THESIS OF PHD DISSERTATION

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Study of grape noble rot caused by *Botrytis cinerea*

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1. Introduction

Botrytis cinerea is a well-known pathogen of grapevine, and also common and widespread ascomycete fungus and necrotrophic pathogen attacking numerous different plant species e.g. grape, tomato, kiwi fruit, strawberry and raspberry, herbaceous, shrub and tree species. However, under certain microclimatic conditions, *Botrytis* infection results in noble rot, the essential process in the production of the world-known Tokaji aszú wines in Hungary and other botrytised sweet wines in the word. The Hungarian *aszú* winemaking method is different from the other botrytised sweet wine making techniques in the world, because the Hungarian *aszú* wine is made from grapes that are selected and picked by hand throughout the long noble rot process. In Hungary the practical definition of high quality *aszú* berries are shrivelled but at the same time is fleshy texture, purple colour and slightly shot through with mycelium, but scientific definition has not determined yet. The occurrence of noble rot is dependent mainly on microclimatic conditions, but it is a complex phenomenon, because the local terroir (heritage, local cultivation methods, pruning type etc.) affect the process. In scientific studies usually the field of population genetic of the grey and noble rot were investigated and there is a lack of information on textural and microbial characteristics of the grape berries during the noble rot process. Despite these earlier works, several important questions remain unanswered related to the connection between the *B. cinerea* morphological type, sampling dates grape cultivars, geographic origin and relationships between the physical, chemical and microbial characteristics of grape berries and their interaction during the maturation and noble rot process.

2. Research aims

There is many questions in the role of *B. cinerea* in the noble rot process. It gives several innovation opportunities and challenges to researcher to find the exact role of *B. cinerea* in noble rot process, then since now the so cited Tokaj-Hegyaljai Album, published in 1867, gives the frame of reference. Due to the above sentences my thesis is structured regarding the following questions:

1) Is there any relation between the environmental parameters (vintage, harvest time) and the composition of *B. cinerea* population?
2) How can the homogeneity of a population be interpreted by morphology and physiology?
3) How does the texture of a grape berry changes through the noble rotting process?
4) How does the chemical composition of a grape berry changes through the noble rotting process?
5) How does the microbiota of a grape berry changes through the noble rotting process?
6) How can the "good *aszú* berry" be defined regarding the noble rot corresponding to the observed external and internal parameters?

3. Materials and methods

Collecting grape berries from Eger vineyard

The study was performed in three consecutive years (2013-2015) in a vineyard located at Szomolya (47° 54' 00" N; 20° 30' 00" E) in the Eger wine region. Two cultivars were grown: the white skinned *Olaszrizling* and red-skinned *Turán*. Cultivar (cv) *Olaszrizling* is declared as suitable for botrytised wine making and cv. *Turán* can be used for unique red botrytised wine. Three sampling months (September, October and November) were chosen according to the 3-month-period of noble rot development. The picked berry samples from the botrytised clusters put in sterile storing boxes, shipped to the laboratory and started the *B. cinerea* isolation.

Collecting grape berries from Tokaji vineyard

The study was performed in 2016-2017 in the Tokaj wine region in Hungary. The sampling vineyard was located in Mád, named to Betsek (48° 11’ 18.6” N 21° 19’ 01.8” E) where the presence of noble rotted (botrytised) berries more usual than other vineyards. Two cultivars were grown, the local white-skinned *Furmint* and *Hárslevelű*. Four representative botrytisation phases were defined before the sampling: 1. healthy berries; 2. starting botrytised, not noble rotten, but purple spotted berries, 3. botrytised, noble rotten and purple berries; 4. noble rotten raisin berries with latent mycelia. Three sampling time were chosen in 2017 (September, October and November) according to the autumn-period of noble rot development.

1. Noble rot phase:
Totally ripe, but healthy grape berries.
2. Noble rot phase:
Light brown or berries with purple-brownish spots.
3. Noble rot phase:
Berries dehydration is starting. Totally purple-brownish berries.
4. Noble rot phase:
High quality, totally dehydrated, purple berries with latent *B. cinerea* mycelia.

Figure 1: Noble rot stages/phases (self-made picture)

Measured grape berries physical properties

In 2016 and 2017 vintages for the testing physical parameters a universal testing machine (UTM) TAxT2i Texture Analyser (Stable Micro System, Surrey, UK) equipped with a HDP/90 platform and a 30 kg load cell was used. Data were evaluated using the Texture Expert Exceed software package. For the berry skin hardness test, the berries were placed on the metal plate of the UTM with the pedicel in a horizontal plane so that it was consistently punctured in the lateral face.

