Effect of early leaf removal and vineyard characteristics on 'Zweigelt' grapevines (*Vitis vinifera* L.) in different sites

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One of the greatest challenges of climate change is to prevent vineyards from the effect of adverse weather conditions by using different viticultural practices. Innovative solutions, such as defoliation at bloom, are often applied in quality vine growing. In addition to the positive changes in quality such as higher contents of anthocyanin and aromatic compounds, defoliation also plays a role in crop soundness due to a more favourable microclimate in the bunch zone. We set up our experiment in 2014 and 2015 at three sites in Hungary (Dunakeszi, Vác, Erdőkertes) with 'Zweigelt' grapevines (*Vitis vinifera* L.). Defoliation was performed at the time of bloom, to examine its effect on yield, quality and botrytis infection. The year 2014 was very rainy, especially from ripening to harvest. Due to the frequent rainfall, the cluster zone did not dry up in either site, but we found significant differences between the sites in infection rates when we examined the characteristics of the plantations. Incidence and severity of botrytis varied between the sites, too. In the drier second year (2015), between the sites significant differences in botrytis incidence and rate could not be confirmed. We found that yield, cluster and berry weight were significantly different between the sites, though the rate of differences was not the same in the two years. The origin of must samples had an impact on titratable acidity and pH, but not on soluble solids in 2014, in the next year, however, there were opposite results. The location significantly affected the vine balance and the macronutrient contents of leaf blades. Based on our results in rainy years, the use of permanent cover crops to control botrytis should be considered.

Keywords: canopy management, rainy year, botrytis, cover crops, nitrogen content

Auswirkung einer frühen Entblätterung auf die Traubensorte 'Zweigelt' (*Vitis vinifera* L.) auf verschiedenen Standorten. Aufgrund des Klimawandels ist es sehr schwierig, den Weinberg vor widrigen Wetterbedingungen zu schützen. Innovative Lösungen wie die Entblätterung bei der Blüte werden häufig im Qualitätsweinbau eingesetzt. Neben den positiven Veränderungen in der Qualität bietet sie auch einen guten Pflanzenschutz durch das günstigere Mikroklima in der Rebzone. Wir haben unser Experiment in 2014 und 2015 an drei Standorten durchgeführt. Die Entblätterung wurde zum Zeitpunkt der Blüte durchgeführt, um ihre Wirkung auf Inzidenz und Schweregrad der Botrytis-Infektion zu untersuchen. Das Jahr 2014 war sehr regnerisch, vor allem von der Reife bis zur Ernte. Aufgrund der häufigen Niederschläge konnte die Rebzone nicht trocknen, so dass der Effekt der Entblätterung nicht beeinflusst wurde. Bei der Untersuchung der Eigenschaften von Rebanlagen stellten wir signifikante Unterschiede in der Infektionsrate fest. Inzidenz und Schweregrad der Botrytis waren an den Standorten unterschiedlich. Dies ist auf die häufige Unkrautbekämpfung im ersten Weinberg zurückzuführen. Die Begrünung zwischen den Reihen bedeutet nämlich eine Konkurrenz bei der Stickstoffaufnahme, die mit der Botrytis-Infektion korreliert. Am zweiten Standort wurde die Unkrautbekämpfung seltener durchgeführt. Am dritten Standort gab es vor langer Zeit eine natürliche Unkrautflora. Im trockeneren zweiten Jahrgang 2015 konnte dieser Standort-Effekt nicht bestätigt werden. Die Herkunft der Mostproben wirkte sich im Jahr 2014 auf die titrierbare Säure und den pH-Wert aus, nicht jedoch auf die löslichen Feststoffe. Im nächsten Jahr gab es jedoch entgegengesetzte Ergebnisse. Der Weinberg beeinflusst das Produktionsgleichgewicht der Reben und den Makronährstoffgehalt der Blattspreite erheblich. Basierend auf unseren Ergebnissen sollte die Verwendung einer dauerhaften Begrünung zur Bekämpfung von Botrytis erwogen werden.

Schlagwörter: Laubarbeit, regnerischer Jahrgang, Botrytis, Begrünung, Stickstoffgehalt

