

# Water regime and its possible impact on expression of Esca symptoms in *Vitis vinifera*: growth characters and symptoms in the greenhouse after artificial infection with *Phaeomoniella chlamydospora*

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## Summary

**Impact of abiotic factors has been recently discussed in grapevine trunk diseases such as Esca. Emphasis of the present study lies on the relation between different water regime ("normal" and "stress") on one side and growth characters as well as Esca-related wood symptoms on the other side. Trials were conducted in the greenhouse with cuttings of the cultivars 'Müller-Thurgau' and 'Riesling', part of which artificially infected with *Phaeomoniella chlamydospora*. During a 12 month incubation period the following characters were evaluated: shoot length, weight of stem, expression of wood symptoms, and survival rate of plants. For evaluation of wood symptoms a numerical system was developed that is applied here for the first time. The obtained results indicate that water supply plays a statistically significant role, with plants under "stress" regime shown to be more affected. No strict spatial correlation was found to exist between wood symptoms and physical presence of the pathogen.**

**Key words:** Water regime, esca, numerical evaluation, *Phaeomoniella*.

## Introduction

In stem transections of Esca affected vines a distinct alteration may be observed: xylem vessels are plugged by tylosis and a black gum-like matter, the latter phenomenon properly described as gummosis or black goo (MORTON 1995). Quite obvious in younger vines, affected vessels are often arranged in a somewhat annular mode, comparable with the browning of young vessel rings in Dutch Elm Disease; radial arrangement of symptoms is less common. Anamorphic fungi have been shown to act as causing agents of gummosis-like phenomena in Esca diseased vines (PETRI 1912, LARIGNON and DUBOS 1997, MUGNAI *et al.* 1999). *Phaeomoniella chlamydospora* (W. Gams, Crous, M.J. Wingf. & L. Mugnai) Crous & W. Gams (CROUS *et al.* 1996, CROUS and GAMS 2000) is a cosmopolitan species and the most frequent Esca related pathogen in Central European vines; an apparently minor role is played by taxa belonging to the genus *Phaeoacremonium*, in Central Europe mostly

represented by the species *Phaeoacremonium aleophilum* W. Gams, Crous, M. J. Wingf. & L. Mugnai (CROUS *et al.* 1996). In the latter context it has to be noted however that taxonomy and diagnosis within *Phaeoacremonium* is still underway and additional taxa have been recently identified as occurring in European vineyards (MOSTERT *et al.* 2006, AROCA and RAPOSO 2007, GRAMAJE *et al.* 2007, ESSAKHI *et al.* 2008).

Gummosis is one of several examples to be classified as a so-called tracheomykosis, *i.e.*, a fungal disease affecting the xylem tissue. Affected hosts comprise both herbaceous and wooden plants. Related fungal pathogens of considerable economic importance worldwide include genera such as *Fusarium*, *Verticillium* or *Ophiostoma*. Heavy infestation with these organisms may result in a dramatic reduction of water transport - down to appr. 1 % of the usual rate - in affected plants (MACE *et al.* 1981). Consequently, gummosis related symptoms like wilting of leaves are more prominent under sunny and warm conditions. In Esca affected wood it is a commonly observed phenomenon that no strict correlation exists between gummosis and physical presence of the fungal pathogens, *i.e.*, plugging of vessels apparently is not solely due to fungal hyphae and/or spores. As an additional factor, defense response of the affected plants results in the production of tylosis and highly viscose compounds such as glycopeptides and/or polysaccharides, which are secreted into the xylem tissue (for instance, see MUGNAI *et al.* 1999, LORENA *et al.* 2001). In this way both the pathogen and the plant contribute to the dysfunction of the xylem tissue.