Measured grape berries basic analytical properties

In the 2016 and 2017 vintages for the measuring basic analytic parameters: sugar content (Brix), treatable acidity (TA) and pH WineScan (FOSS-analytic, Denmark) equipment was used. Before the measuring fresh grape must was made laboratory homogenisator (IUL Masticator, IUL S.A., Spain) and the fresh must analysed (50 ml) which was cleaned by centrifuge.

Grape berries microbiological properties

Eger vineyard: 2013-2015

The first step of *B. cinerea* isolation was put conidia from botrytised berries of *Olaszrizling* and *Turán to* rosebengal chloramphenicol (RBC) medium (Scharlab S.L., Spain). Then cut the monospore isolates from the RBC media and put through the *B. cinerea* single-spore cultures to potato dextrose agar (PDA) (Scharlab S.L., Spain) and maintained on it.

B. cinerea mycelium growing profile measurement and morphological group determination

For growth tests, 5-mm mycelial plug was cut from the margin of an actively growing 4-day-old culture and placed in the centre of a Petri dish containing PDA. Plates were incubated at 20 °C (optimum temperature) and at 15 and 25 °C (sub-optimum temperatures) in dark. Mycelial growth diameter was measured to a 4-day post inoculation. Three replicates were performed for each isolate and the experiment was carried out twice. Morphological features of the same isolates were determined for the three years, three sampled months and the two cultivars in order to investigate their effects on the morphological types of *B. cinerea* isolates. Morphological types were characterized after a 21-day inoculation but only on those isolates that were grown at the optimum temperature (20 °C). Then cultures were macroscopically examined for sporulation, mycelium and sclerotium production.

Tokaji vineyard: 2016-2017

Before the isolation the grape berries (10 g) were crushed with 90 ml physiological sodium solution in a food homogenisator (IUL Masticator homogenisator, IUL S.A., Spain). Then for selecting fungi (yeast and filamentous fungi) from the *Furmint* and *Hárslevelű* grape berries in every noble rot phases and sampling month different selective media were used, Chloramphenicol Glucose Agar (CGA), Czapek Dox Agar (CD) and Trypton Glucose Extract (TGE) for fungi growing. The monospore isolates were maintained in Potato Dextrose Agar (PDA) and Yeast Extract Peptone Dextrose Agar (YEPD).

Yeast morphology

The micromorphology features of the isolated yeasts were examined by fluorescent microscope (ALPHA BIO-5F type fluorescent microscope, 100x objective). Fresh (1-3 days) isolates were used for the measuring. According to the morphological features main micromorphology groups of the yeasts were determined.

Total yeast and filamentous fungi number determination

Total yeast and filamentous fungi number were determined from *Furmint* and *Hárslevelű* berries during the noble rot process by classics cell number method. The following media were used: CD (Czapek Dox Agar, Merck KGaA, Germany) and CGA (Chloramphenicol Glucose Agar, Scharlau Chemie S.A. Spain) and the cells were grown on 22, 25 °C. After three days the appeared cells were numbered.

DNA extraction and rDNA amplification

For the DNA extraction of fungi (2016, 2017) NucleoSpin Plant II DNA extraction kit (Macherey-Nagel, Germany) was used. The following PCR conditions and primer pairs (Table 1.)

Table 1. Conditions of PCR reactions

Microorganism type	Primer Pair	Target rDNA region	PCR condition
Filamentous fungi	ITS 1F/ITS 4	ITS1/5,8S rDNS/ITS2	denature 94°C 3 min, 94°C 45 sec, annealing 55°C 1 min, extension 72°C 2 min, 35 cycles, 72° 10 min
Yeast	NL 1/NL 4	28S D1/D2	denature 94°C 3 min, 94°C 45 sec, annealing 53°C 1 min, extension 72°C 2 min, 35 cycles, 72° 10 min

For the purification of PCR product QIAquick PCR purification kit (Qiagen, Germany) was used, the sequencing was applied by the BaseClear Company (The Netherlands).

Sequencing method

The sequencing was applied by the BaseClear Company, (The Netherlands). High-quality sequences were grouped into operational taxonomic units (OTUs) at 97% sequence similarity with USEARCH v. 11. We assigned OTUs to taxonomic groups based on pairwise similarity searches against the NCBI Nucleotide

database. DNA sequences have been deposited at the NCBI GenBank.