Quality oriented vine growing is highly influenced by microclimatic conditions of the canopy. Optimal radiation, humidity, temperature and airflow influence both architecture and composition of the bunches. Canopy management aims at providing optimal microclimatic conditions and ensuring vegetative and generative balance of the plants (Smart, 1985).Leaf removal is a generally applied treatment. This manipulation has multiple purposes: removing the leaves opens the bunch zone for the air-flow to dry the clusters, while radiation is increased as a consequence of reduced self-shading. Careful performance of this operation improves chemical composition (e. g., sugar content, anthocyanin content, aromatic compounds) of both grape and wine (Hunter et al., 1991; Main and Morris, 2004; Vilanova et al., 2012). On the other hand, removing the leaf results in a different physiological response of the vines as well. Performing defoliation at bloom causes insufficient fruit-set, therefore provides fewer compact clusters (Coombe, 1959; Sabbatini, 2011). This phenomenon occurs because fewer flowers are set due to the lack of carbohydrate in the bunch zone of the shoot. During bloom, flowers get assimilates directly from the leaves opposite to the clusters, thus fruit set depends on the nutrient supply of the flowers (Vasconcelos and Castagnoli, 2000; Caspari et al., 1998). Caused by the less compact clusters, bunch-microclimate is less favourable for pathogen infection. Defoliation in this way is suitable for crop regulation in the case of high-yield varieties, especially with larger and compact clusters (Poni et al., 2006; Sabbatini and Howell, 2010). However, according to Howell et al. (1994), performing defoliation when berries have reached pea size does not cause crop loss, in the same time berries exposed to sun from early developmental stages onwards can develop thicker cuticles because of increased UV radiation that is also relevant for skin thickness (Percival et al, 1993; Verdenal et al., 2017). On the other hand, if defoliation is performed in a later stage of the vegetation period, the berry skin cannot thicken enough and the crop can be damaged by higher UV radiation or hails (Zoecklein et al., 1992).One of the primary fungal diseases of the grape is bunch rot caused by Botrytis cinerea. Infection requires sensitive varieties, dense bunches and high humidity in the bunch zone. As leaf removal provides an open canopy and less dense bunches, it can significantly reduce the risk of infection (Percival et al., 1993; Prior, 2010). Previous studies showed that early leaf removal reduces the risk of botrytis on the berries (Vasconcelos and Castagnoli, 2000; Sabbatini and Howell, 2010; Tardaguila et al., 2010; Mehofer et al., 2019). Abro et al. (2013) found that there is a relation of causality between nitrogen uptake of grapevine and the occurrence of botrytis. If the soil is overloaded by nitrogen, the canopy becomes more dense (Mundy and Beresford, 2007). Therefore, the plant nutrition plan has to take into consideration the plant demands and the cover crops applied in the inter-row (Tan and Crabtree, 1990; Wolpert et al., 1993; Bugg et al., 1996; McGourty and Reganold, 2005; Jacometti et al., 2007; Jacometti et al., 2010). In addition, frequent tillage increases nitrogen uptake and should only be carried out in justified cases (Zanathy et al., 2014). The aim of this study was to evaluate the effect of early leaf removal on the vegetative performance, fruit quality and botrytis infection of the variety 'Zweigelt' in two consecutive years at three growing sites.

Materials and Methods

Vineyards

The experiments were conducted with the grapevine (*Vitis vinifera* L.) variety 'Zweigelt' grafted onto Teleki 5C rootstocks. The investigations were carried out in 2014 and 2015 in three vineyards located in Central Hungary (Dunakeszi, Erdőkertes, Vác,), owned by local farmers. The sites have different soil characteristics but rather similar climatic conditions (hot, dry summer with cold winter) and cultivation practices (Table 1

Table 1: Characteristics of the three vineyards

	Dunakeszi	**Vác “Török Hill”**	**Erdőkertes**
Location	47°39'50.3"N 19°08'45.5"E	47°46'40.1"N 19°09'52.0"E	47°40'58.4"N 19°19'48.9"E
Year of plantation	1982	1983	1986
Soil type	sandy soil	clayey brown forest soil on loess	sandy and clayey brown forest soil
Water-holding capacity	low	high	low
Organic material content*	50-100 t/ha	100-200 t/ha	50-100 t/ha
Row orientation	NE-SW	E-W	NE-SW
Soil management	below vines: chemical weed control between rows: mechanical weed control	below vines: chemical weed control between rows: mechanical weed control	natural weed flora
	modified Moser cordon (VSP)	modified Moser cordon (VSP)	modified Moser cordon (VSP)
Cordon height	1.5 m	1.5 m	1.5 m
Spacings	3.0 m × 1.2 m	3.0 m × 1.2 m	3.0 m × 1.0 m
Bud load	spur pruning with 15 buds per meter of row	spur pruning with 15 buds per meter of row	spur pruning with 15 buds per meter of row

* AGROTOPO Database, 2014

Meteorological data

Meteorological data were obtained from the Hungarian Meteorological Institute (met.hu, 2020). In 2014 and 2015, the hours of annual sunshine were 2086.5 and 2279.2, respectively. The vegetation periods began in the middle of March in 2014 and at the beginning of April in 2015. The average length of growing season in Hungary is 180 to 190 days, that was reached in both years at all sites. The sum of active heat was above 1500 °C in both years in Dunakeszi and Erdőkertes, but in Vác in 2014 and 2015 we measured 1343 °C and 1495 °C, respectively.

In 2014, the annual rainfalls in Dunakeszi, Vác and Erdőkertes were 784.0 mm, 642.5 mm and 566.2 mm, respectively. From the time of ripening (the third decade of July) until harvest (September, 9th), the numbers of rainy days were 20 in Dunakeszi (sum: 291.1 mm), 19 in Erdőkertes (sum: 140.4 mm) and 15 in Vác (sum: 200.7 mm). This means that in this period there were rainfalls almost every second day in Dunakeszi and Erdőkertes and on every third day in Vác. In 2015, the annual rainfall in Dunakeszi, Vác and Erdőkertes was 470.6 mm, 488.7 mm and 508.0 mm, respectively. The numbers of rainy days in the same period were fewer (14 in Dunakeszi, 13 in Vác and 15 in Erdőkertes). In 2015, the highest precipitation was measured in Erdőkertes (110.3 mm) and a much lower amount in Dunakeszi (75.5 mm) and Vác (77.2 mm).

