause this phenomenon. This is somewhat contradicted by the fact that in 2015, despite extremely low rainfall and high summer temperatures, I encountered a statistically invaluable number of shrivelled clusters. In 2016, I experienced significant berry shrivel, but only in Vác. In addition to this disease, botrytis has also occurred on several grapevines. Examining the evaluated vines, I found that only one disease occurred within a cluster, not the two together. Cluster tipping did not reduce the incidence of shrivelling, but it was effective in reducing its severity. However, I did not achieve results with leaf removal. In the case of Zweigelt (which is a particularly high-yielding variety), cluster tipping is a more effective yield-limiting procedure instead of defoliation. This may explain the role of cluster tipping, mentioned in several literature references, in reducing berry shrivel. Since the incidence of botrytis was less high in the case of grapevines growing shrivelled clusters, it can be assumed that the symptoms associated with berry shrivel, at least those related to content indices, appear earlier than botrytis. Based on this, it is also conceivable that high acid and low sugar content are not conducive to fungal disease. Examining the relationship between the two diseases, it can be said that the higher the number of shrivelled clusters, the less rotten clusters were in the area.

In most cases, **climatic factors**, including prolonged dry, warm weather, are associated with berry shrivel. In 2014 and 2015, when the mean temperature was the highest, I did not encounter this disease. In contrast, it occurred when this value was lower (2016). According to my results, it is not possible to draw a clear conclusion based on the average annual temperature on how it may affect the appearance of berry shrivel. In 2014, the number of heat days was low and there were no hot days. There were many heat and hot days in 2015, yet no berry shrivel occurred. In 2016, the number of heat days was again low and there were no hot days, yet the disease appeared. Based on these, I can conclude that high temperatures during the growing season are not, or not the only factors that can affect berry shrivel. However, precipitation can have a serious impact on the disease. In 2014, excessive rain fell on the plantations, and no shrivelling occurred. Although the average precipitation was low in 2015, it is likely that the previous year's amount was well stored in the soil so no shrivelling occurred again. In 2016, enough rain fell in the first half of the year as well as in July, yet I encountered berry shrivel. Based on these, I assume that the disease is more closely related to precipitation than to temperature, and that the rain that fell in the previous vintage also contributes to the onset of the disease.

Soil nutrient conditions are also considered to be a factor in berry shrivel. The nitrogen and potassium content measured in the soil did not differ in 2016 in the case of Erdőkertes and Vác, however, shrivelling appeared at the latter site. Thus, based on my results, the potassium content of the soil did not play a role in berry shrivel. At the same time, significant amounts of sodium were measured from soil samples in Vác compared to Erdőkertes, especially Dunakeszi. It is possible that this element causes a stress in the plant that manifests itself in berry shrivel. The other important observation is that the lime content in the soil of Vác is extremely high. Also in this plantation the amount of zinc and manganese is much lower compared to the other two plantations.

3.3. Yield

In 2014, there was no clear difference in **yield** between the plantations and no significant difference was found between the treatments. In 2015, there was a significant difference in yields between the sites, there was no effect of the treatments. In 2016, there was no significant difference between treatment or sites. It can be that in the case of high-yielding varieties such as Zweigelt, crop regulation can be achieved less with phytotechnical practices than with pruning. However, my results also confirm that this variety is one of the most high-yielding ones, the yield of which is very difficult to reduce to the optimum level.

In 2014, the site had a significant effect on the average **cluster and berry weight**, the treatments did not. In the case of Dunakeszi in 2015, the average weight of the clusters far exceeded that of the other years, but also in the other areas I was faced with data exceeding the variety descriptions.

In the case of the average cluster weight, I did not find any significant difference between the treatments, but the site had a significant impact on it. In respect of the average berry weight, neither treatment nor site had an effect. In 2016, the weight of clusters and berries was significantly modified in only 1-1 cases by treatment and site. Based on my results, I can conclude that during the three examined years the effect of leaf removal at bloom and cluster tipping on cluster weight was not significant for the Zweigelt variety, the differences were more due to differences between plantations.

In the case of the average **berry weight of the shrivelled samples** from Vác in 2016, it can be seen that the average weight of the shrivelled berries is significantly lower compared to the healthy ones. Among the treatments, I was able to influence the weight of the berries by removing the leaves: regardless of their health status, the berries of the clusters that received this treatment were statistically different from the other two (control, cluster tipped) treatment groups.

3.4. Sugar and titratable acidity content and pH-value of must samples

Examining the **sugar content** in the rainy year of 2014, I could not find any significant treatment effect, and nor was there difference between the sites. In 2015, the effect of the treatments did not appear again within the plantation, differences can only be detected between the plantations. In 2016, there was no significant difference between the sites or as a result of the treatments. Based on my results, it is questionable that those yield-limiting methods I have examined can improve the quality of the must, I can rather confirm the experiences that state an indifferent (or just the opposite) effect.

