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Distribution of Esca Disease in Hungary and the Pathogens Causing the Syndrome

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Surveys were carried out in the 11 major wine regions of Hungary from 2003 to 2005 to identify pathogenic grapevine wood fungi. Occurrence of the disease in vineyards younger than 12 years, was studied separately. Sixty-six percent of the vineyards were free, thus symptoms of early decline were present in 34% of the plantations with 0,3–2,6% incidences. All vineyards over 12 years were affected by esca and early decline pathogens. Several fungi were consistently associated with these symptoms: The most frequently isolated species from older stocks were *Fomitiporia* sp., *Phaeomoniella chlamydospora, Phomopsis viticola, Botryosphaeria* sp. and *Eutypa lata*. On younger, 2–4-year-old plants, mostly *Phaeomoniella chlamydospora, Phaeoacremonium* sp., *Cylindrocarpon* sp. and *Fomitiporia* sp.occurred. Several associated species were identified on the declining stocks: *Fusarium* spp., *Penicillium* spp., *Alternaria alternata, Aspergillus* spp., *Trichoderma* spp., *Verticillium* spp., *Pestalozzia pezizoides* and *Monochaetia viticola.*

Keywords: *Vitis vinifera,* grapevine, esca, early decline

Similarly to other vine-growing countries of the world, early decline of grapes is a severe problem also in Hungary.

Ubrizsy (1965) mentions for the first time that the fungi *Stereum hirsutum* and *Phellinus igniarius* have a role in provoking *grapevine apoplexy* = esca. From the 1970s Lehoczky conducted detailed studies on the etiology of early declines (1968, 1972a, 1972b, 1974, 1984, Lehoczky and Moller, 1979). He concluded from his research results that, in 75–80% if the cases, the decline of more than two years old stocks is caused by infection. Several pathogens may provoke alterations in the plants that lead to early decline. Chronic abnormal metabolic processes (e.g. decline of pathogenic origin, tomato black ring virus and chrome mosaic virus), vascular system unable to function in the stem (stem pitting virus), disturbance in the cambial zone, irregular vascular tissues in the stem *(Agrobacterium vitis),* abnormal dying of vascular tissues in the arm and stem (e.g. *Phomopsis viticola, Botryosphaeria* spp*, Eutypa lata*), abnormal dying of root tissues (*Armillaria mellea, Rosellinia necatrix, Phytophthora* spp.) can provoke early decline before the stocks reach thirty years. He wrote in connection with the then diagnosed decline caused by *Stereum* that it may be taken for certain that the disease had already occurred in the vine-growing areas of Hungary a long time before. However, we have data on the 'apoplexy' of grapes only from the second part of the '70s. (Lehoczky and Makó, 1983). It was observed sporadically in the 12–19 years old high-cordon vineyards between 1976 and 1982 in the wine-region of Tokaj on cv. Furmint, in South-Balaton (to the south of the Lake Balaton) on cv. Leányka, Muscat Lunell, Chasselas and Italian Riesling, in the Great Hungarian Plain and Badacsony (on the northern hill of the Lake Balaton) on cv. Italian Riesling. In 1982, in South Balaton, a sudden and unusually high (11.6%) incidence of symptoms was observed in an 18-year-old, 'Red Traminer' vineyard with high cordon training system. Lehoczky and Makó were the first in 1983 to publish an overall description of the esca syndrome. From that time on, in Hungary all the symptoms related to esca were attributed to infection by the fungus *Stereum hirsutum*. Opinions about the role of the fungus from the point of view of forestry and viticulture are diverse even today (Véghelyi et al., 2001, 2003). Forestry pathologists consider the long-ago known wood-decaying fungus of deciduous trees (oak, beech, hornbeam, birch and alder) a saprophyte (Gyarmati et al., 1975). On the other hand, grapevine specialists regard it as a pathogen that establishes on the died wound surfaces of old grapevine stocks as a saprophyte and penetrates deeply in the dead pith, becoming pathogenic when it reaches the central annual rings and causes extensive tissue necrosis. It was observed that fruiting bodies of the fungus seldom develop on died woody parts of grapevine (Viala, 1926; Galet, 1977). Chiarappa (1959) stated that in California vascular necrosis was primarily caused not by *Stereum hirsutum,* but *Phellinus (Fomes) igniarius* and *Cephalosporium* sp. Lehoczky also indicated that the role of other organisms in the development of esca disease also needs to be studied, because e.g. *Cephalosporium* sp. could also often be isolated from the diseased vascular tissues in Hungary. The spreading of the infection did not stop in Hungary. According to the survey conducted in the nine major wine-growing areas in 1997 and 1998, early decline reached 8–21% incidence (Aponyi et al., 1999). A dramatic change occurred in 2000–2001, when the appearance of symptoms of *Eutypa* and esca was massive in various districts in the Great Hungarian Plain, South-Balaton (around Balatonboglár) and the Mátra hills (Mikulás and Lázár 2001a, 2001b, Dula 2003, Mikulás et al., 2004). The number of diseased plants is increasing year by year both in old and young, 4–6-year-old vineyards. Rábai et al. (2005) studied the problems of early grapevine decline in seven smaller districts of county Veszprém, on the northern side of Lake Balaton between 1998 and 2004. They concluded that early decline occurred as a result of various factors. In most cases, the fungal species *Phomopsis viticola, Diplodia mutila* and *Fomitiporia punctata* could be detected on plants showing esca symptoms. Molecular studies (PCR-RFLP) detected 41% phytoplasma infection. The situation has become even worse by the high incidence of early decline observed in new plantations. In Hungary the land ownership fundamentally altered after the social change. Encouraged by the subsidies provided for the establishment of vineyards and preparing for the EU accession, a „plantation fever" began in 1997 and ten thousand hectares of new vineyards were established till 2004. Vineyard reconstruction involves changes both in varieties and training systems. The demand for propagating and planting material, therefore plantation costs also increased by smaller spacing and higher plant density Since the beginning of planting, in the 1–4-year-old vineyards, the number of plants infected by *Agrobacterium,* stem pitting virus and phytoplasma has been high, and the occurrence of stocks showing symptoms of young esca and white rot is not rare either that can be clearly attributed to infected propagating material. Symptoms of Petri disease has been observed on cv. Merlot in the wine-region of Eger since 1997. This is why the need for replacing 30–50% of the plants is not rare (Dula 2003, 2004). According to the available Hungarian literature, this is not a completely new situation, because it was in 1915 when Istvánffy (1915) wrote for the first time about the different causes of grapevine decline. Twenty years after, Barra (1935), and Sántha (1935) dealt with a new pith disease and its possible causes in connection with 'court-noue' (stunted shoot) of grapevine. In 1939 Erdély studied the causes of the dramatic degree of insufficient taking and early decline, observed especially in newly established, grafted plantations in 16 wine-regions. He gave an account of his experiences in his paper titled „A new grapevine disease: black pith".

