Thesis for doctoral (PhD) dissertation

INVESTIGATION OF BIOTIC AND ABIOTIC FACTORS INFLUENCING THE OCCURRENCE OF GRAPEVINE TRUNK DISEASES AND POSSIBILITIES OF USING MICROORGANISMS ISOLATED FROM GRAPE

András Csótó

Supervisor: Prof. Dr. Erzsébet Mónika Karaffa

UNIVERSITY OF DEBRECEN

Kálmán Kerpely Doctoral School

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1. INTRODUCTION AND OBJECTIVES OF THE DOCTORAL RESEARCH

Grapevine trunk diseases (GTD) are mentioned among the most serious problems in grape plant protection, as we do not have an effective control against, even though it causes significant economic damage worldwide (Surico et al., 2008, Hofstetter et al., 2012, De la Fuente et al., 2016, Gramaje et al., 2018). GTD is an umbrella term that includes several diseases and disease complexes, such as esca, black dead arm (BDA), or eutypa dieback (Mugnai, 2011). These diseases appear in plantations older than 8-9 years, are characterized by the fact that they do not express visible symptoms each year, however the pathogens can also be present in the transport vessels without any symptoms (Hevitt et al., 1957, Úrbez - Torres et. al., 2008, Hofstetter et al. al., 2012, Díaz – Latorre, 2013, Kovács et al. 2017). It is very difficult to determine the time of infection, as years can elapse between the infection and the appearance of symptoms (Varga, 2009). In some years, the diseased trunks show tiger-striped or stunted leaf symptoms, these may disappear or return, and with the complete blockage or necrosis of the transport tissues, the plant sooner or later partially or completely dies (Mikulás, 2008, De la Fuente et al., 2016). The symptom complex was already known to the ancient Romans and the medieval Moors (Mugnai et al., 1999), however its first official descriptions were recorded in California by an unknown author (1895) and in France by Ravaz (1898). The development of the disease therefore depends on the balance of the trunk's microbiome and the internal and external environmental factors that affect it (Marchi et al., 2006, Pacifico et al., 2019), so the severity of the infection is described with the the disease incidence (DI), not with the percentage of the infected plants (Kenfaoui et al., 2022).

Disruption of the microbiome can be caused by frost damage, drought, high soil salinity, high temperature, but also arsenic pollution. The spread and symptom expression are influenced by precipitation and water supply, partly the spore dispersal of many pathogens are assisted by, and partly because it promotes the translocation of phytotoxins that cause leaf symptoms in the plant (Marchi et al. 2006, Bruno – Sparapano, 2007, Bertsch et al., 2013). There are significant differences in the general GTD sensitivity of the cultivars. For example Sauvignon Blanc and Cabernet Sauvignon were sensitive, while the Merlot was more resistant in the surveys (Travadon et al., 2013, Murolo - Romanazzi, 2014, Sosnowski et al., 2016, Foglia et al., 2022 Cardot et al., 2019). There are very limited possibilities for protection against the GTD pathogens , due to their diversity and protected habitat (Gramaje et al., 2018). The last effective chemical agent, sodium arsenite, was withdrawn in the early 2000s. Since then, the disease complex has become more and more severe (Surico et al., 2008, De la Fuente et al., 2016). We do not know of any resistant cultivar or wild relative species (Murolo – Romanazzi., 2014). Therefore, plant health, hygiene and agrotechnical tools can reduce the infection of the plantation. Greater efficiency may be achieved by biocontrol agents, which can colonize pruning and other wounds and plant vessels, than chemical pesticides which have difficulties to reach the pathogens (Di Marco et al., 2004, Gramaje et al., 2018). The use of the former group is also favourable because, these treatments meet the requirements formulates in the directives of the European Union as the Green Deal or its Farm to Fork strategy (Európai Bizottság, 2019, 2020). Majority of the tested biocontrol agents to control GTDs are of the *Trichoderma* species (Hunt et al., 2001). They can be used to protect cuts and other wounds, or for preventive colonization of the plants (Di Marco et al., 2004, Pollard-Flamand et al., 2022). Many *Trichoderma* species produce fungal cell wall-degrading enzymes and secondary metabolites that inhibit the germination of pathogen spores, and their presence also triggers and stimulates plant defense reactions (Harman 2000, Howell, 2003, Vinale et al., 2008). In addition, they can support the plant's general fitness and nutrient uptake by triggering hormonal actions, producing biostimulant metabolites, and the mobilization of nutrients (Harman et al., 2006, López-Bucio et al., 2015, Pascale et al., 2017). We have very limited epidemiological data of domestic plantations and the sensitivity of cultivars from the Carpathian Basin. It is essential to recognize the factors that promote the spread and the appearance of symptoms, as well as to develop protection technology to control the disease.

The goals of my research work were to examine biotic and abiotic factors favouring the symptom expression of GTD and the possibility of using microorganisms isolated from grapes as follows:

1: Assessment of the incidence of GTDs in several Hungarian wine regions; 2: Analysis of the effects of environmental factors on disease incidence; 3: Revision of the order of sensitivity of the cultivars, and determination of the sensitivity of the cultivars with importance in the Carpathian Basin, and exploration of sources of resistance; 4: Investigation of the biocontrol and biostimulant effect of *Trichoderma* species isolated from grapes in laboratory and field conditions.

2. MATERIALS AND METHODS

2.1. Disease incidence in Hungarian vineyards and affecting environmental factors

In order to determine the prevalence Hungarian GTDs, and the effects of certain environmental factors, we conducted a survey in 2021-2022, as well as analyzed the results of long-term experiments. In the examined areas (Table 1), we examined 100 capitals per row and 400 or a multiple of this per variety, depending on the maximum number of plants per row and per cultivar. In the case of a smaller plant number than, all plants of the cultivar was assessed.

Table 1: Plant numbers and cultivar composition of the assessed vineyards

wine region	plant number	site	plant number	cultivars[1]
Tokaj	2003	Szarvas	2003	H
Szekszárd	3601	Lajvér	3601	CF, CS, C, BF, M, WR, PN, SB,
Villány	2985	Bocor	712	WR, PN, BF, CS, WR,
		Göntér	141	M, PN, P, SB
		Zuhánya	855	CF, CS, BF
Badacsony	4599	Badacsonytomaj	4599	FR, CS, CF, PN, WR, J, PG, KNY, KB, OD, PF+germplasm collection
Kunság	2280	Kecskemét	2280	germplasm collection
Pécs	724	Pécs	724	germplasm collection
Eger	9357	Almagyar	800	FA
		Kőkötő	616	FA
		Nagy-Eged	800	BF
		Nagy-völgy	1600	CF, CS, M, BF
		Pajados	1141	CF, BF, M
		Posta út	1200	CF, M, T
		Szarvas	2800	OC, CF, CS, M, WR, PN, S
		Zsidó-szél	400	BF
Pallag	860	Pallag	860	germplasm collection
Total	**26409**			

[1] BF: Blaufraenkisch, C: Chardonnay, CF: Cabernet Franc, CSF: Cserszegi fueszeres, CS: Cabernet Sauvignon, FA: Feteasca Alba, FR: Feteasca Regala, H: Harslevelue, J: Juhfark, KB: Korai Bibor, KNY: Keknyelue, M: Merlot, OC: Odesskii Chernyi, OD: Odysseus, P: Portugieser, PF: Pannon frankos, PG: Pinot Gris, PN: Pinot Noir, S: Syrah, SB: Sauvignon Blanc, T: Turan, WR: Welschriesling. Prime names of cultivars based on the database of the VIVC (Maul – Töpfer, 2003).

The results only included data from survey of 2022, and data of the previous year was served to separate the annual and earlier plant death. Three categories were determined for GTDs symptom expression in 2022: 1: healthy plant; 2: leaf symptoms in 2022 or apoplexy, or no budburst of the plant, which was living at previous year; 3: the plant died earlier (before 2022).

The effect of the spatial proximity of the diseased plants was examined in Harslevelue cultivar in a long-term experiment took between 2013 and 2019 in the Szarvas vineyard of the Tokaj Wine Region. The individual plants of the vineyard were classified based on the number of years they showed the classic foliar or dieback GTD symptoms. The neighbours of the plants with frequent symptom expression (4 or more years) were classified with the same method.