While gummosis plays a significant role in younger vines, white rot becomes a major and eventually the dominant factor in older Esca diseased vines. In European viticulture Esca related white rot is caused by a basidiomycete, *Fomitiporia mediterranea* M. Fischer (FISCHER 2002), which in the stem head of older vines may occupy 80 % or even more of cross sections (some basic information on white rot phenomena in Central European grapevine is provided by FISCHER and KASSEMEYER 2003, KUNTZMANN *et al.* 2010). Water transport is severely affected or non-existing in such areas. It has to be noted however that also in non-affected vines water transport is usually restricted to the youngest growth ring; this is brought about by a rich formation of tylosis in older sections (ESAU 1960). Due to a negative water balance, lack of water often results in a re-

versible wilting of affected plants. More severely, an irreversible wilting may be caused by a more massive production and secretion of toxins. As for trunk diseases of grapevine, hydroxybenzaldehydes (TABACCHI *et al.* 2000) and/or naphthalenone derivatives such as scytalone and isosclerone (EVIDENTE *et al.* 2000, ABOU-MANSOUR *et al.* 2004; for a recent review, see ANDOLFI *et al.* 2011) have been discussed as possible phytotoxic compounds.

Grapevine trunk diseases are perennial; still, the major part of infected plants is symptomless on the leaves and berries. Outbreak of external symptoms is not strictly correlated with inner symptoms, and may be in some relation to cultural practices or environmental stress such as temperature, water shortage or overstock (DI MARCO *et al.* 2000, 2008, EDWARDS *et al.* 2007, FISCHER 2009, LECOMTE *et al.* 2009).

Unusually hot and dry weather periods have been repeatedly noted in Germany during the last ten years. Among these, the most noteworthy has been the summer of 2003; additional examples are represented by spring periods in 2006 and 2011.

In German viticultural areas the first signs of the so-called Petri disease (also called young esca; for definitions see SURICO 2001) were evident in 2003: in this year young vines infected by Esca related pathogens, mostly *Phaeoconiella chlamydospora* (= *Pch*) and, more rarely, *Phaeoacremonium aleophilum* (= *Pal*) showed distinct signs of wilting and dieback (FISCHER 2003). As a puzzling phenomenon, differing rates of disease severity were noted among vineyards, even though planted with plant material of the same origin. Besides, the exact impact of possible infections with *Pch* and/or *Pal* could not be clarified - no strict correlation was found between the occurrence of wilting symptoms and the extent of physical presence of the pathogens within the plant (this observation later on confirmed by EDWARDS *et al.* 2007). A possible background of this somewhat confusing situation was thought to lie in the different amounts of water available in the plantations, related to differing natural water resources and/or irrigation management.

The amount of water supply and its exact impact on the vines is difficult to evaluate under field conditions; besides, field conditions do not easily allow clear statements with respect to the pathogen incidence in the particular vines. Both for water management and pathogen inoculation, the present study has been conducted under fairly defined conditions in the greenhouse. In particular, the following topics should be addressed: i) with *Pch* as pathogen in a „close to nature“ infection protocol, is it possible to differentiate between treated and non-treated plants? ii) In this context, is it possible to induce external symptoms such as leaf discoloration (“tiger stripes”) after a limited incubation time? ii) In case of differing water regime, how does artificial infection influence the growth characters and internal symptoms of the plants? iii) Is it possible to develop some criteria which can be used for a numerical and reproducible evaluation of the particular disease severity of plants? And, iv), are the obtained results likewise applicable to different cultivars, or are there indications of a cultivar-specific variability?

## Material and Methods

**Plants and pathogens:** Sixty “two bud”-cuttings each of ‘Müller-Thurgau’ (usually considered as highly susceptible against Esca) and ‘Riesling’ (somewhat less susceptible to Esca) were visually inspected for symptoms, weighed and numbered as 1-120 before potting. On the average, cuttings were about 10-13 cm long. Rooting of cuttings was performed in 7 cm diameter jiffy pods. After two months the plants were transferred to 1.3 liter-pots; the substrate was composed of ½ compost (Floragard, “FG Kompost”) mixed with soil (“FG Blumenerde”), ¼ of sand, and ¼ of peat (“Floratorf”).