From the late '80s, the high increase in the number of plants showing esca symptoms not only in old, but also in young, 3–6-year-old vineyards has been conspicuous in several other European wine-growing countries (Italy, France, Portugal, Spain, Greece, Austria), the United States, South Africa and Australia (Mugnai et al., 1999, Surico 2000). The global problem called for and launched a wide international research programme and co-operation. In spite of the fact that esca is one of the grapevine diseases with the oldest history, the related research in etiology began only at the end of the 19th century and most of the new information was generated by studies conducted in the past 10–15 years (Chiarappa, 2000). During this time it was revealed that esca was an extremely complicated complex of grapevine diseases where, as a result of the joint effect of biotic and abiotic factors, physiological and structural changes provoked the symptoms. The pathogenic fungi associated with the syndrome, their pathogenicity and relationships were clarified. The most frequently isolated fungi were *Fomitiporia mediterranea (F. punctata)* earlier named *Phellinus punctatus* a basidiomycete fungus causing white rot and *Phaeomoniella chlamydospora (Cephalosporium), Phaeoacremonium aleophilum*. A clear relationship was found during the detailed studies of esca and Petrie disease, observed in the newly planted vineyards in South Africa and later in America in the early '90s, described under different names at the beginning (early decline, black goo). *Phaeomoniella chlamydospora* and various *Phaeacremonium* species were most frequently isolated from such plants (Ferreira et al., 1994, Morton, 1995, Crous et al., 1996). Intensive studies are conducted on the species not closely associated with esca but similarly affecting the woody parts of grapevine plants, causing partial dieback, such as e.g. *Eutypa* spp. (Safodien et al., 2005, Schwappach and Grimm, 2005) *Botryosphaeria* spp., *Phomopsis* spp. (Van Niekerk et al., 2005a, 2005b) and *Cylindrocarpon* spp., causing the „black foot" disease (Rego et al., 2001, 2005). After having been found in Australia, the sexual form of *Phaeoacremonium aleophilum, Togminia minima* producing perithecia was identified also in California (Pascoe et al., 2004, Rooney-Latham et al., 2005). In addition to the species known so far, 14 new *Phaeoacremonium* species were isolated from grapevine, e.g. *Phaeoacremonium alvesii, P. australiensis, P. krajdenii, P. venezuelense,* (Mostert et al., 2005) Epidemiological studies revealed that cold and rainy conditions highly influence the intensity of the appearance of leaf symptoms (Braccini et al., 2005). The biochemical changes following the infections were clarified, the accumulation of the so-called stress metabolites (phenols, polyphenols) was detected in the diseased plants (Amalfitano et al., 2000, 2005). Studying the options for control resulted in the consensus of stating that the diseased plants could not be cured. The effective control is the prevention of the disease, planting of healthy propagating material and the exclusion of infection via wounds within the vineyard, the application of integrated management programmes (Fourie and Halleen, 2005). Several studies confirmed that the primary source of infection was propagating material, therefore in South Africa and Australia hot water treatment (50 °C for 30–45 minutes) of rootstocks and scions was recommended as the most feasible and effective means of control (Wait and May, 2005). At the same time, it is efficient against latent virus, phytoplasma and *Agrobacterium* infections of shoots (Szegedi and Süle, 2005). Treatment of stocks showing symptoms did not provide acceptable results, irrespective of the active substance (fosetyl-Al, triazoles), mode of application (spraying, drench, and brushing, soil and stem injection) and the number of treatments (Di Marco at al., 2000, Di Marco and Osti, 2005a). It was proved that crop quality and quantity could be improved by applying certain plant conditioners and biostimulants to diseased stocks (Di Marco and Osti, 2005b).