Treatment

We selected 24 treated and 24 control vine stocks per site: 4 in each of the 6 investigated rows. Defoliation was carried out at full bloom (BBCH 57 to 65, on May, 22nd, 2014 and on June, 4th, 2015). Four to five leaves per fruiting shoot were removed by hand (each leaf on the opposite site of the clusters, one leaf below the first and one leaf above the last cluster). The details of plant protection in the experimental plots are shown in Table 2.

Table 2: Regime of plant protection in 2014 and 2015 in Dunakeszi, Vác and Erdőkertes

2014	**Product**
14. April	Vegesol eReS (5 l/ha) (BVN Növényvédő Kft., Budapest, Hungary)
03. May	Kupfer Fusilan WG (2 kg/ha) (Kwizda Agro GmbH, Wien, Austria), Folpan 80 WDG* (1.25 kg/ha) (Adama Makhteshim Ltd., Beer-Sheva, Israel), Kumulus S (3 kg/ha) (BASF S.E., Ludwigshafen, Germany), Polyram DF* (1 kg/ha) (BASF S.E., Ludwigshafen, Germany)
19. May	Kumulus S (2 kg/ha) (BASF S.E., Ludwigshafen, Germany), Polyram DF (2 kg/ha) (BASF S.E., Ludwigshafen, Germany), Topas 100 EC (0.25 l/ha) (Syngenta AG, Basel, Switzerland), Pergado F (2 kg/ha) (Syngenta AG, Basel, Switzerland), Ortus 5 SC (1 l/ha) (Nihon Nohyaku Co. Ltd., Tokyo, Japan)
05. June	Kumulus S (4 kg/ha) (BASF S.E., Ludwigshafen, Germany), Polyram DF (2 kg/ha) (BASF S.E., Ludwigshafen, Germany), Topas 100 EC (0.25 l/ha) (Syngenta AG, Basel, Switzerland), Mildicut (2 l/ha) (ISK Biosciences Europe N.V., Diegem, Belgium),
21. June	Kumulus S (2 kg/ha) (BASF S.E., Ludwigshafen, Germany), Folicur Solo (0.4 l/ha) (Bayer AG, Leverkusen, Germany), Kupfer Fusilan WG (2.5 kg/ha) (Kwizda Agro GmbH, Wien, Austria), Mavrik 24 EW (0.2 l/ha) (Adama Makhteshim Ltd., Beer-Sheva, Israel)
09. July	Rally Q (1 l/ha) (Dow AgroSciences Italia S.R.L., Milano, Italy), Kumulus S (2 kg/ha) (BASF S.E., Ludwigshafen, Germany), Kupfer Fusilan WG (3 kg/ha) (Kwizda Agro GmbH, Wien, Austria)
14. August	Kumulus S (2 kg/ha) (BASF S.E., Ludwigshafen, Germany), Teldor 500 SC (1 kg/ha) (Bayer AG, Leverkusen, Germany)
2015	
04. May	Kumulus S (2 kg/ha) (BASF S.E., Ludwigshafen, Germany), Polyram DF (2 kg/ha) (BASF S.E., Ludwigshafen, Germany), Karathane Star (0.6 l/ha) (Dow AgroSciences Italia S.R.L., Milano, Italy)
02. June	Kumulus S (3 kg/ha) (BASF S.E., Ludwigshafen, Germany), Polyram DF (2 kg/ha) (BASF S.E., Ludwigshafen, Germany), Karathane Star (0.6 l/ha) (Dow AgroSciences Italia S.R.L., Milano, Italy)
25. June	Kumulus S (2 kg/ha) (BASF S.E., Ludwigshafen, Germany), Luna Experience (0.5 l/ha) (Bayer AG, Leverkusen, Germany), Pergado F (2.5 kg/ha) (Syngenta AG, Basel, Switzerland)
21. July	Kumulus S (4 kg/ha) (BASF S.E., Ludwigshafen, Germany), Rally Q (1 l/ha), (Dow AgroSciences Italia S.R.L., Milano, Italy), Pergado F (2 kg/ha) (Syngenta AG, Basel, Switzerland)
10. August	Kumulus S (2 kg/ha) (BASF S.E., Ludwigshafen, Germany)

Survey of incidence and severity of botrytis

To identify the incidence and severity of botrytis, we used visual inspection (performed at harvest) similar to Hill et al. (2010). The incidence was measured in the following way: Every sound cluster was considered as "sound” and every cluster with at least 1 % of botrytis was considered as "diseased”. We evaluated the percentage of severity of botrytis in steps of 10 %. The sound clusters were classified as 0 %, and every infected cluster was classified with 10, 20 … 90, 100 % according to the severity of the rot.