Statistic analyses

Data on mycelial growth rate were analysed by using analyses of variance in order to determine the effect of year, sampling month, cultivar, temperature and their interactions. Means were separated by using an LSD test ($\alpha = 0.05$). Frequency distribution (FD%) of each morphological type was calculated for each year, sampling month and cultivar as FD% = (isolate numbers in a given classes of morphological type / total numbers of isolates) x 100. Frequency distributions were then analysed by using analyses of variance in order to determine the effect of year, sampling month, cultivar, morphological group and their interactions. Means were separated by using an LSD test ($\alpha= 0.05$). Differences in morphological groups, vintages and sampling month were visualized using non-metric multidimensional scaling (NMDS). Determine the NMDS „stress" value too. For each group of variables, we performed perMANOVA to estimate the amount of variation.

Analysis of variance was applied to all the variables studied. The groups of botrytisation phases (1-4), the grape cultivars (*Furmint* and *Hárslevelű*) and the collecting times (September, October, November) were compare according to the F_{sk}, E_{sk}, W_{sk}, Berry Hardness (BH), sugar content (Brix), pH, treatable acid (TA) tartaric acid equivalent and microbial composition. Statistical analyses were performed with the statistical software R (version 4.03) using packages: Vegan, mixOmics, and FactoMineR. Data were filtered for outliers using Rosner outlier test, scaled and standardized to zero mean and unit standard deviation, normalized data were use where it is mathematically necessary. To estimate how quantitative dependent variables changes according to the levels of categorical independent variables (e.g. botrytisation phase, collecting time and cultivar) several ANOVA models were tested in one-way, two-way and two-way-interaction cases. Akaike information criterion (AIC) was calculated to find the information value of each model by balancing the variation explained against the number of parameters used. To find the significant groupwise differences where the 95 % confidence interval doesn't include zero Tukey's HSD test were carried out, the group labelling and the spread of the distributions are visualized. It is beneficial to

illustrate the multidimensional distribution in reduced space thus Principal Component Analysis (PCA) and Partial Least Square Regression were obtained. Differences in fungal community composition among samples were visualized using non-metric multidimensional scaling (NMDS) with *metaMDS* function in the *vegan* R package with Bray-Curtis distance measure. Community turnover (beta diversity) within noble rot phases were calculated with *betadisper* function. The distribution of fungal OTUs among noble rot phases in each cultivar was visualized using the *sna* package based on presence-absence matrices. The independent quantitative variables were merged into variable groups: texture, analytics and microbial community. For each group of variables, we performed perMANOVA to estimate the amount of variation explained by the botrytisation phase, sampling month, and cultivar with 9999 permutations. In texture and analytical case Euclidean distance matrices were framed, in occasion of microbial community Bray-Curtis dissimilarity matrix was performed from the fungal community composition matrix of the samples. To estimate the correlation between the different variable groups, pairwise Mantel-tests were carried out, using Pearson's correlation coefficient.

4. Results and discussions

During three vintages (2013-2015) growing profile and morphological features of *B. cinerea* isolates originated noble rot grape berries were examined, then in the following two vintages (2016, 2017) the physical, chemical and microbiological features of the whole noble rot process were investigated. My results show new parameters of the high quality *aszú* berries and new statements in connection with the noble rot process.

Strain collection

In the three vintages (2013-2015) 696 monospore *B. cinerea* isolates were stored at -80 °C. These isolates originated from vineyard of Szomolya from two grape cultivar, *Olaszrizling* and *Turán*. In 2016 and 2017 345 yeast and filamentous fungi isolates were stored at -80 °C from Betsek vineyard of Mád village of Tokaji wine region from two grape cultivar, *Furmint* and *Hárslevelű* from four botrytisation phases.