The effect of the vertical position on the slope on symptom expression was investigated in Blaufraenkisch and Feteasca Alba varieties in the Nagy – Eged vineyard and in the Kőkötő vineyard of the Eger Wine Region, with a slope steeper than 15° and signs of significant water erosion. In the former vineyard, the rows are perpendicular to the slope, in the latter, they are in the direction of the slope.

The impact of the surrounding forest areas on the appearance of the disease complex was evaluated by analyzing the data of the 2022 survey discussed above, excluding terraced cultivation areas and variety collections. The symptom appearance values were compared between four forest cover groups based on the method of Csótó et al. (2022), respectively: 1. without forest or forest strip; 2. neighbouring full hedge; 3. in the immediate vicinity of a forest; 4. surrounded by forest or there is a forest directly at the highest point of the area's slope.

The effect of waterlogging was investigated on the appearance of symptoms using data from the grape germplasm collection of the University of Debrecen, Institutes for Agricultural Research and Educational Farm, Pallag Experimental Station of Horticulture in four years (2013-2015 and 2019). The soil type of the plantation is sandy loam, but there is a waterproof layer at a depth of approximately 70 cm, so the deeper parts of the area are prone to waterlogging. There are five individuals of one grape cultivar in the plantation, so we measured the rate of symptom appearance per cultivar, and then in our analysis we compared with the four-year average of disease incidence in the group of varieties affected by waterlogging and those not affected.

2.2. Cultivar sensitivity studies

Based on the data of the 2022 survey, the GTD resistance of the cultivars was also determined in the four investigated cultivar collections under different environmental conditions (i. University of Debrecen, Institutes for Agricultural Research and Educational Farm, Pallag Experimental Station of Horticulture; ii. and iii. Badacsonytomaj and Kecskemét Researh station of the Hungarian University of Life Sciences, Research Institute for Viticulture and Oenology, iv. University Wine Estate Pécs (Table 2).

Table 2: Characteristics of the surveyed germplasm collections (Csótó et al., 2023a).

	Badacsonytomaj	Kecskemét	Pallag	Pécs
[1]Soil	erubase/ Eutric histosol	Sand/ Haplic arenosol	Sand/ Haplic arenosol	Brown earth/ Chromic cambisol
Relief	Mountain slope (top-valley row direction, terrace cultivation)	Lowland	Lowland	Mountain slope (terrace cultivation)
Cultivation type	Grafted	Own rooted	Own rooted	Grafted
Climate	szubmediterrán, száraz meleg nyárral	kontinentális	kontinentális	szubmediterrán, száraz meleg nyárral
Relative climate sector[2]	IIIc	Ib	Ia	IIIb
Average temperature fluctuation (°C)	21–22	23–24.5	23–24	21–22
Annual precipitation (mm)	600–800	500–550	550–700	600–800
Annual sunshine duration (h)	1950–2050	2000–2150	1900–2050	2000–2100

[1]Nomenclature of soil types based on official Hungarian (National Land Centre, 2023)/ WRB (IUSS WRB, 2015) and European Comission (European Comission, 2005). [2]Relative climate sectors by Bartholy – Weidinger (2010).

The BDA, esca and Eutypa-like symptoms were considered a GTD symptom. The sequence of sensitivities found in the Hungarian variety collections was set up on the basis of disease incidence, similar to the relevant literature sources discussed in the antecedents of the thesis, i.e. the rate of foliar symptoms and death were included together.

Table 3: Groups of cultivars based on lineage (Csótó et al., 2023a).

Ancestry on parent and grandparent level	Categorization I.	Categorization II.
monofiletic *Vitis vinifera*	*Vitis vinifera* (Vv)	*Vitis vinifera* (Vv)
occurrence of American species[1]	*Interspecific (I)*	*American origin (Ao)*
occurrence of Vitis amurensis		*Vitis amurensis origin (Va)*

[1]*V. labrusca*, *V. riparia* or *V. rupestris*

Many of the surveyed cultivars had non-*V. vinifera* ancestry. The different *Vitis* spp. in the pedigree of a cultivar were certified based on data from the Vitis International Variety Catalogue (VIVC) (Maul – Töpfer, 2003). The cultivars were grouped for further analysis based on their ancestry from different *Vitis* spp. (Table 3). Varieties were categorized based on a new method for determining disease severity (i.e. severity of visible symptoms). Four GTD sensitivity groups were created for categorization based on the symptom type (annual foliage symptoms and death or complete death) and the frequency of the various symptoms. Highly sensitive (HS), where all symptomatic plants of the cultivar are dead; sensitive (S), where both dead plants (resulting from apoplexy of the trunk) and fresh GTD leaves and dieback symptoms are detected. The cultivar was considered resilient (R) if only foliar symptoms were present, while neither apoplexy nor annual GTD leaf and dieback symptoms were detected in unsusceptible (U) cultivars (table 4.)

Table 4: GTD susceptibility categories (Csótó et al., 2023a).

Sensitivity Categories		**GTD Symptoms**	
Two Groups	**Four Groups**	**Apoplexy (Dead Plant)**	**Leaf Symptoms and Fresh Dieback**
More sensitive	highly sensitive	+	-
	sensitive	+	+
Less sensitive	resilient	-	+
	unsusceptible	-	-

To reveal the potential differences in pathogen sensitivity among the different ancestry groups, the four original groups were re-appreciated, where the two more sensitive (HS and S) and the two less sensitive (R and U) categories were merged. The ratio of the lineage groups within each of these two redefined sensitivity categories was compared to the theoretically expected distributions with the binominal test.

The tendency of the GTD to kill the host plant was determined in parallel by calculating the proportion of individual plant losses within the disease incidence of the lineage groups and comparing the lineage groups in pairs. Monophyletic European *V. vinifera* (Vv) cultivars against the (1) interspecific (I) ones and (2) hybrids with American *(V. rupestris*, *V. riparia*, *V. labrusca*–Ao) and Asian (*V. amurensis*–Va) species co-origin.

2.3. Laboratory studies of endophytic *Trichoderma* strains isolated from grapes

In our studies, we worked with ten endophytic *Trichoderma* strains isolated from Furmint grapes in the Tokaj Wine Region by Kovács et al. (2017). We performed their species-level identification using the latest accepted reference databases (ex-type strain sequences) and methods (based on tef1 sequences) (Druzhinina et al., 2008, Chaverri et al., 2015, Bissett et al., 2015, Cai et al., 2021). Sequencing of purified amplification products was performed in all cases by Microsynth Austria GmbH (Vienna, Austria). The sequences were deposited in GenBank (OK560824-OK560833 és OK655885-OK655894). The MEGA 7.0 program (Kumar et al., 2016) was used to create the phylogenetic tree, using the Maximum Likelihood method, with 1000-fold Bootstrap analysis.

The mycelial growth of *Trichoderma* isolates at different temperatures (5; 18.5; 20; 22.5; 25; 30 and 37 °C) was determined in three replicates, our experiment and analysis includes the partial results of (Kovács 2017). A 10 mm diameter mycelial disk was cut from the growth zone of the fungal colonies and placed in the middle of potato dextrose agar (PDA, Scharlau, Barcelona, Spain) in a 60 mm diameter Petri dish. Two colony diameters were measured regularly for 4 days, or until the colonies reached the edge of the Petri dish, and their average values were used for the analysis. To test the resistance of isolated *Trichoderma* strains to fungicides in vineyards, fungicides used at the time of the study were tested in their permitted upper doses (Table 5).

Table 5: The fungicides and concentration of their active ingredients in the culture medium used in the fungicide tolerance test (Kovács et al., 2021).

Target pathogen	Product name	Active ingredient	Concentration (mg/L or ml/L)
Plasmopara viticola (Berk. & M.A. Curtis) Berl. & De Toni	Orvego	Ametoctradin Dimetomorph	399 299.25
Erysiphe necator Schwein.	Rally Q SC	Miclobutanil Quinoxifen	45 45
	Sercadis	Fluxapiroxad	225
	Talentum 20 EW	Miclobutanil	80
Botrytis cinerea Pers.	Chorus 50 WG	Ciprodinil	469
	Teldor 500 SC	Fenhexamid	835

Growth inhibition was performed in triplicate, compared to mycelial growth on PDA without fungicide, after Bouanaka et al. (2021) method.

$$\mathrm{I}\ \% = \frac{(\mathrm{dc} - \mathrm{df})}{dc} \times 100$$

where: I%: percentage of inhibition df: diameter of fungicide treated colony; dc: diameter of control colony.