Yield and berry composition

Harvest was carried out on September, 9th, in both years at all sites. Yield and bunch numbers of 24 control and 24 treated plants were evaluated at each location. We measured the yield of each vine with a portable digital scale (Nevis Digital Scale 40 kg, Spro N.V., Vianen, Netherlands). At this time, we collected 24 sound bunches from the control and 24 sound bunches from the treated plants (1 representative cluster per selected plant) for further examination. Average berry weight (g) was measured with a digital scale (Sartorius Basic 210, Sartorius AG, Göttingen, Germany) according to the weight of 30 berries per cluster. The must samples – obtained from individual clusters (24 control and 24 defoliated must samples) – were analysed separately. In must samples, we measured Brix-content (°Bx) (Atago Pocket Refractometer Pal-1, Atago Co. Ltd., Fukaya, Japan) and pH values (Thermo Scientific Orion 3-Star pH Portable, Thermo Fisher Scientific Inc., Waltham, USA). Titratable acidity (g/l) was measured with wet chemistry (alkaline titration with 0.1 mol/dm^3 NaOH).

Nutritional content of leaf blades and soil samples

We determined the nitrogen status of the vines and soils. Soil samples were collected in 2014 at stage BBCH 77 according to the recommendation of the National Agricultural Research and Innovation Centre (NARIC) Badacsony, Hungary: pooled soil samples from depth of 0 to 30 cm and 30 to 60 cm (20 point samples resulting in 1 kg soil from each location). Leaf blade samples were collected from the nodes opposite to the bunches (in the case of defoliated grapevines we collected the leaves nearest to the bunches) at the time of harvest. We collected 50 leaves from the control and 50 leaves from the treated vines from each site and divided into 3 sub-samples – thus we sent for analyzation a total of 18 samples yearly. The measurements of nutritional content of leaf blades and soil samples were carried out by means of atom absorption spectrophotometer, Kjeltec N analyser and FIAstar 5000 analyser (according to MSZ 20135:1999).

Cane weight and Ravaz index

Cane weight measurement was carried out with a portable digital scale (Nevis Digital Scale 40 kg, Spro N.V., Vianen, Netherlands). We pruned (spur pruning with 15 buds per meter of row) every examined vine: 24 control and 24 treated plants from each location. Cane mass was determined based on the individual plant: We collected all the pruned shoots and measured with the scale. Based on the fruit weight and cane weight of each investigated plant, the Ravaz index was calculated both years: yield/cane mass.

Statistical analysis

The effect of sites and defoliation on botrytis incidence and severity was analysed by 2-way MANOVA model with random block design (year effects were considered as blocks). Significant overall effect was detected according to the unexplained variance rate described by Wilk's lambda. In case the overall MANOVA test was significant, we ran follow-up one-way ANOVA test with Bonferroni's Type I error correction. To normalize the skewed data, the frequency and the severity of infection were transformed by $arsin\sqrt{x}$. To detect the effect of different growing sites and defoliation on the yield, cluster weight and berry weight, as well as on soluble solid (°Bx), pH values and titratable acid and finally, on the nutritional contents of leaves (nitrogen, phosphorus, potassium), we also carried out two-way random block design MANOVA models with follow-up analysis. Cane weight and Ravaz values depending on sites and defoliation were examined by two-way random block design ANOVA models. The normality of the residuals of all models was proved by d'Agostino's test while the homogeneity of variances was checked by the ratio of maximal and minimal variances. We performed Games-Howell's post-hoc test to separate significantly different group means in each year. Statistical analysis was performed using IBM SPSS v25 (Armonk, NY: IBM Corp., 2017). As a graphical representation, for the investigated parameters incidence, severity of botrytis, yield, cluster weight, berry weight, °Bx, pH, titratable acidity, N%, P%, K%, we performed a normalization with transformation

$$x_{j,\text{ normalized}}=\frac{\left(x_j-\min_i x_i\right)}{\left(\max_i x_i-\min_i x_i\right)}$$

where i and j run through Dunakeszi-defoliated; Dunakeszi-control; Vác-defoliated; Vác-control; Erdőkertes-defoliated; Erdőkertes-control. In this way, the minimal value among the six values takes zero and the maximal one takes 1. All the other values have their normalized values between 0 and 1. We visualize these values in a spider-web diagram (Fig. 1). In this diagram, it is easy to compare the sites and treatments with their higher or lower values. The figures were prepared in Excel 2016.

Results

Incidence and severity of botrytis

As illustrated in Table 3, in this study, we found that both site and treatment have significant effect on the incidence of botrytis (%) with significant year effects as block effect. In 2014, the highest percentage of infected clusters was found at Dunakeszi in the control parcels (96.9 %) while the lowest (57.1 %) was found in defoliated plants in Erdőkertes. In contrast with this in 2015, the highest value was observed in Vác in the control parcels (37.3 %), the lowest at Erdőkertes when defoliation (13.6 %) was carried out. Based on two-way random block design MANOVA, we found that site (Wilk's lambda: 0.60; $P < 0.001$), defoliation (Wilk's lambda: 0.94; $P < 0.05$) and site × defoliation interaction (Wilk's lambda: 0.93; $P < 0.05$) had all significant overall effects on the incidence of botrytis (% of the infected clusters) and severity of botrytis (% of diseased berries within cluster) with a significant year effect as block effect (Wilk's lambda: 0.31; $P < 0.001$).