*Phaeoconiella chlamydospora*, strain HT5 (Baden, Germany, from *Vitis vinifera* ‘Müller-Thurgau’) was used as pathogen. Mycelial cultures were incubated on 2 % PDA (potato dextrose agar) under room conditions. After several weeks a conidial suspension was obtained by floating the Petri dishes with 250 µL of sterile tap water, which was transferred to 100 ml Erlenmeyer flasks containing 50 mL of liquid ME (malt extract). After sufficient growth, usually six weeks after inoculation, the culture was filtered using glass filters with pore sizes of 40-100 µ and 16-40 µ respectively. For artificial infection of plants the filtrate was adjusted to a conidial concentration of appr.  $1 \times 10^4$  per mL.

**Artificial infection:** Appr. 7 d after outgrowth pruning was simulated by removing the uppermost portion of the shoot; the upper node was not affected. The resulting pruning wounds were inoculated with 40 µL of the above conidial suspension (Fig. 1). Control plants were treated with sterile water. For each cultivar, 40 plants were assigned for infection, 20 plants remained non-infected.



Fig. 1: Artificial infection of pruning wound with conidial suspension of *Phaeoconiella chlamydospora*.

**Growth conditions of plants:** After infection, plants were cultivated for 10 months in the greenhouse (August 2010 through June 2011). Throughout the experiment daylight conditions were applied. Temperature range was in relation to overall weather conditions except for winter time, when plants were transferred to cabins with temperatures between appr. 20 and 25 °C. For plant protection reasons spraying of leaves was carried out against powdery mildew (3 treatments) and downy mildew (1 treatment). Water regime was as follows: For each cultivar the 40 infected plants were subdivided in groups of 20; these subgroups were kept under "normal" and "water stress" regime respectively. The non-infected plants were divided in groups of 10; these subgroups were treated in the same way. "Normal" plants were supplied with 100 mL of water per day, "water stress" plants had a water supply of 100 mL once a week under warmer (> appr. 25 °C) and once in two weeks under cooler conditions (< appr. 25 °C).

**Assessment of growth characters and wood symptoms.** Growth characters: Shoot length was measured for all vines 10, 12 and 14 weeks after infection. Evaluation of leaf symptoms was carried out every two weeks. Vines were harvested after appr. 12 months of cultivation, *i.e.*, ten months after infection. Remaining soil was carefully removed from the roots by washing in tap water, and formation of roots was visually evaluated. Before weight determination of the plants, both shoots and roots were trimmed to their basal part and remaining water droplets were removed with a paper towel.

**Internal characters:** Wood symptoms were examined by cross sections placed below the upper node, in a distance of 2-4 cm from the pruning site. The total number of xylem vessels per cross section was estimated for some representatives of each cultivar and was found to be usually between 400 and > 500; usually about 50 -≥ 60 parenchyma rays separate xylematic strands containing appr. 5-12 vessels of different size (proto- and metaxylem). Based on this observation a numerical system was developed in relation to the number of affected xylem vessels per section: 1 = no symptomatic vessels; 2 = appr. 10 affected vessels (~ 2 % of total vessel number); 3 = appr. 25 affected vessels (~ 5 %); 4 = appr. 50 and more affected

vessels (≥ 10 %); 5 = dead plant. Ratings of 1.5, 2.5, and 3.5 were assigned to intermediate numbers of affected vessels. For each experimental assay the results were pooled and the average means and standard deviation calculated. Some samples were also examined by longitudinal sections, but no further evaluation was conducted here.

**Statistical analysis:** Due to normality and homogeneity of data, the comparisons of growth characters and wood symptoms were analyzed by ANOVA followed by post-hoc-tests of Least Significant Difference between means (LSD). In addition, Student's t-test at the 5 % significance level was used to compare weight means of cuttings before and after the trial. Statistical analyses were done with SPSS statistics software 19 (IBM 2011).

Parametric tests were applied after testing the normality and homogeneity of data ( $\alpha = 0.05$ ).

## Results

**Growth characteristics:** A regular outgrowth was observed for all of the 120 cuttings. For each experimental assay the average length of the main shoots after 10, 12 and 14 weeks is given in Tab. 1.