The mycoparasitic ability of *Trichoderma* isolates was examined by Szekeres et al. (2006) method, with BCI (biocontrol index):

$$\mathrm{BCI}\ \% = \frac{(\mathrm{dA} + \mathrm{dP})}{\mathrm{dA}} \times 100$$

where dA: diameter of antagonist on the line of confrontation on PDA; dP: diameter of pathogen on the line of confrontation on PDA.

Pathogens used in biocontol efficacy tests are summarized in Table 6.

Table 6: The pathogens in the biocontrol efficacy tests (Kovács et al., 2021).

Reference number[1]	**Pathogen**	**Host (isolation)**	**Acession number**[2]
CBS 337.29	*Pythium acantophoron*	*Ananas sativus* (L.) Merr.	HQ665212
JT2015	*Botryosphaeria dothidea*	*Juglans regia* L.	MN706192
J2034	*Diaporthe eres*	*Juglans regia* L.	MT111103
HUT01	*Diplodia seriata*	*Vitis vinifera* L.	KU377167
R.3	*Eutypa lata*	*Vitis vinifera* L.	OK178559
B.CS.5.4.20.1.B	*Neofusicoccum parvum*	*Vitis vinifera* L.	OK178560

[1] Reference number in the CBS, or in the strain collection of the Microbiological Laboratory of Food Science Institute, University of Debrecen, Hungary.
[2] Accession number of the ribosomal DNA region.

2.4. Efficacy tests of endophytic *Trichoderma* strains isolated from grapes in field

The inoculum were produced by two different methods. For the treatment of experimental site I, a conidium suspension was prepared by washing off the colonies growing on PDA medium. A. II. the mass production of *Trichoderma* conidiospores to be used in the experimental area was carried out by submerged bioreactor cultivation in the Department of Biochemical Engineering of the University of Debrecen. For the treatment, the conidium suspensions were diluted to 10^6 orders of magnitude. The strains used and the concentration of their conidium suspensions are summarized in Table 7.

Table 7: Inoculum used in the experiments (Csótó et al., 2023b).

Experimental field	***Trichoderma* strains**	**Inoculum production method**	**Spore concentration (spore mL−1)**	
			following production	**in soaking treatments**
I. (Siklós, Zuhánya)	*T. simmonsii* (TR05)	culture on PDA medium	1.5×10^9	10^6
	T. orientale (TR06)		1.1×10^9	
	T. gamsii (TR08)		1.4×10^9	
II (Szálka, Lajvér)	*T. afroharzianum* (TR04)	submerged liquid culture	4.3×10^7	10^6
	T. simmonsii (TR05)		6.7×10^7	

The I. experiment was set in the Zuhánya vineyard of the Villány Wine region, the II. experiment was set in the Lajvér vineyard of the Szekszárd Wine Region. The cultivar and clone composition of the experimental sites is discussed in Table 8. During treatment, the grafts were soaked in the spore suspension for 48-72 hours and then planted with a hydrodrill. The control was soaked with well water also used for dilution.

Table 8: The scion-rootstock combinations used in the experiments (Csótó et al., 2023b)

Experimental field	Scion cultivar (clone)	Rootstock (clone)	Planting date
I (Siklós, Zuhánya)	Blaufraenkisch (Kt.1.)	5BB (K21)	2015.4.17–20.
	Blaufraenkisch (A4/1)	5BB (We48)	
	Cabernet Sauvignon (337)	K5BB (ISV1)	
	Cabernet Franc (GM/Trv)	K5BB (101)	
	Cabernet Franc (E11)	K5BB (ISV1)	
	Cabernet Franc (ISV5)	K5BB (GM13)	
II (Szálka, Lajvér)	Cabernet Sauvignon (E153)	K5BB (ISV1)	2017.5.2.–4.

The effect of *Trichoderma* treatment was surveyed on budding in April 2019, four (experimental area I) and two (experimental area II) years after *Trichoderma* treatment. A total of 98-150 grapevine plants were assessed for their budburst and bud development.

Bud burst ratio (BB%) was calculated as follows:

$$\text{BB \%} = \frac{\text{no. buds with detected burst}}{\text{total no. of buds on the cane}} \times 100$$

The bud burst vigor was evaluated based on a four-grade scale described in Table 9.

Table 9: Bud burst vigor scale applied in bud development survey (Csótó et al. 2023b).

scale	BBCH value *	Description of phenological stage
0	0	The winter buds are dormant or aborted
1	01–05	Start of bud swelling to “wool stage”
2	07–09	Bud burst
3	11–15	Starting of leaf development

* BBCH: scale system describing the developmental stages of various economic plants (Meier, 2018)

Bud burst vigor index (BBVI%) was calculated as follows:

$$\text{BBVI \%} = \frac{\sum \text{class frequency x score of rating class}}{\text{total number of observations} \times 3\ (\text{maximal rating class})} \times 100$$

The grape yield was measured only in experimental site I, on Blaufraenkisch clones four years after the *Trichoderma* treatment. The weight of the harvested grapes was measured row by row using a Demandy TCS-B H45x60 scale (Hungary Mérleg Ltd., Budapest).

The experimental yield per plant was calculated as follows:

$$\text{Experimental yield} = \frac{\text{weight of harvested grapes}}{\text{no. planted grapevines}}$$

The potential yield per plant was calculated as follows:

$$\text{Potential yield} = \frac{\text{weight of harvested grapes}}{\text{no. living grapevines}}$$

After planting, samples were taken annually to verify the presence/absence of *Trichoderma* strains in the treated and untreated plants from both I. and II. experimental site. At each sampling, two control and four treated plants were selected along with a significant part of the root system. We also removed four plants from the II. experimental site in the fifth month after the treatment and processed them for sampling. From these, the edophyton fungi were isolated after surface disinfection based on the method of Kovács 2017. The re-isolated *Trichoderma* strains were identified based on morphological characteristics and ITS or *tef1* sequences. The sequences were deposited in GenBank (ON931231-ON931232 és ON937623-ON937629).

The software and statistical methods used in the analysis

The groups formed in the analyzes were compared with the non-parametric Mann–Whitney U-test, as our data typically did not meet the conditions of parametric tests: normal distribution and homogeneity of variances. Normality was tested with Q-Q diagrams, while homogeneity of variance was tested with Levene's test. In the analyses, a significance level of 5% was determined. The StatSoft Statistica 7 program package and the MS Excel 2016 program were used for the analysis and graphical display. The binomial test was performed with the Stat Trek online calculator (Berman, 2023). The Sankey chart was created using the Sankeymatic online chart maker.

3. RESULTS

3.1. Disease incidence in Hungarian vineyards

The average GTD symptom expression showed around 16 plant loss of % and around 11% annual leaf symptoms in the investigated plantations, based on the survey data in 2022.

Table 10: Symptom incidence per wine region and per vineyard

Wine Region	Plant loss before 2022 (%)	Annual symptom expression in 2022 (%)	Healthy plants (%)	Site	Plant loss before 2022 (%)	Annual symptom expression in 2022 (%)	Healthy plants (%)
Tokaji	0.06	5.09	89.37	Szarvas	5.99	5.09	89.37
Szekszárdi	9.55	2.83	88.34	Lajvér	9.55	2.83	88.34
Villányi	8.38	4.89	87.27	Zuhánya	5.26	5.96	89.12
				Göntér	11.21	2.54	86.53
				Bocor	6.46	8.29	86.52
Badacsonyi	15.66	11.61	72.91	Badacsonytomaj	15.66	11.61	72.91
Kunsági	19.56	8.68	73.20	Kecskemét	19.56	8.68	73.20
Pécsi	20.58	6.91	72.51	Pécs	20.58	6.91	72.51
Egri	24.01	9.23	67.36	Posta út	8.00	6.67	86.00
				Pajados	16.56	4.12	80.11
				Zsidó-szél	16.75	7.00	76.75
				Nagy-völgy	19.81	6.75	73.50
				Kőkötő	18.83	14.77	66.88
				Almagyar	31.25	8.75	60.50
				Szarvas	35.89	9.64	55.54
				Nagy-Eged	25.88	21.25	52.88
Pallag	22.67	15.81	62.09	Pallag	22.67	15.81	62.09
Total	16.19	10.81	73.56				

The cumulative symptom occurrence rate of 27% can be considered severe even compared to the previously reported disease incidence from different countries. Therefore it is recommended to accurately determine the national damage with a random selection of plantations for representative survey, similarly to the French or Californian model (Siebert, 2001, Hofstetter et al., 2012, Lorch, 2014). Dula (2011) reported a continuous increase in the GTDs symptoms appearance in the decade. The methodology of the survey is not clear, however the 2022 result is more than double of the 12.29% DI in Dula’s research, so we can assume a rapid increase of the symptoms in Hungary. The annual symptom expression of some plantations is higher than the percentage of the detected plant loss during whole lifespan of the plantation. These data anticipates the futher increase of the plant loss. The highest values of plant loss and annual symptom appearance were detected in the plantations where dead plants were not removed underlying the importance of plant hygiene operations during the annual management practices (OIV, 2006, De la Fuente et al., 2016 , Gramaje et al., 2018). The Hungarian grape germplasm collections show a particularly severe picture, with over 10 % of annual symptom expression. However, the plant loss is even higher (almost double - 19.6%), the mainly caused by fungal destruction occurring after planting or in established plantations. In the germplasm collections, the propagating material has a diverse origin, so the chance of introducing pathogens is higher, and it is difficult to solve the optimal cultivation for the different varieties, so the difference in plant condition can also be significant.