In 2014, there were differences between the plantations in respect of the content of **titratable acidity**, but there was no significant treatment effect. In 2015, there was no significant treatment effect in any of the cases. There was a statistically significant difference between the plantations only between the cluster tipped samples of the Dunakeszi and Vác. In 2016, again, the effect of the sites was verifiable, but there was no significant treatment effect. Based on these, it can be seen that the acid content-reducing effect of cluster tipping can be questioned based on my results.

In the case of **pH-value**, in 2014 I did not find a significant difference between the treatments. In contrast, location significantly affected this indicator. In Erdőkertes, there were significantly lower pH-values compared to the other two sites in all three treatment groups. In 2015, no treatment effect occurred again. However, there is already a difference in respect of locations: in Erdőkertes cluster tipped samples had a significantly lower pH-value compared to the Dunakeszi samples. Similarly, the value of berries picked in Erdőkertes if they were not treated had also significantly lower pH-values compared to the other two sites. In 2016, neither the treatments nor the sites had

an effect on pH. In general, I could not significantly affect the pH-value with any of the treatments, the differences were at most between the plantations.

In the case of **berry shrivel** in Vác in 2016, there was a significant difference between healthy and shrivelled clusters in respect of sugar and titratable acidity content and pH-value: I measured lower sugar content and pH-value as a result of berry shrivel, while titratable acidity content was very high in the must. The deteriorating values I also experienced support the results reported in the literature. Examining berry shrivel, it can be concluded that the treatments do not modify the above content indicators.

3.5. Amounts of biogenic amines

In the analysis of biogenic amines, methylamine and tyramine were not present in the samples in evaluable amounts. Treatment and location significantly affected the amount of biogenic amines in all three years. However, the sampling time of the samples did not significantly affect their concentration, which is in contrast to that previously reported. While in 2014 the sites differed less, in the second year, when I compared the plantations, more differences appeared, and these differences peaked especially in the last examined vintage.

In 2014, there was no clear effect of treatments for b-phenylethylamine, the result depended on the site. In Dunakeszi and Erdőkertes as well, cluster tipping was the method by which the histamine concentration could be significantly reduced. In the case of serotonin, the effect of the treatments was plantation-dependent, and leaf removal increased the amount. In 2015, I obtained a significantly higher value of b-phenylethylamine in Dunakeszi and Vác in case of defoliation compared to the other two treatments. Meanwhile, in Erdőkertes, this amine was present in the highest amount in the controls. Serotonin was significantly increased in Dunakeszi and Vác by leaf removal, although at the latter site I also improved the value with cluster tipping. Histamine was not significantly affected by the treatments in any of the plantations. In 2016, compared to the previous year, the effect of the treatments was more marked. Again in Dunakeszi and Vác, b-phenylethylamine was significantly higher due to leaf removal. In Dunakeszi, serotonin could be significantly increased with the help of defoliation, and in Vác, the treatment significantly improved the amount in all cases. Histamine was only affected by the treatments in Dunakeszi, where both interventions significantly reduced its value.

I also compared the biogenic amines by vintage: in this case I examined the results of each treatment within one site, comparing the three years. It can be seen that a vintage effect occurred during the evaluation of b-phenylethylamine, cadaverine and putrescine.

3.6. Result of phytoplasma test

In my experiment, while walking through the plantations, I encountered phytoplasmic symptoms in many cases. This observation, as well as the symptoms often similar to berry shrivel, I tested the grapevines showing signs of shrivel for this pathogen. During the research period, the symptoms reminiscent of phytoplasma were practically the shrivelled and dried clusters. In addition, the characteristic leaf coloration appeared on only two designated vines. Leaf drift, poorly ripened cane did not occur on the tested grapevines. As a result of phytoplasma testing, only 4 of the 50 samples were positive, and for these PCR positive samples, Stolbur phytoplasm (‘Candidatus Phytoplasma solani’) belonging to subgroup 16SrXII-A was identified. Based on this study and the absence of known symptoms of phytoplasma, it can be concluded that the Zweigelt-specific berry shrivel is not a phytoplasmic pathogen.

3.7. Chemical composition of leaves

Based on my results, I concluded that the nutrient content measured in the leaf blade is not usually affected by the treatments, but especially in the case phosphorus, potassium and iron, the sites have a significant effect on them. Zinc is often adversely affected by defoliation.

4. CONCLUSIONS AND PROPOSALS

During my field experiment in three areas (Dunakeszi, Vác, Erdőkertes) between 2014 and 2016, I examined the effect of cluster tipping and leaf removal at bloom on the yield and quality of the Zweigelt variety. I also expanded the knowledge about berry shrivel: I tried to clarify how yield, climatic factors, and nutrient supply are related to this disease. Based on my observations, I draw the following conclusions and suggestions:

- Based on my results, it can be established that berry shrivel does not appear in all vintages, and its appearance also differs from plantation to plantation. Most authors associate it with warm-dry weather. In my view, in years with high rainfall (2014), no shrivelled clusters can be encountered at all, however, symptomatic clusters may already appear in dry and warm-year, but due to the soil’s favorable water-status not in significant proportions (2015). Accordingly, it may be worth considering irrigation.