According to the follow-up two-way ANOVA with Bonferroni's correction, the site effect was significant for both on the incidence and on the severity of botrytis infection (F(2;125) = 24.96; P < 0.001, F(2;125) = 40.23; P < 0.001, respectively). Defoliation resulted in also significant differences in incidence and severity of botrytis infection (F(1;125) = 7.40; P < 0.05; F(1;125) = 5.95; P < 0.05, respectively; Table 3). Though the treatment resulted in a lower percentage of infection and severity at all sites in both years, in 2014, they were not notably different irrespective of treatment. In 2015, however, defoliation caused a significant infection reduction in Erdőkertes.

Yield, cluster weight, berry weight

MANOVA with yield, cluster weight and berry weight showed significant growing site (Wilk's lambda: 0.44; P < 0.001) and year (Wilk's lambda: 0.57; P < 0.001) effect with insignificant defoliation effect (Wilk's lambda: 0.96; P = 0.21) and site × defoliation interaction (Wilk's lambda: 0.93; P = 0.21). In 2014, the highest yield was harvested from the control plants in Vác (1.5 kg), the lowest from the defoliated vines in Erdőkertes (0.8 kg). In contrast with this in 2015, control plants in Dunakeszi showed the highest yield (2.6 kg) and the defoliated vines in Erdőkertes the lowest (0.9 kg). The differences between the sites were significant (F(2;126) = 12.02; P < 0.001), albeit they were more conspicuous in 2015 than in 2014. Defoliation caused minor differences in the yield while the year effect was also insignificant (F(1;126) = 2.58; P = 0.11). In Dunakeszi and Erdőkertes irrespective of the treatment, the yield was higher in the second year of the experiment (Table 3). Cluster weight showed significant differences between the sites (F(2;126) = 45.57; P < 0.001). In 2014, the lowest bunch weight was observed in Erdőkertes with the control vines (110.9 g), the highest in Vác in the control parcels (257.2 g). Differences between the investigated years are noticeable (F(1;126)=66.41; P < 0.001) as the highest average cluster weight in Dunakeszi was 176 g and 531.8 g harvested from the control vines in 2014 and 2015, respectively. Like the previous year, in 2015 the smallest bunches were observed in Erdőkertes, but in this year from the defoliated vines (176.4 g) (Table 3). Berry weight also showed significant differences between the sites (F(2;126) = 17.54; P < 0.001). Lowest values were observed in Dunakeszi with the defoliated plants, where berry weight was 1.8 g, while the largest berries were harvested in Vác, where berry weight was 2.3 g irrespective of the treatment. In 2015, berry weight was 2.4 g with the control plants harvested in Dunakeszi while the smallest berries were 1.9 g irrespective of the treatment in Erdőkertes (Table 3).

Berry composition

MANOVA revealed a significant effect of site (Wilk's lambda: 0.42; P < 0.001) and year (Wilk's lambda: 0.34; P<0.001) with insignificant defoliation effect (Wilk's lambda: 0.98; P = 0.44) and site × defoliation interaction (Wilk's lambda: 0.96; P = 0.60) on berry composition. Analysis of the berry composition showed that the site had significant effect on °Brix (F(2;124) = 9.65; P < 0.001) which was manifested in 2015. In 2014, the lowest value was observed in Dunakeszi with the defoliated parcel (17.9 °Bx), and the highest was harvested from the control plants in Vác (19.6 °Bx). In contrast with this in 2015 the highest °Bx was observed in the case of the defoliated plants in Vác (22.4 °Bx), the lowest in Dunakeszi with 20.6 °Bx, irrespective of the treatment. Significantly lower must pH was measured in Erdőkertes in 2014 (F(2;124) = 41.23; P < 0.001). As for the titrable acidity, the differences between the sites were significant (F(2;124) = 5.80; P = 0.14). In 2014, acidity was the highest in Dunakeszi (defoliation: 8.8 g/l), while the lowest mean value was 7.1 g/l in the control parcels at Vác. In 2015, we got a lower titratable acidity at all sites of the experiment between 6.5 g/l and 6.9 g/l (Table 3).

Cane weight and Ravaz index

Two-way random block design ANOVA resulted in a significant defoliation effect (F(1;112) = 20.54; P < 0.001) on cane weight, but site, year and site × defoliation interaction effect were not significant (F(2;112) = 1.49; P = 0.23, F(1;112) = 2.16; P = 0.14, F(2;112) = 0.69; P = 0.51, respectively). In 2014 in Dunakeszi and Vác we measured significantly lower cane weights with the defoliated plants (0.18 kg/m^2 at both sites) than on the control vines (0.28 kg/m^2 and 0.26 kg/m^2) (Table 3.). In the following year, the cane weights measured on the treated vines were significantly lower in Erdőkertes (control: 0.2 kg/m^2, treated: 0.27kg/m^2), but they did not differ significantly in other sites. The vine balance of the plantations was expressed by the Ravaz index (the ratio of yield and cane weight). This value was influenced by the growing site significantly (F(1;110) = 3.12; P < 0.05), but not by the defoliation (F(1;110) = 0.21; P = 0.65). The site × defoliation interaction was not significant (F(2;110) = 0.09; P = 0.91). In 2014 in Erdőkertes (4.99) and Dunakeszi (5.41), the values did not differ significantly from each other, while, in the case of defoliation, mean was significantly higher in Vác (7.96). In the next year, none of the examined factors affected the results notably.