3.2. Effects of environmental factors on symptom expression

3.2.1. The effect of adjacent diseased plants on symptom expression

Neighbors of plants with high frequency of GTDs symptoms were not more likely to be symptomatic (Figure 1). Based on our results, the spatial proximity of infected plants or direct transmission with cultivation tools probably does not play as a big role in the spread as the movement of the inoculum with water or erosion.

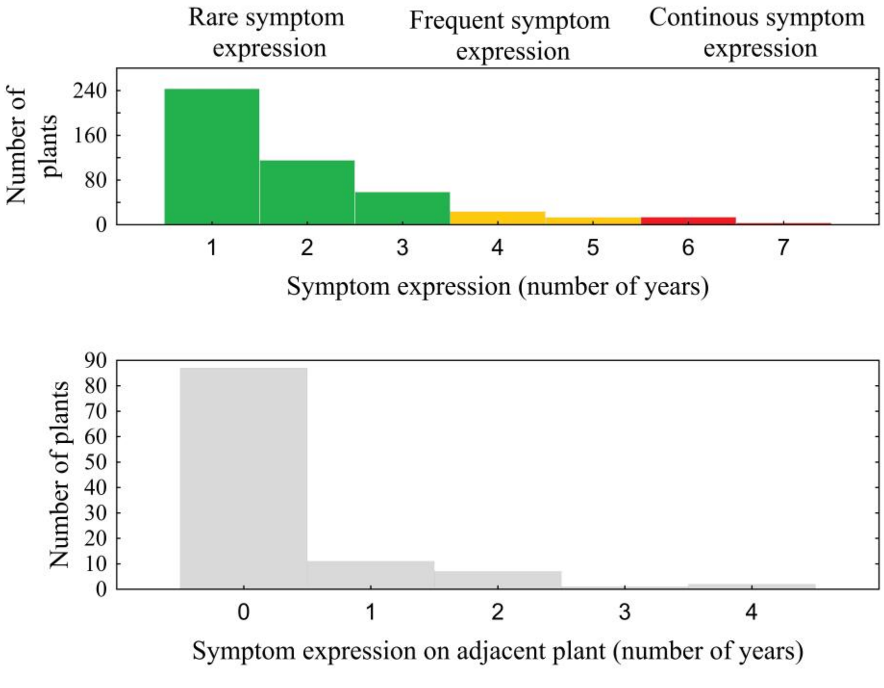

Figure 1: Distribution of the plants showing symptoms and the neighbors of plants with frequent and continuous symptoms according to the number of years they expressed symptoms.

Previous research also highlighted the higher role of water in the spread of GTDs or in the symptom expression, than the spatial proximity (Marchi et al., 2006, Calzarano et al., 2018, Bortolami et al., 2021) . This is supported by the random distribution of atic plants in the surveys of Redondo et al. (2001) and Sofia et al. (2006). Li et al. (2017) examined the spatial relationships of symptomatic plants and weak effect was found to the symptom expression of the neighboring ones.

3.2.2. The effect of the vertical position on a slope

Higher annual and total mortality rate was detected in the bottom parts of the slope, than in the higher areas, with 9-11% and 12.5-29% incease. The symptoms were especially frequent where alluvium and plant remains accumulated on the plateaus at the bottom of the slope. Kovács et al. (2017) showed a higher incidence of symptoms in terraced areas, which may be related to the effect of plateaus.

3.2.3. The effect of forest proximity

Beyond the significant slopes, the proximity of forests (often above the vineyard) is also a typical characteristic of the grapevine plantations. The symptom appearance rate was 16.61% without neighboring a forest; 23.40 % next to a full hedge; 43.31% in the immediate vicinity of a forest, and 42.41% in an area surrounded by a forest. Stronger afforestation is therefore associated with an increase in the rate of plant loss. The movement of water and water erosion can not only transport the inoculum in the plantation, but commuter or pathogen fungi in forest woody plants and decomposers of dead wood (which can also act as pathogens of grapes) can also enter with it (Mugnai et al. , 1999). BDA disease complex isthe dominant in Hungary, and their pathogens from the *Botryosphaeriaceae* family are also considered to be significant pests of ornamental and forest trees (Zlatkovíc et al., 2016). Scaling the neighbouring afforestation, we showed that a stronger forest effect, accompanied by a higher rate of plant loss the symptom appearance.

3.2.4. The effect of waterlogging on DI

In Hungary, grape cultivation is also carried out in lowland areas, and waterlogging may appear here. Within the plantation, we observed a higher symptom occurrence of 35.40% in the waterlogged spots than in the parts of the plantation not prone to waterlogging (21.62%). The water level that exceeds the soil surface can bring the inoculum to the root neck parts, which are often damaged as a result of mechanical works in the plantation or frost damage, and the abundant water supply starts the dispersal of pathogens, so the conditions for infection are met (Marchi et al., 2006, Van Niekerk et al., 2011, Billones-Baaijens et al., 2023). The condition of the grapevines on waterlogged spots is also weaker, further predisposing them to GTD (Goldammer, 2018, Ruperti et al., 2019). In their work, Halleen et al. (2007) also recommended avoiding clay soils with poor drainage to prevent GTD.

3.3. Cultivar susceptibility against GTD

Although no grape species or cultivar is known to be completely resistant to GTD, determining their sensitivity order has a primary importance in order to discover the properties related to resistance, the genes coding for possible tolerance, or for the sustainable utilization of growing areas severely affected by GTD (Sosnowski et al., 2016). The simplest measure of sensitivity is the disease incidence (DI%), on the basis of which the varieties are also ranked in the literature. Sauvignon Blanc showed the highest DI with 61.25%, while Syrah showed the lowest with 3.33% and the fully asymptomatic Merlot (Figure 2). Among the signature cultivars of Hungary Furmint showed slightly higher DI (40,00%), than Juhfark (19,44%).