Environmental factors effect the *B. cinerea* mycelial growth

During my study the vintage (2013, 2014, 2015), grape cultivar (*Olaszrizling, Turán*) and collection time (month: September, October, November) as environment factors effect investigated to the mycelial growth rate of *B. cinerea* isolates originated noble rot grape berries in different incubation temperatures. The aim of my analysis was to study that the differences of the different varieties (vintage, cultivar, collection time) how can change the distribution of the measured mycelial growth rate. Analyses of variance (ANOVA) indicated significant differences among years, months, and temperatures for mycelial growth rate but the differences among cultivars were non-significant (mean square= 138.7, P= 0.164). As cultivar being non-significant, data of mycelial growth rate were averaged for the two cultivars and not shown separately for temperatures, years and months. Across the three years and the three sampling months, mycelial growth rate ranged from 59 to 79 mm, from 75 to 84 mm, and from 55 to 77 mm for incubation

temperatures of 15, 20, and 25 °C, respectively. Regarding to the average growth it can be declared that the highest value is measured in vintage 2013 (76 mm), contrary to the 2014 and 2015 values (65 mm and 69 mm). There were significant difference between September and November sampling. The observed white (*Olaszrizling*) and red (*Turán*) grape varieties showed no difference concerning the optimal temperature of mycelial growth (20 °C) of isolates collected. The average of the daily maximum temperature was 22,7 °C in 2013, which is close to the optimal, contrarily in 2014 and 205 it was even higher (23.1 °C and 24.0°C), causing slower growth rate in those vintages consequently.

One can conclude from the above statements that 2013 gave the opportunity of high quality *aszú* berry creation, then the autumn weather was optimal for *B. cinerea* growth. The extreme absolute precipitation distribution in 2014 inhibited the growth of the fungi turned the noble rotting process to grey rot, which is proofed by the highest growth rate measured in this vintage. In 2015 the temperature were far from optimal, in practice the botrytization does not appeared in that fall.

Environmental factors effect to the *B. cinerea* morphological types

ANOVA test was performed to estimate the significance regarding to vintages, harvest months and morphologic types, where those three factors with p-value less then 0.05 found, in contrast to the grape variety which has no effect on morphologic composition. The observed three vintages gave average mycelial ratio values between 51.4% and 91.7% (70.5% as average), which was significantly higher than sclerotial types.

NMDS ordination was calculated to determine the dissimilarities regarding to morphological groups (Figure 2.), which shows visual separation in vintages.

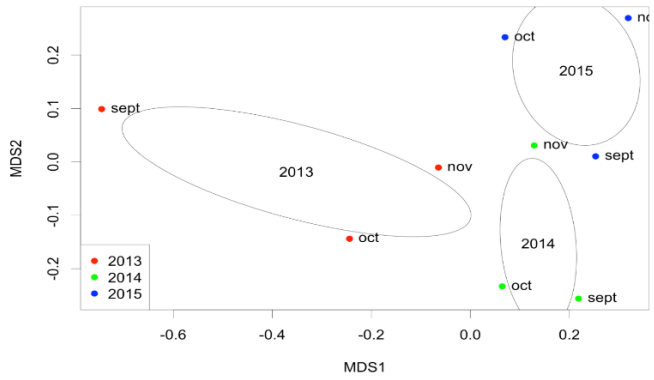

Figure 2. NMDS ordination plot of samples, grouped by morphologic types. The colours indicates the vintages as denoted in the legend.

Permutation multivariate ANOVA was made to estimate how much the morphologic type is described by the multidimensional distributions. As result it is clear that different morphologic types are belonging to overlapping sample groups, contrary vintages gives significant differences in ordination ($p=0.048$, $R^2=0.28$). The findings clears out that vintage and harvest time have significant effect on botrytization development due to the caused differences in morphologic composition.

Grape berries physical properties changing during the noble rot process

The changing of physical parameters of grape berries during the noble rot were demonstrated in the figure 3. With respect to measured physical variables, we observed a strongly significant decline in berry skin brake force (F_{sk}, in mN) and berry skin break energy (W_{sk}, in mJ) and during the noble rot process in both cultivars, with significant differences observed between the cultivars as well. This latter may be driven by the fact that decreases in both F_{sk} and W_{sk} were more gradual in *Furmint*, with a perceptible, but non-significant increase between phase 3 and phase 4, while the measured properties showed a steep decline already by phase 2 and remained low in *Hárslevelű*. Despite these moderate differences among cultivars, the pattern of F_{sk} during the process of the botrytisation is very similar in both cultivar. The

elastic modulus of berry skin or skin elasticity (E_{sk}, in N/mm) and berry hardness (BH, in N) showed similar patterns to those observed above, with exception that differences between the cultivars were negligible, with only the noble rot phases having significant effect in them. Overall, the drop-like decrease between phase 1 and phase 2 can be regarded as an indicator of the beginning of the rotting process.