A four-year Hungarian-Italian Intergovernmental Research Cooperation was started in 2004. Participants from Hungary are the Plant Protection and Soil Conservation County Services, Central Service for Plant Protection and Soil Conservation (CSPPSC) and ABASZÖV (Association of Hungarian Producers of Grapevine Propagating Materials), while the Italian partners are CNR University, Toscana; DIBA (Dipartimento di Biotechnologie Agrarie), Institute of Biometerology, Bologna (National Research Council /C. N. R./ The Institute of Biometerology), ARSIA-regione Toscana.

This paper deals with the Hungarian results on the survey for the spreading of esca and young vine decline, on studies to identify the pathogens causing the disease complex and to determine their dominance relations in the Hungarian wine-regions.

Materials and Methods

The surveys were carried out in the 13 major wine-regions of Hungary (Ászár-Neszmély, Csopak-Balatonfüred, Badacsony, Balatonboglár, Balatonmelléki, Eger, Kunság, Mátra, Pannonhalmi, Somló, Sopron, Tokaj and Villány), on cultivars Cabernet Sauvignon, Cabernet Franc, Chardonnay, Chasselass, Chenin Blanc, Ezerjó, Furmint, Hárslevelű, Kékfrankos, Muscat Lunel, Italian Riesling, Pinot Noir, Rhein Riesling, Pinot Gris, Zengő, Green Veltelini and Zweigelt, both in vineyards younger than and over 12 years.

Three hundred plants were inspected in each vineyard, using a common method. We looked for the different types of symptoms, counted the plants died in the particular years, and the ones missing, replaced and renewed. Samples were taken from the stocks showing ambiguous symptoms and were tested with different laboratory methods. After harvest, 10-cm parts were cut from a woody part and their surfaces were disinfected with Neomagnol (chloramine B). A little piece was taken from the internal part with the leading edge of the discoloured, affected zone and was placed in a moisture chamber and in parallel on PDA medium for incubation.

The surveys were carried out in late summer from 2003 to 2005, and the sampling data were recorded on the map.