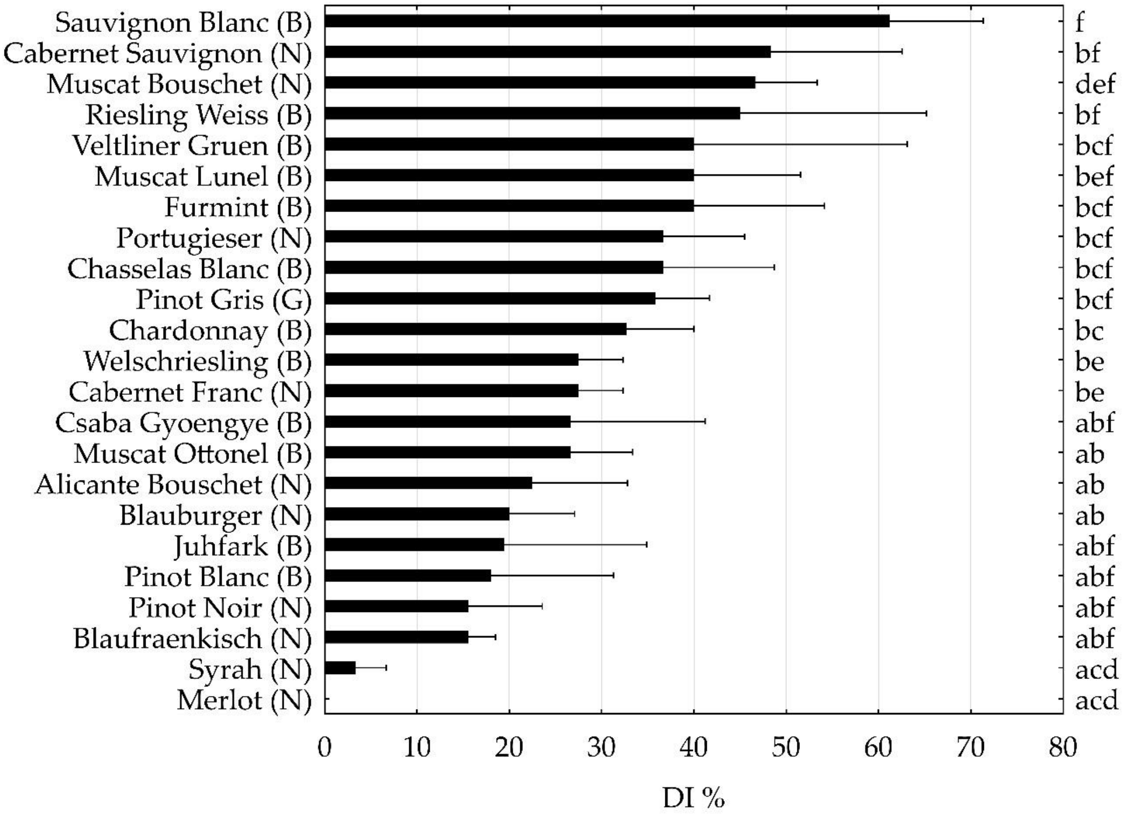

Figure 2: Disease incidence (DI) of grapevine trunk diseases of the most important international and national grape cultivars, surveyed in three or four Hungarian germplasm collections. The capital letters between brackets indicate the berry skin color: (N): noir, (B): blanc, and (G): gris, as defined in the VIVC database (Maul – Töpfer, 2003). Small letters show significant differences based on the Mann–Whitney U-test ($p < 0.05$). (Csótó et al., 2023a).

One of the most significant hybrids in the world, mainly for table grape production, the Hungarian bred Csaba Gyoengye has medium DI (26.66%) (Hajdu, 2013). In the literature regarless of berry skin color Cabernet Sauvignon (Quaglia et al., 2009, Borgo et al., 2016, Sosnowski et al., 2016, Cardot et al., 2019, Foglia et al., 2022) and Sauvignon Blanc (Billones-

Baaijens et al., 2014, Sosnowski et al., 2016) are among the most sensitive cultivars. Riesling Weiss (Travadon et al., 2013, Billones-Baaijens et al., 2014, Sosnowski et al., 2016) is also very sensitive, although not to the same extent to all disease complexes.

In our case, even the Muscat Bouschet was among the varieties with the highest DI. Merlot has a good resistance against all major GTD complexes (Quaglia et al., 2009, Travadon et al., 2013, Murolo - Romanazzi, 2014, Cardot et al., 2019), and Syrah has also good resistace against esca (Borgo et al., 2016). In our survey, they also showed the lowest DI.

Our results are therefore in line with previous research, Regarding the most commonly cultivars worldwide. One of the possible reasons for the difference in sensitivity is the higher lignin content of Merlot (compared to the sensitive Cabernet Sauvignon). The production of this substance is mainly caused by biotic stress in the variety (Rolshausen et al., 2007, Smith et al., 2019).

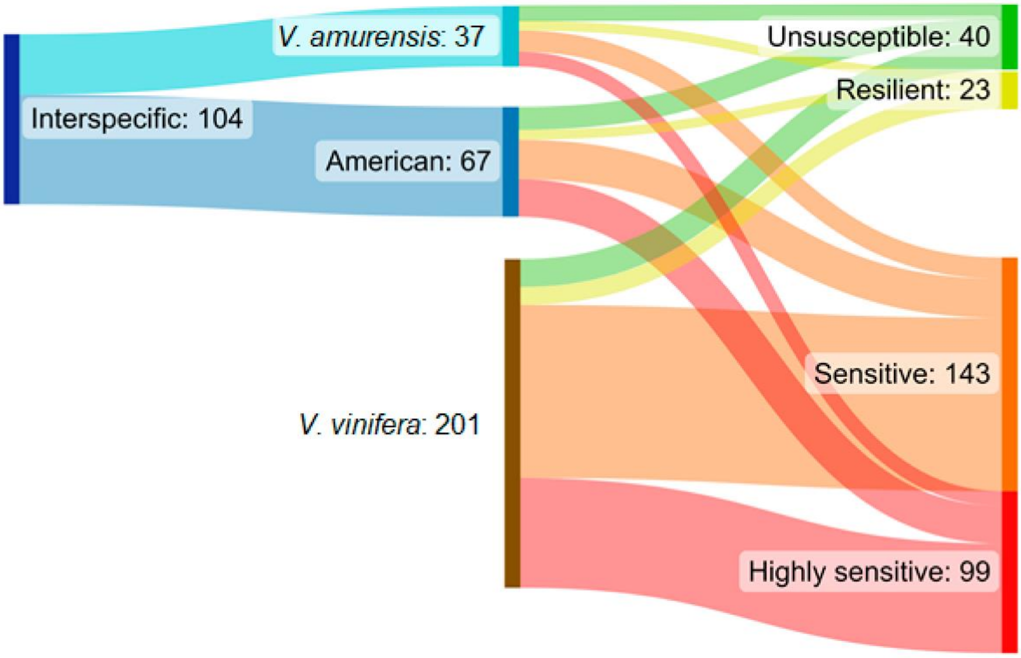

Figure 3: Distribution of the studied cultivars regarding their *Vitis* pure or mixed ancestry and the GTD pathogens sensitivity groups. Diagram created by SankeyMATIC (Csótó et al., 2023a).

We established new sensitivity categories, based on the cultivars reactions to GTD, the appearance of chronic symptoms, or the tendency to die. In this categorization, the varieties and hybrids bred from monophyletic *V. vinifera*, Asian (*V. amurensis*) and various American species are remarkably unevenly distributed (3. ábra). By treating the unsusceptible-resilient and sensitive-highly sensitive groups together, the differences in distribution in the two new groups thus formed can be verified with a binomial test. The varieties of monophyletic *V. vinfera* origin are more sensitive, than the interspecific ones, regardless of Asian or American species origin, compared to the expected distribution. The higher sensitivity of the monophyletic *V. vinifera* than the interspecific hybrids and hybrids of Asian origin was demonstrated by the higher mortality expressed as a proportion of the disease incidence. Treating the group of hybrids of American species origin separately, we could not reveal a statistically verifiable difference, because it is of very inhomogeneous species origin: *V. riparia*, *V. labrusca*, *V. berlandieri*, etc., so further investigations can be recommended by dividing the group by species origin (Maul – Töpfer, 2003). In general, the better disease resistance of interspecific hybrids is due to the better genetic diversity. The resistance of *V. amurensis* can be derived from several beneficial properties. The basic reason for its breeding is its good cold tolerance (Zhang et al., 2012, Wang et al., 2021) and its resistance to several pathogens (Kozma – Dula, 2003, Liu et al., 2013, Venuti et al., 2013, Foria et al., 2022). Resistance to other diseases ensures better condition, and cold tolerance is associated with a lower chance of developing frost damage, as well as a more vigorous start of vegetation, so the chance of infection is also lower. Infected individuals of *V. amurensis* origin were not damaged to the same extent as the monophyletic *V. vinifera* cultivars, this may be due to the very small xylem diameter (Guo et al., 1987, Jacobsen et al., 2015). In the narrower xylem, the fluid flow is weaker, thus the translocation of toxins (Foglia et al., 2022). Pouzulet et al. (2014) proved that varieties with wider xylem have stronger compartmentalization in response to biotic stress (however, it is easier for pathogens to avoid this). In the process, the plant closes the transport wessels with tyloses and gum-like substances to prevent the spread of microorganisms, but in the case of GTD pathogens, this backfires, because with the help of their enzyme systems, these pathogens utilize their substances as substrates (Pouzoulet et al., 2014).

3.4. Laboratory studies of endophytic *Trichoderma* species isolated from grapes

3.4.1. Moleclular identification on species level

The ten *Trichoderma* strains isolated by Kovács et al. (2017) were identified at the species level based on the methodology and references of Carbone - Kohn, 1999, Druzhinina et al. 2008, Bissett et al. 2015, Chaverri et al. 2015, and Cai et al. 2021 (Figure 4). The species affiliation and accession numbers of the strains are summarized in Table 11.