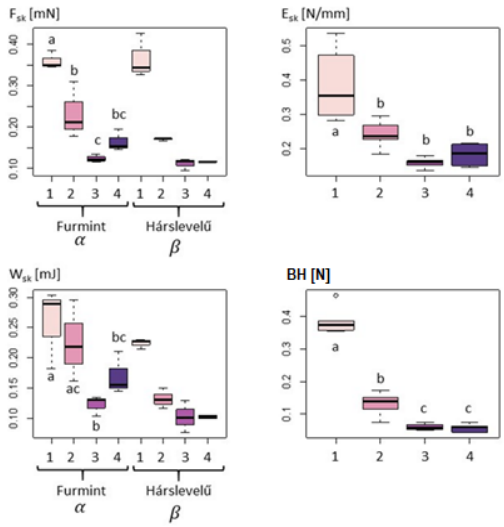

Figure 3. Physical variables of grape berries undergoing noble rot as compared with ANOVA according to noble rot phase (arabic numeral from 1 to 4), sampling dates (September-November), and cultivars (*Furmint* and *Hárslevelű*). For each dependent variable, only the best fit model showing the most influential categorical variables is shown. Letters indicate significant differences in post hoc Tukey HSD tests ($p < 0.05$), with lowercase letters referring to phases, uppercase letters to collecting months and Greek-letters to grapevine cultivars.

In general, the berry skin parameters indicate the berry skin becomes weaker, less elastic and easier to break during the noble rot, likely due to the degradation of cellulose in the plant cell walls by fungi. The only exception of this trend is the slight increase in berry hardness in the last phase in *Furmint*, which may be explained by the excessive drying of the grape berries. It is noteworthy, that berry skin parameters were almost entirely influenced by the botrytisation phases and to a small extent by cultivar, but not by sampling month. This means that the visually defined phases can indeed inform growers about the textural characteristics of the berries throughout the harvest season. In addition, the measurable prediction points we detected can help growers to monitor and forecast the onset and development of noble rot, based on the clear drop in textural parameters and the appearance of other grapevine pathogens on the grape berries.

Grape berries chemical properties changing during the noble rot process

Regarding chemical properties, sugar content (soluble solids in brix) was strongly influenced by the interaction of noble rot phase and sampling month (Table 1). The emerging trend is an overall increase in sugar content during the noble rot process, upon which superimposed is another increasing trend during the harvest period from September through November, particularly in phase 3 and phase 4. The sugar content in collected phase 4 *aszú* berries is highest in November and with small variation, which gives highest quality berries for *aszú* wine in general. There were significant differences in pH among the cultivars, with *Furmint* generally being somewhat more acidic than *Hárslevelű*. In addition, there was a significant effect of sampling month in *Furmint*, that showed a continuous increase in pH during the harvest period.

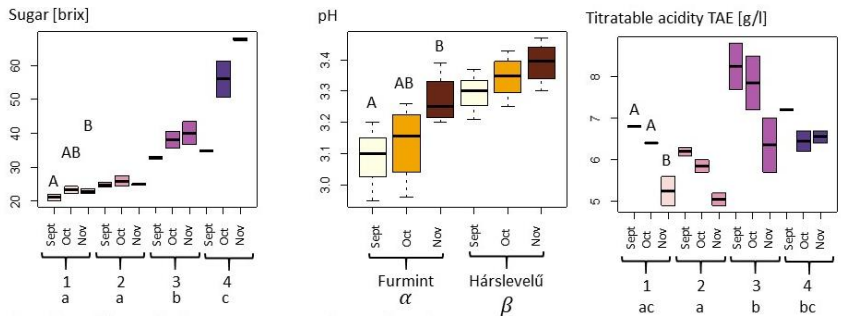

Figure 4. Chemical variables of grape berries undergoing noble rot as compared with ANOVA according to noble rot phase (arabic numeral from 1 to 4), sampling dates (September-November), and cultivars (*Furmint* and *Hárslevelű*). For each dependent variable, only the best fit model showing the most influential categorical variables is shown. Letters indicate significant differences in post hoc Tukey HSD tests ($p < 0.05$), with lowercase letters referring to phases, uppercase letters to collecting months and Greek-letters to grapevine cultivars.

My investigation shows that in the later harvest time the sugar level is the highest, especially in phase 3. and 4., which means it is essential to collect *aszú* berries in later months (October or November).