Nutritional content of leaf blades and soil samples

Considering the nutritional content (N, P, K %) in leaves, MANOVA resulted in significant site, defoliation and year effect (Wilk's lambda: 0.02; P < 0.001, Wilk's lambda: 0.37; P < 0.001, Wilk's lambda: 0.14; P < 0.001, respectively) together with significant site × defoliation interaction (Wilk's lambda: 0.10; P < 0.001). In the case of each of the three macronutrients, we found significant differences between sites (N%: F(2;29) = 28.40; P < 0.001); P%: F(2;29) = 214.74; P < 0.001; K%: F(2;29) = 7.04; P < 0.01). We found significant results for P%, due to defoliation (P%: F(1;29) = 37.18; P < 0.001). The differences, however, were not significant for N% and K% (F(1;29) = 3.44; P = 0.21; F(1;29) = 1.63; P = 0.06, respectively). The nitrogen content was the lowest in Erdőkertes in both 2014 and 2015 (1.66 % and 1.57 %, respectively), while the highest values were observed in Dunakeszi (2014: 2.16 %; 2015: 2.21 %). In the case of phosphorus, the lowest values were observed in Vác (2014: 0.18 %; 2015: 0.16 %), the highest in Erdőkertes (2014: 0.34 %) and in 2015 in Erdőkertes and Vác we measured 0.22 %. In both years, the lowest potassium value was recorded in Dunakeszi (2014: 0.84 %; 2015: 0.7 %), the highest in Erdőkertes (0.99 %) and Dunakeszi (1.18 %). According to the analysis of the samples (Table 3), in 2014 in Dunakeszi and Vác, the amount of nitrogen in the soil was almost the same, but in Erdőkertes, we found much lower values.

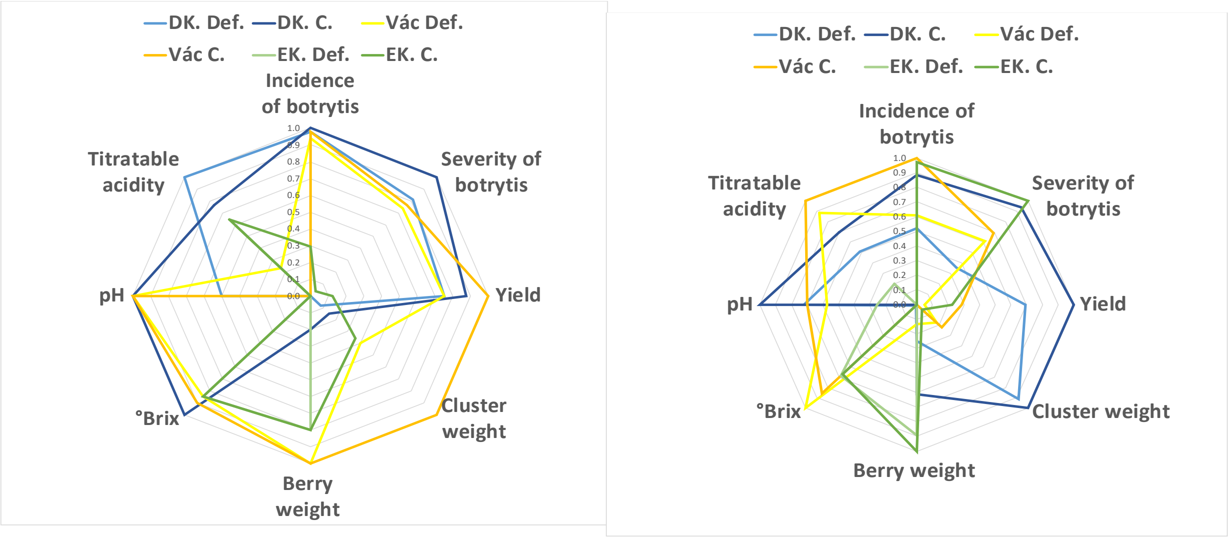
Table 3: Descriptive statistics (mean±St.Dev) of the investigated parameters in Dunakeszi, Vác and Erdőkertes on the defoliated and control vines in 2014 and 2015