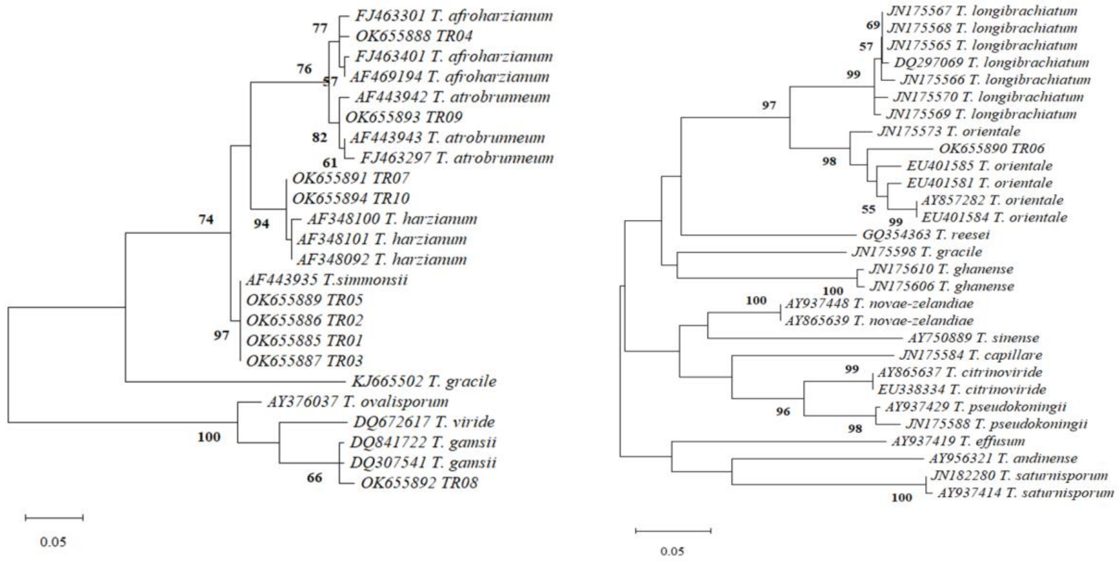

Figure 4: Maximum Likelihood phylogenetic tree generated from *tef1* of *Trichoderma* isolates TR1-TR10 and deponated sequences with accession number before species name. The length of branches is proportional to the number of nucleotide differences in the sequences, the scale is under the dendrogram. The numbers above branches show the results of the bootstrap analysis values higher than 50, from 1000 replicates.

Table 11: *Trichoderma* strains from endophytic woody tissues of 'Furmint' grapevine from the Tokaj Wine Region, Hungary in 2014 (Kovács et al., 2021).

Species	**Strain number**	**NCBI GenBank Accession Number**	
		ITS[1]	***tef1***[2]
Harzianum clade			
T. afroharzianum	TR04	OK560827	OK655888
T. atrobrunneum	TR09	OK560832	OK655893
T. harzianum	TR07	OK560830	OK655891
	TR10	OK560833	OK655894
T. simmonsii	TR01	OK560824	OK655885
	TR02	OK560825	OK655886
	TR03	OK560826	OK655887
	TR05	OK560828	OK655889
Longibrachiatum clade			
T. orientale	TR06	OK560829	OK655890
Viride clade			
T. gamsii	TR08	OK560831	OK655892

[1]ITS: Internal Transcribed Spacer. [2]*tef1*: Translation elongation factor 1-α.

3.4.2. Mycelial growth at different temperatures

We investigated the mycelial growth of the species at different temperatures. Most species showed the fastest growth at 30 °C, however, the growth curve of T. orientale also showed an upward trend at 37 °C, as a result we excluded it from further studies as a potential human pathogen. A *T. simmonsi*, *T. afroharzianum* és a *T. atrobrunneum* also grew well at 5 °C.

Similar cold tolerance was reported by Longa et al. (2008) in connection with the strain *T. atroviridae* SC1.

3.4.3. Fungicide tolerance

The fungicide tolerance tests was on strain TR04 (*T. afroharzianum*) and TR05 (*T. simmonsii*) (12. táblázat). TR04 was the most resistant, here three systemic fungicides did not inhibit growth at all.

Table 12: Testing the fungicide tolerance of *Trichoderma* strains to preparations used in viticulture (2019) (Kovács et al., 2021)

Product name	**active ingredient**	**target pathogen**	**mycelial growth inhibition (%)**	
			TR04	**TR05**
Orvego	dimetomorph, ametoctradin	downy mildew	0.00	0.00
Rally Q SC	miclobutanil, quinoxifen	powdery mildew	28.82	41.18
Sercadis	fuxapiroxad	powdery mildew	0.00	0.00
Talentum 20 EW	miclobutanil	powdery mildew	57.6	58.43
Chorus 50 WG	ciprodinil	grey mould	43.33	51.96
Teldor 500 SC	fenhexamid	grey mould	0.00	7.25

In the case of TR05, the active ingredient fenhexamide inhibited growth to a negligible extent. TR04 and TR05 and their combination are technologically compatible with the active substances fenhexamid, fluxapiroxad, dimethomorph and ametoctradin in the application concentrations found in the products we examined. The latter two are mainly used against downy mildew in grapes, however, fehexamid and fluxapiroxad are effective against Ascomycota fungi, i.e. the resistance of the tested *Trichoderma* strains against them is also outstanding within the phylum. Previously, several studies showed dfferent degrees of fungicide tolerance among *Trichoderma* strains (McLean et al., 2001, Khirallah et al., 2016, Silva et al., 2018, Maurya et al., 2020). The mycelial growth of both strains was significantly inhibited by fungicids containing cyprodinil, myclobutanil and myclobutanil + quinoxifen. The stronger inhibition of the pure myclobutanil active ingredient can be explained by its higher myclobutanil concentration.

3.4.4. Biocontrol efficacy

The biocontrol efficiency of strains TR04 and TR05 was studied in *in vitro* confrontation tests. All but one of the examined GTD pathogens showed 100% effectiveness against the Ascomycota fungi, BCI was higher than 90% against *Neofusicoccum parvum* characterized by rapid growth. *Pythium acantophoron*, as a reference Oomycota species, was also completely inhibited by the two strains.

3.5. Investigations of endophytic *Trichoderma* species isolated from grapes in field

3.5.1. The effect of *Trichoderma* treatment on grape budburst

Regarding the biostimulator effect of the tested *Trichodema* strains, we achieved a significant improvement in the budburst ratio and budburst vigor index (Figure 5). Andreini et al. (2009) observed delayed, slow bud break and initial development due to esca infection. The suppression of pathogens may also play a role in that effect, in addition to the biostimulatory metabolites of the *Trichoderma* strains.

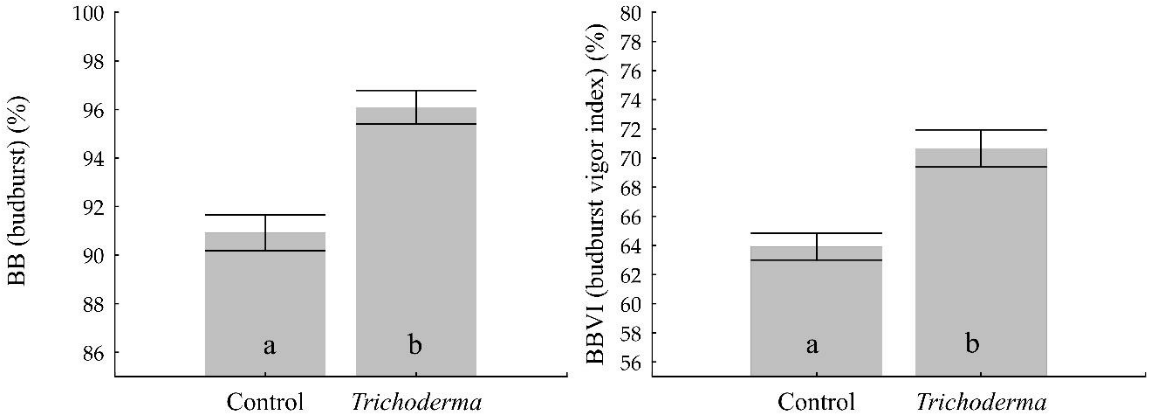

Figure 5: The effect of *Trichoderma* treatment on the budburst ratio (a) and the budburst vigor index (b) (Csótó et al., 2023b). Lowercase letters indicate statistical differences based on the results of Mann-Whitney U tests.