Examination of the microbiota of grape berries during botrytization

Morphology richness of yeasts

Before determining the richness of yeasts and filamentous fungi and performing molecular identifications the morphologic research was carried out of isolated from 2016 to reduce the number of sequencing processes. From the surface of grape berries in different phase of botrytization the total number of yeast and filamentous fungi cells ware monitored in year 2016 and 2017. It was found out that both total numbers showed continuous increase regarding to the two vintages and two grape varieties.

Result of molecular identification

In all phases the genus *Aureobasidium* sp. and *Hanseniaspora* sp. were found, with the highest diversity in pahse 1. In phase 4. beside the *Botrytis* sp., *Penicillium* spp., *Alternaria* spp., *Aspergilus* spp., *Curvibasidium* spp. and *Cladosporium* spp.,filamentus fungi were found. In both vintages, 2016 and 2017, *Aureobasidium* sp., *Hanseniaspora* sp., *Cryptococcus* sp. and *Metschnikowia* sp. genus were the highest in numbers regarding to all the phases in both varieties. To assume, one can declare that regarding to different phases there is variation in the yeast and fungi composition of samples, which might be caused by the different physiologic condition of the berries in different phases.

During the *aszú* development the total number of yeast and fungal cells is increasing contrary the diversity shows significant decrease. It can be effected by the larger relative surface ratio of a rushed grape berry, otherwise the growing sugar concentration reduce the number of microorganisms. Regional grape varieties as *Furmint* and *Hárslevelű,* shows different microbial community. In vintage 2017 198 isolated were collected and sequenced. The DNA sequences was assigned to OTUs (operational taxonomic unit, with higher than 97% similarity in DNA sequence) where 26 of them represent the 198 isolates.

Figure 5 shows that the microbial composition is significantly different between varieties and harvest times but show homogeneity in botrytization phases. In *cv. Furmint the* total number of OTUs are increasing by the harvest time but with lower diversity in November. In *cv. Hárslevelű* the total number of OTUs has no significant increase by the harvest time. From the results one can say that the botrytization phase has no effect on the total number of OTUs during noble rot.

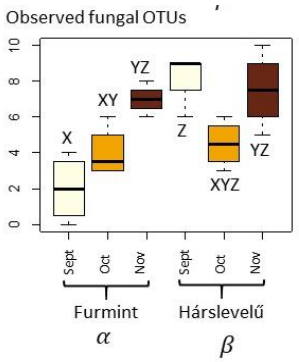

Figure 5. Distributions of microbial communities of noble rot: 1-4 denotes botytization phases, sept-nov indicates the harvest time and Greek letters (α and β) indicates the cultivars *(Furmint* and *Hárslevelű*) respectively. Letters shows significant differences in post hoc Tukey HSD test, capitals regarding harvest time. Subfigures indicate only the significant variations.

Relationships among physical, chemical and microbiological variables and noble rot phases

In order to explain the differences between our berry samples, which were selected according to these factors: noble rot phases, collection times and cultivars, principal component analyses was performed to provide partial visualisation of the data set in reduced dimensions. Three principal components with eigenvalues higher than 1 were obtained. From the variables, PC1 (26.27% total variance) was most highly correlated with TA, BH and Es_k. Berry skin break force, sugar content and W_{sk} dominated in PC2 (22.2% total variance). The projection of the samples along the directions is identified by the first two PCs (Figure 6.). The separation of different categories of samples from this scatterplot indicates that the first three botrytisation phases were separated and there is an overlap between the third and fourth phases.

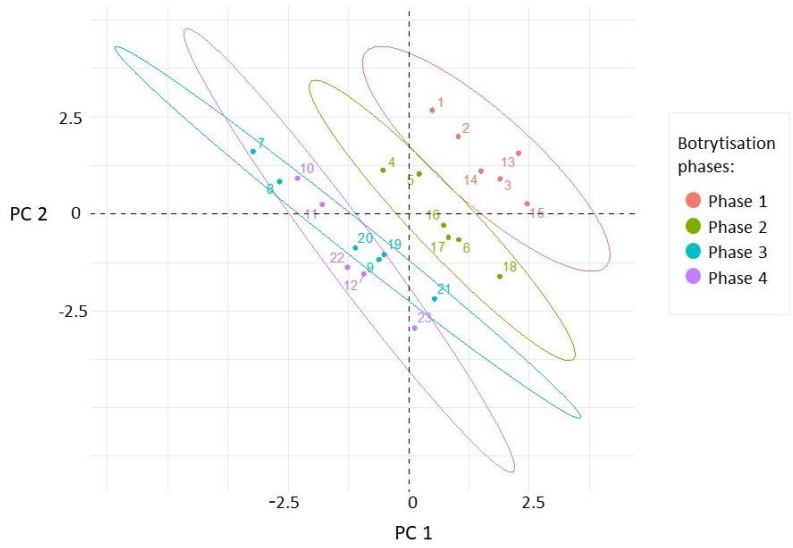

Figure 6. Scatterplot of the samples in the plane defined by the first two principal components calculated from the four textural (F_{sk}, E_{sk}, W_{sk}, BH), three analytical (Sugar, TA, pH) and fungal richness variables. The sample points are coloured in accordance with the botrytisation phases.