Results

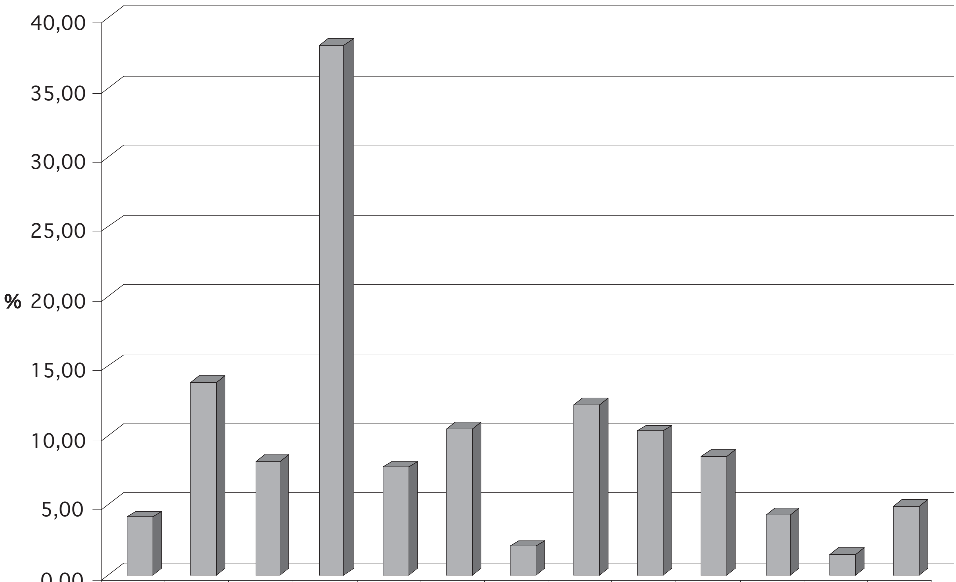
Forty-four and 18 vineyards over and below 12 years, resp. were involved in the study.

Fig. 1 shows the share of plants with esca symptoms for the particular wine regions in the average of the survey years.

The three years' data were compared to get a picture of the infections established in the surveyed wine regions.

The occurrence of the disease in vineyards younger than 12 years was studied separately. Sixty-six percent of the vineyards were free, thus symptoms of early decline were present in 34% of the plantations with 0.3% -2.6% incidences.

Each of the vineyards over 12 years were affected by early decline.



	Ászár-Neszmély	Csopak-Balatonfüred	Badacsonyi	Balatonboglári	Balatonmelléki	Egri	Kunsági	Mátrai	Pannonhalmi	Somlói	Soproni	Tokaj	Villányi
%	4,10	13,80	8,10	38,10	7,70	10,50	2,00	12,20	10,30	8,50	4,30	1,40	4,90

Fig. 1. Share of plants with esca symptoms for the particular wine regions in the average of 3 years in Hungary (2003–2005)

At the end of the study we analysed the data of the 3-year survey to reveal the causes of grapevine decline. We traced the declining process by isolating the pathogens from the dying plants.

We hereby present the causes of the decline, focusing on the wine regions of Somló and Badacsony.

In the first region, during the survey in older vineyards, 20% of the stocks with decline symptoms were sampled for laboratory testing. The fungus *Fomitiporia* sp. was isolated from 57% of the samples taken from the stems. Among fungi affecting woody parts, *Phomopsis viticola* and *Botryosphaeria* sp. were most frequently present in the arms.

In the same wine region, 1% of early decline was found in a young vineyard planted with cv. Furmint. *Fomitiporia* sp. was isolated from the stems of the 5–6 years old stocks in this plantation.

In the other wine region, at Badacsony surveys were conducted in older vineyards. Twenty-nine percent of symptomatic plants were sampled for laboratory testing. Ten percent of the samples proved positive for each of the three fungi *Phaeomoniella chlamydospora, Eutypa lata* and *Fomitiporia* sp. In the remaining samples, the presence of *Phomopsis viticola* alone or together with *Botryosphaeria* sp. was detected.

In the wine region of Eger, *Phaeomoniella chlamydospora, Phaeoacremonium* sp. and *Cylindrocarpon* sp. were isolated from the rootstocks of young Merlot plants with symptoms of Petri disease. Testing of plants showing early decline in a 3-year-old vineyard of the wine region in the northern side of Lake Balaton resulted in identifying *Phaeoacremonium* sp.

The survey revealed that, in addition to the pathogens, the role of nutrient deficiencies (e.g. of iron) and climatic factors (rainfall shortage, drought, heavy winter frosts) was also decisive in provoking the decline. During extremely rainy seasons, such as of 1999, 2001, 2004 and 2005, the appearance of esca symptoms is consistently conspicuous, and in parallel, chlorotic decline is also severe. Probable the similarly extremely warm and droughty years, such as 2000, 2002 and 2003 before the rainy ones may also play a role in the continuous worsening of plant state.