3.5.2. Reisolation of the applied strains

The *Trichoderma* strains used in soaking treatment were successfully re-isolated from different parts of the plant 5 and 15 months and 4 years after application. In the treatment of grape with *Trichoderma*, colonization around the treated wound has been described so far (Pollard-Flamand et al., 2022, Carro-Huerga et al.,2020). *T. harzianum*, *T. atroviride*, *Trichoderma virens* (Jaarsveld et al., 2020) and *T. asperellum* ICC 012, T. gamsii ICC 080 (Di Marco et al., 2004) were isolated seven months after inoculation. After successful wound inoculation with *T. atroviridae* strain AG1, John et al. (2008) reported colonization of the entire plant after 20 months. The *T. orientale, T. gamsii* and *T. afroharzianum* investigated in our experiment are therefore able to colonize the vines even in the longer term than those described so far. Compared to chemical treatment, in addition to internal protection, the possibility of treatment extending beyond the vintage is an advantage.

4. NEW SCIENTIFIC RESULTS OF THE DISSERTATION

1. The vineyards surveyed in seven wine regions of Hungary in 2022 showed an average of 27% GTD incidence. The highest disease incidence, 38.48%, was measured in the Nagy-Eged vineyard in the Eger Wine Region.
2. We have shown that the spatial proximity of plants showing symptoms has no effect on the appearance of symptoms. In the long-term experiment, the neighbors of the plants with frequent symptom expression were healthy on average in 87.81%, which is a better ratio than the 76.08% symptom appearance average of the plantation.
3. The rate of plant loss is higher in the lower parts of the plantations. The annual plant loss is 9-11% higher, the total plant loss measured in the plantation is 12.5-29% higher.
4. The GTD incidence increases with the degree of forest cover in the areas surrounding the plantation. Without a forest neighborhood, the symptom appearance rate is 16.61%; next to a full hedge 23.40 %; 43.31% and 42.41% in the categories with significant forest cover.
5. The rate of symptom occurrence is increased by waterlogging of the area, we measured 35.40% DI in waterlogged wineyard parts and 21.62% in non-waterlogged wineyard parts.
6. We found that the order of sensitivity of the cultivars established in the international literature is also correct under the conditions of our country. In our survey, Sauvignon Blanc showed the highest disease incidence at 61.25%, while Syrah with 3.33% disease incidence and symptom-free Merlot showed the lowest. Among the Hungarian signature cultivars, Juhfark shows 19.44% and Furmint 40.00% disease incidence.
7. Monophyletic *V. vinifera* varieties are more sensitive to GTD than interspecific hybrids. The mortality of diseased plants is 76.39% for monophyletic species, 64.30% for interspecific ones. Varieties of *V. amurensis* origin are highly resistant with a mortality rate of 52.18%.
8. By identifying the previously isolated *Trichoderma* strains based on the *tef1* marker sequence, one *T. afroharzianum*, one *T. atrobrunneum*, one *T. gamsii*, two *T. harzianum* and four *T. simmonsii* species were differenciated.
9. They are completely resistant to the doses of ametoctradin, dimethomorph and fluxapiroxad specified in the label, while fenhexamide only causes a negligible 7.25% inhibition only in the case of TR05.
10. Biocontrol index of TR04 and TR05 strains are 100 % against *Pythium acantophoron*, *Diaporthe eres* and *Eutypa lata*.
11. Pre-plant soaking *Trichoderma* treatment improved rate and vigor of budburst by more than 5%.

12. The *Trichoderma* strains used persisted for 5 months to 4 years in different parts of the grape plant.

5. PRACTICAL USE OF THE RESULTS

1. When planting grapes, environmental factors promoting the appearance of GTD symptoms, the location at the bottom of a slope, tendency to waterlogging, and the proximity of forest areas must be taken into account.
2. The planting of more resistant varieties, such as Merlot or Shiraz, as well as species hybrids primarily of *V. amurensis* origin, is recommended for areas with presumably greater GTD symptom expression.
3. Among the Hungarian cultivars, Juhfark is moderately resistant, Furmint is moderately sensitive, and Csaba Gyöngye (Csaba Gyoengye) has medium tendency to espress the symptoms.
4. Orvego, Sercadis, and Teldor 500 SC do not inhibit the mycelial growth of strains TR04 and TR05, so it is possible to apply them with a short period, even in a tank mixture. Rally Q SC, Talentum 20 EW and Chorus 50 WG significantly inhibit the growth of strains, their joint application is not recommended.
5. TR04 and TR05 also have a 100% biocontrol index against non-grape pathogenic Oomycota and Ascomycota fungi, so they can also be used in a wider range of cultivated plants.
6. Bud fertility and early development of grapes can be improved with endophytic *Trichoderma* species.
7. The persistence and biostimulant effect of the endophytic *Trichoderma* strains can be detected even 4 years after application.

6. LITERATURE

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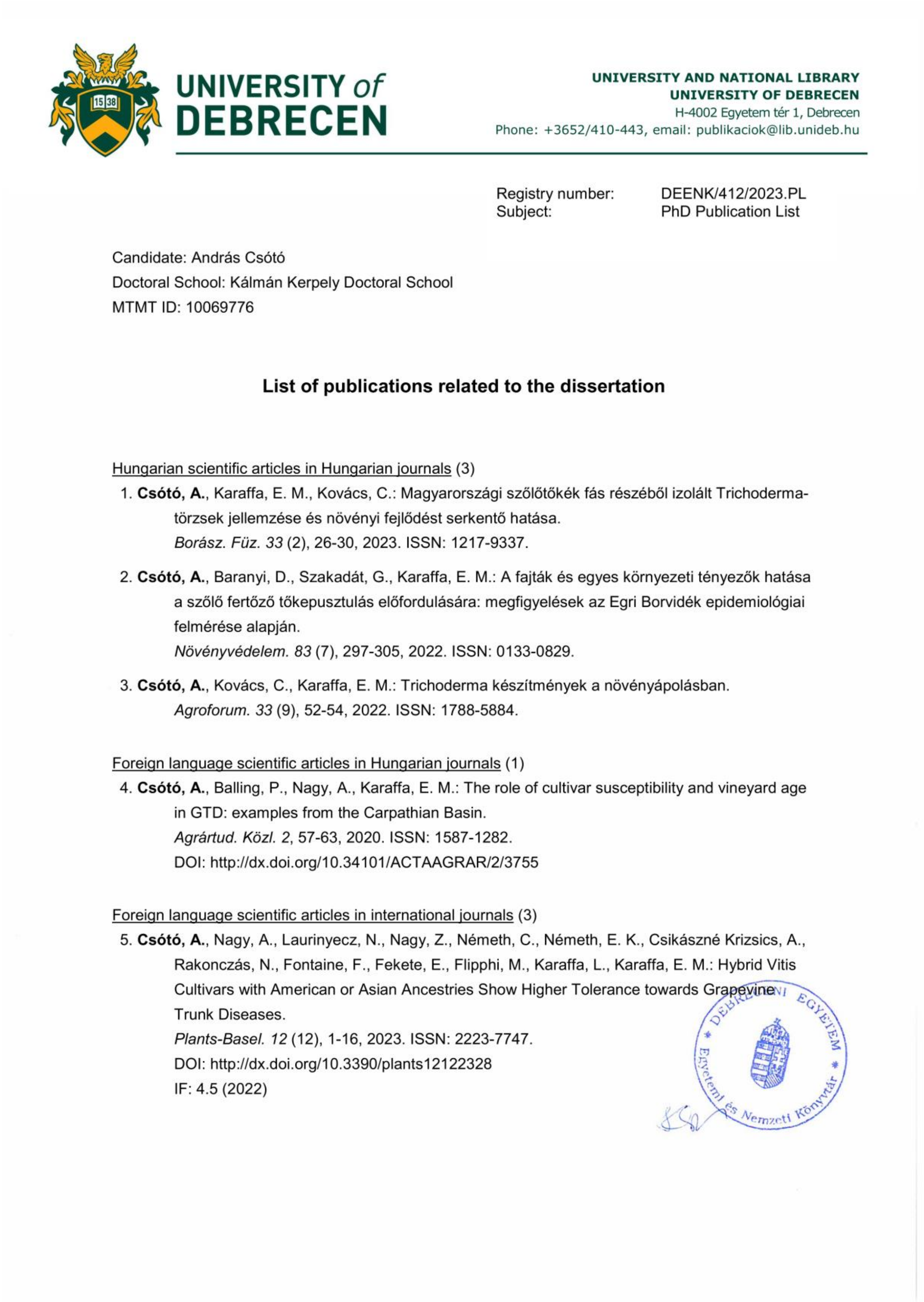
7. LIST OF PUBLICATIONS RELATED TO THE DISSERTATION