According to see the correlation between the measured variables the berry texture indicators correlate with each other positively, the pH and the number of fungal OTUs do not correlate with texture parameters, but do correlate with each other. Sugar was negatively correlated with berry texture indicators, while acidity has no correlation with any other variables. Beside the grouping and factor analysis of the samples permutational multivariate ANOVA (permANOVA) was made to compare the dispersion of point scores and centroids of the sample groups concerning factorial parameters. According to the permANOVA results, the variable groups of texture parameters, analytical properties and fungal community are significantly different in distribution between botrytisation phases, so the defined phases can be separated by all the measured data.

Table 2: Proportion of variation in physical (texture), chemical (analytics), fungal community variables explained by noble rot phase, sample collecting time and grapevine cultivar using permutational multivariate analysis of variance (PerMANOVA).

	Noble rot phase		**Sampling month**		**Cultivar**	
Variable	Var. (%)	*p*	Var. (%)	*p*	Var. (%)	*p*
Texture	**57**	**0.001**	**31**	**0.001**	12	0.057
Analytics	**72**	**0.001**	11	0.12	0	0.835
Fungal community	**21**	**0.031**	**22**	**0.014**	**25**	**0.014**

In table 2, the significance p-values and explained variance show that all the physical, chemical, and microbial variable groups have significant differences among the four botrytisation phases. For texture and analytical parameters, a relatively high explained variance values are given (57% and 72%, respectively), highlighting the importance of these in the process of noble rot. In case of sample collection time, texture and fungal community have higher, analytics has lower correspondence, but it is necessary to declare, that collection time plays an important role in the chemical properties of fully botrytised berries. Finally, cultivars do not have strong influence on the texture and measured chemical properties, but cultivar has a significant effect on fungal community composition. In addition, we observed lower compositional turnover (beta diversity) among samples of fully botrytised berries in phase 4 than in the partially botrytised berries of the preceding phases. Mantel tests were used to detect possible correlation can be detected between variable groups. In the case of physical and chemical parameters, we used Euclidean distance, while for the fungal community data, we generated a Bray-Curtis distance matrix. The only significant correlation was between physical parameters and fungal community composition (Mantel statistic R: 0.1624, p = 0.0429), while no significant correlation was found between the chemical and fungal, nor between chemical and physical parameters. In other words, as samples become more dissimilar in terms of physical parameters, they also become more dissimilar in terms of fungal community composition, while chemical variables appear to change somewhat independently from physical parameters and resident fungi. The lack of significant changes in fungal richness among the botrytisation phases indicates that the complexity of fungal communities is comparable along the noble rot process. Nonetheless, the significant effect of noble rot phase on the composition of the

fungal community suggests some deterministic component of community dynamics that likely is related to the predictable physical and chemical changes in the berries.

5. Conclusion

As outcome new aspects were recognised in the relationship between *B. cinerea* and noble rot concerning the above detailed results. Profession winemakers and viticulturists define the high quality *aszú* berry as high sugar and acid content in a well-balanced ratio, having low enough pH to a stabile fermentation process, have special *B. cinerea* related aroma compounds and have shrivelled texture with soft tissue inside. Behind the scene of noble rotting process physico-chemical and microbiological characteristics were not completely described yet. Examinations and hypothesis' presented in this thesis are originated from these lack of definition, then the vintage and the harvest time play an important role in the morphological composition of *B. cinerea* population. Moreover the botrytisation process itself was examined taking the fungal microbiome into account. Multivariate statistical analyses were performed to determine the textural analytical and microbiological changes as a complex multivariate model during the noble rot. The most relevant variables are the treatable acid, berry hardness, Young modulus of berry skin, berry skin brake energy and sugar content which define a proper model to separate four botrytisation phases significantly. As a so called "good *aszú* berry" was put on a new footing multidimensional model can describe the quality of a noble rotten berry. Assuming the results high quality *aszú* berry might be harvested in late October or November from *Furmint* variety, picking only the fourth phase completely shrivelled berries. Using the model in practice the properties of a certain viticulture conditions and vintage climate parameters should be taken into account therefore the above statements will help producers to enhance quality and economic benefits.