The relationship of esca and early decline with training system was also studied, as the differences in the disease incidences in the particular wine regions may be attributed to the different ages, cultivars and training systems in the studied vineyards.

About 50% of Hungarian vineyards are too old, over 25 years, much older than the age planned at their planting. The vigour of the stocks decreases, the number of died plants increases and the profitability of vine growing gets worse in direct ratio with the age of the vineyards. (See *Table 1* with the share of vineyards in the particular age categories in Hungary). The earlier trend was to apply training systems suitable for heavy individual plant load to achieve high yields (Moser, single curtain, high cordon). Continuously heavy load of the stocks and the high number of pruning wounds were characteristic of that growing practice. In the older vineyards there is a relationship between the training system and the disease incidence *(Fig. 2)*.

The training systems applied in the studied vineyards are as follows: cordon, Sylvoz, Lenz-Moser, Guyot, single curtain, umbrella and twin-vine system.

Table 1

Area and share of vineyards in the particular age categories in Hungary

Below 5 years	6–10 years	11–20 years	21–30 years	31–40 years	41–50 years	Over 50 years	Together
9057 ha	3258 ha	35 665 ha	23 426 ha	9526 ha	3767 ha	6721 ha	91 421 ha
9,9%	3,6%	39%	25,6%	10,4%	4,1%	7,4%	100%

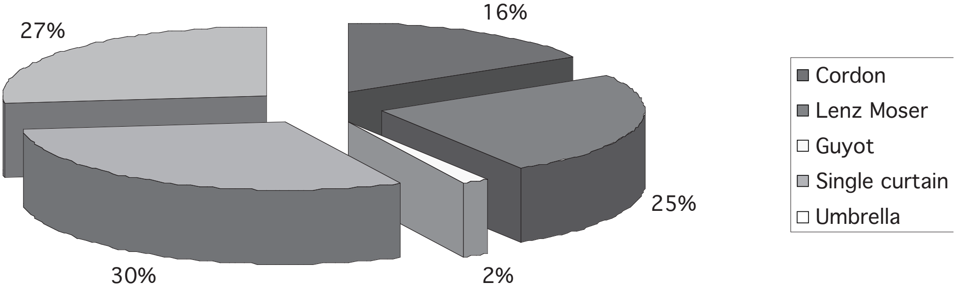


Fig. 2. Share of plants with symptoms of esca early decline under the particular training systems

Comparing the different training systems, the symptoms of esca and early decline were most frequently observed in single curtain, umbrella and Lenz Moser systems (30%; 27%, and 25% respectively), while applying the cordon system involved only 16%. Incidence of the disease was insignificant in vineyards using the again up-to-date Guyot training systems allowing less pruning wounds.

Discussion

The problem of early decline in young and bearing vineyards occurred also in Hungary due to the effects of the „replanting fever" and the intemperate rainfall and temperature conditions (drought) provoked by the climate change in the recent 15 years. Researches indicate that we face a disease of complex etiology.

Different fungal pathogens play a role in the infection. The species most frequently isolated from older stocks are *Fomitiporia* sp., *Phaeomoniella chlamydospora, Phomopsis viticola*, *Botryosphaeria* sp. and *Eutypa lata*. On younger, 2–4-year-old plants, in most of the cases *Phaeomoniella chlamydospora, Phaeoacremonium* sp., *Cylindrocarpon* sp. and *Fomitiporia* sp., were present.

Several associated fungal species were identified on the declining stocks: *Fusarium* spp., *Penicillium* spp., *Alternaria alternata, Aspergillus* spp., *Trichoderma* spp., *Verticillium* spp., *Pestalozzia pezizoides* and *Monochaetia viticola.*

The high rate of plants showing esca symptoms or white rot in non-bearing plantations and of esca, phytoplasma, virus and *Agrobacterium* in already bearing vineyards but below 10 years clearly proves that the source of infections is the affected propagating material. In

Hungary it is difficult to take out only the esca syndrome from the current young grapevine decline problem, we need to deal with different pathogens in the near future. Focusing on the prevention of the disease, the most important tasks are to produce healthy propagating material and to mitigate the role of stress factors that can be influenced by man.

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