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Registry number: DEENK/412/2023.PL
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Candidate: András Csótó
Doctoral School: Kálmán Kerpely Doctoral School
MTMT ID: 10069776

List of publications related to the dissertation

Hungarian scientific articles in Hungarian journals (3)

1. **Csótó, A.**, Karaffa, E. M., Kovács, C.: Magyarországi szőlőtőkék fás részéből izolált Trichoderma-törzsek jellemzése és növényi fejlődést serkentő hatása.
   Borász. Füz. 33 (2), 26-30, 2023. ISSN: 1217-9337.
2. **Csótó, A.**, Baranyi, D., Szakadát, G., Karaffa, E. M.: A fajták és egyes környezeti tényezők hatása a szőlő fertőző tőkepusztulás előfordulására: megfigyelések az Egri Borvidék epidemiológiai felmérése alapján.
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4. **Csótó, A.**, Balling, P., Nagy, A., Karaffa, E. M.: The role of cultivar susceptibility and vineyard age in GTD: examples from the Carpathian Basin.
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5. **Csótó, A.**, Nagy, A., Laurinyecz, N., Nagy, Z., Németh, C., Németh, E. K., Csikászné Krizsics, A., Rakonczás, N., Fontaine, F., Fekete, E., Flipphi, M., Karaffa, L., Karaffa, E. M.: Hybrid Vitis Cultivars with American or Asian Ancestries Show Higher Tolerance towards Grapevine Trunk Diseases.
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6. **Csótó, A.**, Kovács, C., Pál, K., Nagy, A., Peles, F., Fekete, E., Karaffa, L., Kubicek, C. P., Karaffa, E. M.: The Biocontrol Potential of Endophytic Trichoderma Fungi Isolated from Hungarian Grapevines, Part II, Grapevine Stimulation.
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7. Kovács, C., **Csótó, A.**, Pál, K., Nagy, A., Fekete, E., Karaffa, L., Kubicek, C. P., Karaffa, E. M.: The Biocontrol Potential of Endophytic Trichoderma Fungi Isolated from Hungarian Grapevines. Part I. Isolation, Identification and In Vitro Studies.
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11. **Csótó, A.**, Hegymegi, F., Pál, K., Kovács, C., Szakadát, G., Karaffa, E. M.: Biológiai ágensek a szőlő fás betegségei elleni védekezésben.
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20. Csüllög, K., Tóth, G., Vartek, C., Piti, A. N., Nagy, A., **Csótó, A.**, Riczu, P., Biró, G., Tarcali, G.: Spatial distribution of Macrophomina phaseolina (Tassi) Goid. in the Carpathian Basin and its damage on sunflowers.
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Növényvédelem. 84 (5), 193-200, 2023. ISSN: 0133-0829.

22. Kövics, G., Tarcali, G., Csüllög, K., Rácz, D., Biró, G., **Csótó, A.**, Szarukán, I., Nagy, A., Szanyi, S., Szilágyi, A., Kovács, G. E., Radócz, L.: A szója integrált védelme.
Növényvédelem. 81 (6), 251-275, 2020. ISSN: 0133-0829.

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23. Csüllög, K., Biró, G., Gonsalves, J. D., Sanga, S. M., Tuly, N. M., Abushawish, A. K., Tóth, G., Vartek, C., Ernhardt, N., Tarcali, G., **Csótó, A.**: Examination of the efficacy of different fungicides against Macrophomina phaseolinaand Sclerotinia sclerotiorum in laboratory conditions.
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25. Kovács, C., **Csótó, A.**, Karaffa, E. M.: Hazai kutatócsoport egy euphresco basics projektben.
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    In: LVIII. Georgikon Napok, [Pannon Egyetem, Georgikon Kar], [Keszthely], 184-191, 2016.

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27. Csüllög, K., Seres, E., Tarcali, G., Tóth, G., **Csótó, A.**: A proklóráz hatóanyag hatékonysága in vivo körülmények között a Macrophomina phaseolina növénykórokozó gombára napraforgó állományban.
    In: 27. Tiszántúli Növényvédelmi Fórum : Program és Összefoglalók. Szerk.: Kövics György, Tarcali Gábor, Debreceni Egyetem Mezőgazdaság-, Élelmiszertudományi és Környezetgazdálkodási Kar, Debrecen, 45-46, 2022.

28. Kecskés, I., **Csótó, A.**: Kukorica szártő megbetegedés mértékének meghatározása különböző módszerekkel, eltérő talajművelési rendszerek esetében.
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29. **Csótó, A.**, File, M., Piti, A. N., Ellmann, B., Pál, K., Szakadát, G., Karaffa, E. M.: Szőlőből izolált potenciális antagonista és biostimulátor hatású mikrogombák minősítési vizsgálatai.
    In: 68. Növényvédelmi Tudományos Napok 2022. Szerk.: Haltrich Attila, Varga Ákos, Magyar Növényvédelmi Társaság, Budapest, 37, 2022.

30. Szőke, L., **Csótó, A.**, Makleit, P.: Hagyományos és biológiai növényvédő szerek hatékonyságának vizsgálata, valamint a ciklikus hidroxámsavak mennyiségének meghatározása különböző őszi búza fajtákban.
    In: 24. Tiszántúli Növényvédelmi Fórum. Program és Összefoglaló, Debreceni Egyetem Mezőgazdaság, Élelmiszertudományi és Környezetgazdálkodási Kar, Debrecen, 52-53, 2019.

31. Kovács, C., **Csótó, A.**, Rakonczás, N., Karaffa, E. M.: A szőlő tőkebeteségeinek vizsgálata a Debreceni Egyetem Pallagi Kertészeti Kísérleti Telepének fajtagyűjteményében.
    In: 62. Növényvédelmi Tudományos Napok. Szerk.: Horváth József, Haltrich Attila, Molnár János, Magyar Növényvédelmi Társaság, Budapest, 87, 2016

32. Kovács, C., **Csótó, A.**, Rakonczás, N., Karaffa, E. M.: Mikroklimatikus viszonyok szerepe a szőlő tőkebetegségeinek tünet megjelenésére.
    In: LVIII. Georgikon Napok : Kivonat-kötet : Programfüzet, valamint az elhangzó és poszter előadások rövid kivonatainak gyűjteménye, Pannon Egyetem, Georgikon Kar, Keszthely, 90, 2016. ISBN: 9789639639843

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33. Kovács, C., **Csótó, A.**, Pál, K., Nagy, A., Fekete, E., Karaffa, L., Kubicek, C. P., Karaffa, E. M.: Endophytic Trichoderma spp. from Hungarian grapevines with biocontrol potential. In: 16th Congress of the Mediterranean Phytopathological Union : Book of abstracts, [s.n.], [s.l.], 1, 2022.

34. Cheradil, A. E. B. D., Bákonyi, N., **Csótó, A.**: Microalgae: a biological tool for plant protection. In: 27. Tiszántúli Növényvédelmi Fórum : Program és Összefoglalók. Szerk.:: Kövics György, Tarcali Gábor, Debreceni Egyetem Mezőgazdaság-, Élelmiszertudományi és Környezetgazdálkodási Kar, Debrecen, 46, 2022.

35. **Csótó, A.**, Kovács, C., Karaffa, E. M., Rakonczás, N.: Survey and examination of GTD-s and isolation of pathogens in the grapevine variety collection of the University of Debrecen. In: Meeting of Young Researchers from V4 Countries Abstract book. Ed.: Monika Wesołowska, University of Rzeszow, Rzeszow, 20, 2016.

Total IF of journals (all publications): 15,831
Total IF of journals (publications related to the dissertation): 12,731

The Candidate's publication data submitted to the iDEa Tudóstér have been validated by DEENK on the basis of the Journal Citation Report (Impact Factor) database.

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