		Dunakeszi		**Vác**		**Erdőkertes**	
		Defoliation	**Control**	**Defoliation**	**Control**	**Defoliation**	**Control**
Incidence of botrytis (% of diseased clusters)	**2014**	96.0±11.2^{b}	96.9±9.1^{b}	94.5±8.1^{b}	96.1±5.2^{b}	57.1±25.2^{a}	68.8±22.7^{a}
	2015	25.9±12.9	34.6±15.9	28.0±21.8	37.3±17.2	13.6±13.6	36.7±27.6
Severity of botrytis (% of diseased berries)	**2014**	70.9±23.2^{b}	83.5±20.8^{b}	65.7±13.1^{b}	67.8±17.2^{b}	16.2±13.9^{a}	18.8±12.1^{a}
	2015	4.6±3.8	9.2±5.2	6.6±7.5	7.2±6.1	1.8±1.9^{A}	9.7±9.9^{B}
Yield (kg/m^2)	**2014**	1.3±0.5^{b}	1.4±0.6^{ab}	1.3±0.3^{b}	1. 5±0.5^{b}	0.8±0.2^{a}	1.0±0.5^{a}
	2015	2.1±0.8	2.6±1.4	1.0±0.6	1.4±0.5	0.9±0.3	1.3±0.4
Cluster weight (g)	**2014**	171.1±43.5^{b}	176.5±30.0^{b}	205.0±30.8^{b}	257.2±50.6^{c}	127.5±19.1^{a}	110.9±30.7^{a}
	2015	501.5±172.4^{b}	531.8±208.5^{b}	238.5±87.5^{a}	255.9±51.6^{a}	176.4±28.6^{a}	194.1±57.8^{a}
Berry weight (g)	**2014**	1.8±0.2^{a}	1.9±0.4^{a}	2.3±0.1^{b}	2.3±0.2^{b}	2.2±0.2^{b}	2.2±0.1^{ab}
	2015	2.0±0.2	2.4±0.2	2.1±0.1	2.1±0.4	1.9±0.2	1.9±2.4
°Bx	**2014**	17.9±2.3	19.9±1.6	19.6±0.5	19.7±0.8	19.6±1	19.6±0.6
	2015	20.6±0.8^{a}	20.6±0.8^{a}	22.4±1.0^{b}	22.1±2.1^{b}	21.8±1.3^{b}	21.8±1.2^{ab}
pH	**2014**	3.2±0.1^{b}	3.3±0.1^{b}	3.3±0.1^{b}	3.3±0.1^{b}	3.1±0.1^{a}	3.1±0.1^{a}
	2015	3.3±0.1	3.3±0.1	3.3±0.1	3.3±0.1	3.2±0.1	3.2±0.1
Titratable acidity (g/l)	**2014**	8.8±1.4^{b}	8.4±1^{b}	7.5±0.8^{a}	7.1±0.7^{a}	8.1±0.9^{ab}	8.2±0.7^{b}
	2015	6.7±0.7	6.8±0.6	6.9±0.5	6.9±1.1	6.6±0.5	6.5±0.7
N (%) in leaf blades	**2014**	2.16±0.03^{b}	1.98±0.17^{a}	1.86±0.11^{ab}	1.87±0.18^{a}	1.66±0.03^{a}	1.87±0.13^{a}
	2015	2.21±0.06^{b}	1.95±0.06^{b}	2.12±0.13^{b}	1.90±0.09^{b}	1.62±0.1^{a}	1.57±0.02^{a}
P (%) in leaf blades	**2014**	0.25±0.01^{Bb}	0.23±0.01^{Ab}	0.18±0.01^{Aa}	0.20±0.01^{Ba}	0.34±0.01^{Bc}	0.26±0.01^{Ab}
	2015	0.21±0.01^{b}	0.22±0.01^{b}	0.16±0.00^{a}	0.18±0.01^{a}	0.21±0.01^{c}	0.22±0.01^{ab}
K (%) in leaf blades	**2014**	0.84±0.03^{a}	1.02±0.06^{a}	1.08±0.02^{b}	1.11±0.05^{a}	1.18±0.07^{b}	1.05±0.06^{a}
	2015	0.99±0.04^{b}	0.70±0.06^{a}	0.87±0.04^{a}	0.86±0.06^{ab}	0.96±0.01^{ab}	0.94±0.03^{b}
N (mg/kg) in soil	**2014**	2.9		2.85		1.3	
Cane weight (kg/m^2)	**2014**	0.18±0.06^{A}	0.28±0.06^{B}	0.18±0.056^{A}	0.26±0.07^{B}	0.21±0.07^{A}	0.23±0.05^{A}
	2015	0.24±0.04^{A}	0.28±0.07^{A}	0.22±0.07^{A}	0.24±0.04^{A}	0.20±0.02^{A}	0.27±0.05^{B}

Means and standard deviations followed by different letters indicate a significant difference if there was any; upper case: comparison of treatment within sites ($p < 0.05$); lower case: comparison of sites within treatments (Games-Howell's $p < 0.05$)