6. New scientific results (Thesis)

During the study the noble rot process was analysed according to i) *B. cinerea* plays the most important role in *aszú* development ii) physico-chemical and microbiological characteristics define the quality of a certain berry. The examinations were highlighted that the noble rot process is more then *B. cinerea* metabolism, it has a complex multivariate dynamics. The following new scientific results are stated:

Thesis 1.:

It was clearly demonstrated that the vintage characteristics effect the mycelial growth rate and morphology of *B. cinerea* during the noble rot process, according to disoptimal temperature and which fall weather with less total amount and hectic distribution of precipitation higher growth rate type strains are forming the population. Regarding to morphology mycelial types show decrease significantly by later harvest times.

Thesis 2.:

It was revealed that the physico-chemical characteristisc of aszú berry change indipendantly by the vintage and cultivar therefore botrytisation phase determine those parameters. A well-defined brake point was appointed (*E-drop*) at the berry skin elastic modulus which can indicate the initial state of *aszú* berry development.

Thesis 3.:

It was demonstrated that harvest time has the most relevant effect on the chemical composition of aszú berry thus a high quality aszú berry should be harvested in late October or November from *Furmint* cultivar picking only the fourth phase berries.

Thesis 4.:

It was presented that the noble rotting phase has no effect on the microbial composition of *aszú* berry contrary cultivar and harvest (collection) time is determining it. Furthermore in average *Hárslevelű* has a more diverse microbiome but in later harvest (collection) times *Furmint* shows such a diversity.

Thesis 5.:

Analysing the relationship between the examined physico-chemicals and microbial variables it was showed that there is strong connection between berry texture and fungal microbiome, hence the background of the botrytisation process is more complex then so far assume.

7. List of publications in the field of studies

Publications in peer-reviewed journals (with impact factor)

Júlia, Hegyi-Kaló; Imre, J. Holb; Szabina, Lengyel; Ákos, Juhász; Kálmán, Zoltán Váczy

Effect of year, sampling month, grape cultivar and incubation temperature on mycelial growth and morphological type of Botrytis cinerea isolated during noble rot development

EUROPEAN JOURNAL OF PLANT PATHOLOGY (2019)

2019 IF = 1,466

Júlia, Hegyi-Kaló, Ádám István Hegyi, József Geml, Zsolt Zsófi, Xénia Pálfi, Kálmán Zoltán Váczy

Physico-chemical characteristics and culturable microbial communities of grape berries change strongly during noble rot development

MDPI Plants (2020)

2020 IF = 2,762

Hungarian language publications

Hegyi-Kaló, Júlia; Lengyel, Szabina; Szalóki, Nikoletta; Szén, Orsolya; Juhász, Ákos; Váczy, Kálmán Zoltán

Különböző aszúsodási fázisokban gyűjtött szőlőbogyók felületén előfordulható élesztő és fonalasgomba közösség vizsgálata

NÖVÉNYVÉDELEM 78: 11 pp. 507-512., 6 p. (2017)

International conference participations

Hegyi-Kaló, J; Pomázi, A; Váczy, K Z

Diversity of Botrytis cinerea population of "Aszú" berries compared within two different vintages

In: Engelhardt, Tekla; Dalmadi, István; Baranyai, László; Mohácsi-Farkas, Csilla (szerk.) Food Science Conference 2015 - Integration of science in food chain: Book of proceedings

Budapest, Magyarország: Corvinus University of Budapest, (2015) pp. 94-97. , 4 p.

Júlia, Kaló-Hegyi; Andrea, Pomázi; Zsuzsanna, Váczy; Kálmán, Zoltán Váczy

Genetic and morphologic characterisation of noble rotted Botrytis cinerea isolates from Eger wine - growing region

In: Dalmadi, I; Engelhardt, T; Bogó-Tóth, Zs; Baranyai, L; Bús-Pap, J; Mohácsi-Farkas, Cs (szerk.) Food Science Conference 2013 - With research for the success of Darányi Program: Book of proceedings

Budapest, Magyarország: Budapesti Corvinus Egyetem, Élelmiszertudományi Kar, (2013) pp. 204-207., 4 p.