- Based on my results, temperature is not the only factor in inducing berry shrivel. Just as mean temperature data and the number of heat and hot days are not appropriate indicators, precipitation, especially in addition to those listed above, can be effective data to link the disease.

• On grapevines where berry shrivel occurs, botrytis may be encountered at the same time. Within a cluster, however, there are no berries that carry either one or the other symptom, only one disease appears on the cluster. In light of these, I hypothesize that the low carbohydrate content due to shrivelling may explain why botrytis does not settle on shrivelled clusters. Therefore, from the onset of veraison, if symptoms of shrivelling have appeared, treatments against botrytis may become unnecessary. However, proving this should be the subject of further research.

• There are many publications linking berry shrivel to a lack of certain nutrients (mostly potassium and magnesium). Based on my results, in 2016 the potassium and magnesium content of the soil on the shrivelled site in Vác did not differ (and their ratio was optimal) from the other two areas where the disease did not occur. At the same time, the lime content of the plantation is extremely high and also rich in sodium. Furthermore, its zinc and manganese content lags behind that of the samples taken in Dunakeszi and Erdőkertes. Based on this, I assume that these elements are related to berry shrivel. In the future, it would be expedient to carry out such research.

• The effect of the treatments on berry shrivel in 2016, in Vác confirmed the experience of the previous experiment: by cluster tipping, the disease was reduced in each cluster. Based on this, it is worth considering that in a plantation where this problem tends to appear frequently and over a large area, the growers should this procedure. However, the treatment does not change the number of clusters affected. Leaf removal, according to my observations, has no positive effect on berry shrivel.

• Berry shrivel has a negative effect not only on quality but also on yield. According to my results, this disease significantly reduces the sugar content and pH-value of the must in addition to the weight of the berries, and also increases the titratable acid content. Because of these, I do not recommend the use of shrivelled bunches in winemaking.

• Berry shrivel is thought to be a physiological disease. In research to this effect, testing for several pathogens has yielded negative results. In my study, it was found that this disease is not caused by Stolbur phytoplasma.

• The amount of biogenic amines can be influenced by the crop regulating methods I studied. The advantage of both treatments is that the ratio of b-phenylethylamine and serotonin is significantly increased in the must, while histamine’s is significantly reduced. Since in my

experiments the clustering tipping increased the putrescine content in several cases, it is more recommended to use the defoliation at bloom to positively influence the content of the biogenic amines in the must.

• The most common problem during the study period was botrytis. Zweigelt is particularly susceptible to this fungal disease. Based on literature data, the methods I also studied (cluster tipping and leaf removal at bloom) affect the structure of the cluster, they become looser, so the incidence and severity of botrytis infection can be reduced. I found that in 2014, which was a particularly rainy vintage, the number of rotten bunches and the proportion of berries covered with mold could not be reduced by neither cluster tipping nor defoliation. Cluster tipping can usually lead to better results, but the effect of treatments is not always valid in drier vintages (2015, 2016). However, the nitrogen removal capacity of a continuous inter-row cover vegetation significantly helps to counteract the negative consequences. For this reason, in the case of varieties that are specifically prone to rot, it may be advisable to growing cover crop as well in the plantation.

• Cluster tipping and leaf removal at bloom did not contribute to a significant reduction in yield in either year. Based on this, the view that these interventions help to curb the harvest quantity can be questioned. In addition, they do not have a more significant effect on the average weight of the bunches and berries. I suggest that for varieties such as Zweigelt, it is advisable to adjust the load during pruning.

• The quality of the must, according to the generally accepted view, is inversely proportional to the yield. Therefore, it is often suggested to limit yields for better content indicators. According to my results, none of the studied methods improved the sugar content of the berries. Similarly, I have not been able to improve the titratable acidity content. When measuring the pH-value, I found that the above treatments had no effect on this either.

5. NEW SCIENTIFIC RESULTS

1. According to my research, the appearance of berry shrivel cannot be explained by the the annual mean temperature. Similarly, I found no correlation between the number of heat days and hot days and the occurrence of berry shrivel. Simultaneously with the onset of this phenomenon, the soil in the affected area had a very low manganese content and a lower amount of zinc compared to the control, while the soil content of sodium and carbonated lime was many times higher than in healthy sites.

2. I found that berry shrivel is not a consequence of botrytis, and as the incidence of berry shrivel increases, the number of rotten fruits decreases.
3. Based on my research, it can be ruled out that berry shrivel is caused by currently known phytoplasma species.
4. Leaf removal in most cases increases the amount of serotonin and b-phenylethylamine, while it reduces putrescine and histamine.
5. Cluster tipping increases the amount of b-phenylethylamine, ethylamine, serotonin, and putrescine, while decreases histamine.

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