Discussion

Botrytis is one of the most important fungal diseases in viticulture. Infection is highly linked to the canopy microclimate which can be modified by different canopy management techniques (Elmer and Michailides, 2007). One of the main operations is leaf removal providing open canopy and air-flow in the bunch zone. Several authors reported a favourable effect of defoliation regarding botrytis. Prior (2010) for example found that bilateral defoliation can reduce botrytis infection by up to 50 %. In our study, in 2014 we observed that defoliation had no effect on the incidence of botrytis. Due to weather conditions favouring growth (high mean temperature, high precipitation), the berry development started and berry touch occurred earlier resulting in the fact that spraying against botrytis in all three areas was missed. Plant protection performed later proved to be ineffective due to heavy rainfalls. In addition, the vines trained on modified Moser cordon (VSP) had only one pair of catch wires and – due to the high vigour – the shoots folded back in many cases, causing self-shading in the bunch zone. The large number of rotten clusters found in 2014 suggests that defoliation may not be effective against botrytis in all cases (Fig. 1). This contradicts other results (Zoecklein et al., 1992; Tardaguila et al., 2010), where the authors observed that especially in the case of late ripening varieties, defoliation at full bloom can reduce the risk of botrytis even under rainy conditions. However, in our case, the weather significantly influenced the results. Nor could we confirm in 2015 (apart from Erdőkertes) that leaf removal would improve the soundness of the bunches. In 2014, in contrast to the treatment, the growing site played an important role on the incidence and severity of botrytis, which is in accordance with English et al. (1989). In our study in Erdőkertes, bunch rot occurred significantly less frequently and to a lesser extent compared to the other two sites. However, in the drier year of 2015, neither the site nor the treatment had pronounced effect on the incidence of botrytis. In contrast, the severity of botrytis was significantly lower in Erdőkertes due to defoliation. In our study, Erdőkertes was the least infected compared to Dunakeszi and Vác. In both years, the site had significant impact on yield, but not the defoliation treatment. This confirms other experiments, for example the one of Zoecklein et al. (1992). According to our results and previous literature (Bledsoe et al., 1988; Hunter et al., 1991; Main and Morris, 2004; Bavaresco et al., 2008), defoliation at bloom does not reduce yield significantly in any case. The variety 'Zweigelt' is not prone to flower shattering. In 2014, the weight of clusters usually reached 180 g which is the average weight of the variety. In 2015, regardless of the treatment the values exceeded the typical cluster weight of this variety. This means that we did not get looser 'Zweigelt' bunches using defoliation. Compared to other results (Verdenal et al., 2017), we could not find significantly lower 'Zweigelt' berry weights due to defoliation. Berry components such as sugar content and acidity are basic indicators of the time of harvest and all oenological operations. Sugar accumulation and acid degradation are influenced by, for example, climatic conditions (temperature, radiation) and cultivation practices (Bonada and Sadras, 2015). Since defoliation of the bunch zone directly modifies bunch microclimate, it is expected to have significant effect on the composition of the berries. Bogicevic et al. (2015) reported that defoliation can cause an increase in the sugar content of the must while Zhang et al. (2017) found that this method also can decrease it. Since different grapevine growing sites have different climatic conditions, consequently the time of the harvest or the berry composition at the same time would differ. In our study, °Brix, pH and titratable acidity of the must were influenced by both the site and year. In contrast with this, defoliation had no effect on berry composition. As in the case of the previously reported investigations, year-to-year effect had a noticeable effect on berry composition, too. We found higher sugar content at all sites in 2015 than a year before. Differences between the sites were significant in the second year of the experiment while we observed no remarkable differences in 2014. In our experiment, defoliation did not increase the quality, while in other cases it did (Hunter et al., 1991; Main and Morris, 2004; Vilanova et al., 2012). In contrast to Vasconcelos and Castagnoli (2000), in 2014 we found that leaf removal reduces cane weight. However, we also found that defoliation does not affect the Ravaz index. In 2015, we did not experience either the effect of this treatment on pruning weights or vine balance. According to Abro et al. (2013), there is a relation between nitrogen uptake and the incidence of botrytis. This suggests that if we had found less rotten clusters at a certain site, there should have been a weaker nitrogen uptake. The comparison of our measurements with the reference (Lőrincz et al., 2015) we found that nitrogen content in the leaf samples was optimal in each site in both years. Still, according to our results, higher nitrogen content in leaf samples was not in conjunction with less rotten clusters and vice versa: We did not observe higher incidence and severity of botrytis in the case of lower nitrogen content of leaves. We also could not prove what Hepner and Bravdo (1985) found, namely, that the nutritional content of the leaves increases due to defoliation. In 2014, in Erdőkertes, where we found fewer rotten bunches, the nitrogen content of the soil samples was only half compared to the other two sites. In 2014, the lower nitrogen content of soil could also have caused lower yield in Erdőkertes.



2014 2015

Fig. 1: Spider chart representing the normalized average values of the investigated parameters at the three sites (legends: DK. Def. – Dunakeszi, defoliated; DK. C. – Dunakeszi, control; Vác Def. – Vác, defoliated; Vác C. – Vác, control; EK. Def. – Erdőkertes, defoliated; EK. C. – Erdőkertes, control).

Conclusion

Leaf removal is a widely applied canopy management practice aiming at providing higher crop quality and optimal microclimate in the bunch zone aiming at reducing botrytis infection. In this study, we found that defoliation had a marginal effect on the incidence and severity of bunch rot, but year-to-year effect significantly modified the rate of infection. At the three investigated sites, 'Zweigelt' grapevine variety showed differences in yield, cluster and berry weight, highlighting the importance of the terroir research. According to our results, in the case of this variety, leaf removal at bloom is not the most appropriate method to reduce yields. In our investigation, qualitative traits of the must samples (°Bx, pH and titratable acidity) also showed alteration in the different years and sites, but not in defoliation.

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