General growth behavior was widely identical between 'Müller-Thurgau' and 'Riesling' in that development of the plants was best under "normal" water regime, with no significant difference between the variants "infected" and "non-infected" after 14 wks of cultivation. Under "water stress", development of plants was distinctly reduced, but again no significant difference between "infected" and "non-infected" was recorded after 14 wks. Differentiation between "normal" and "water stress" conditions was indistinct after 10 weeks, but became significant with ongoing cultivation.

**Weight of cuttings. Beginning and end of trial:** The average weight of plants, without the roots and for all water-infection combinations, is summarized in Tab. 2.

Between cultivars, a distinct discrepancy was evident between cuttings of 'Müller-Thurgau' and 'Riesling', with the former being ~ 20-40 % heavier than the latter, both in the beginning and in the end of the experiments. This observation was irrespective of the applied growth condi-

Table 1

'Müller-Thurgau' and 'Riesling': mean shoot lengths (cm) 10, 12 and 14 wks after pruning under different water und infection (*Phaeo-  
monia chlamydospora*) regime

Cultivar		Müller-Thurgau			Riesling		
Growth conditions		Mean of length main shoot (cm)			Mean of length main shoot (cm)		
Infection	Water	10 wks <sup>1</sup>	12 wks	14 wks	10 wks	12 wks	14 wks
no (n = 10)	"normal" (n = 10)	20.3 ± 9.5 a <sup>2</sup>	33.8 ± 12.7 a	74.5 ± 11.8 a	20.1 ± 4.6 ab	35.4 ± 10.2 a	75.1 ± 14.6 a
no (n = 10)	"stress" (n = 10)	22.9 ± 9.1 a	27.8 ± 11.7 a	39.5 ± 14.8 b	18.4 ± 5.4 a	31.0 ± 11.1 a	54.2 ± 22.0 b
yes (n = 20)	"normal" (n = 20)	25.4 ± 12.6 a	45.8 ± 24.3 b	79.6 ± 29.8 a	26.4 ± 12.6 b	45.9 ± 19.2 b	84.7 ± 26.1 a
yes (n = 20)	"stress" (n = 20)	25.5 ± 7.0 a	32.1 ± 8.8 a	46.4 ± 7.7 b	20.3 ± 6.0 a	28.9 ± 7.4 a	49.3 ± 12.3 b

<sup>1</sup> Time interval after pruning/infection.

<sup>2</sup> Comparison of all water-infection combinations within cultivars and within defined time interval; LSD ( $\alpha = 0.05$ ). Means followed by the same letter are not significantly different ( $P > 0.05$ ) from control (infection "no", water "normal").

Table 2

'Müller-Thurgau' and 'Riesling': weight (g) of cuttings determined in the beginning and in the end (12 mts) of trials

Cultivar		Müller-Thurgau		Riesling	
Growth conditions		Weight cuttings (g)		Weight cuttings (g)	
Infection	Water	Beginning trial	End trial	Beginning trial	End trial
no (n = 10)	"normal" (n = 10)	12.8 ± 5.8 a <sup>1</sup>	17.2 ± 5.6 a	8.3 ± 1.8 ab	11.7 ± 2.2 ab
no (n = 10)	"stress" (n = 10)	11.2 ± 3.9 a	11.9 ± 4.6 b	9.5 ± 2.8 a	10.5 ± 2.3 bc
yes (n = 20)	"normal" (n = 20)	10.9 ± 3.7 a	14.3 ± 3.9 ab	7.6 ± 1.8 b	11.9 ± 3.4 ab
yes (n = 20)	"stress" (n = 20)	11.6 ± 3.7 a	12.3 ± 3.6 b	9.5 ± 2.5 a	9.5 ± 2.3 c

<sup>1</sup> Comparison of all water-infection combinations within cultivars and within defined period of trial; LSD ( $\alpha = 0.05$ ). Means followed by the same letter are not significantly different ( $P > 0.05$ ) from control (infection "no", water "normal"). See text for inner-cultivar comparison between different periods of trial.

tions. Within cultivars and between different infection-water combinations, after 10 mts. differential cultivation the weight was found to be significantly lower under "water stress" conditions and this applies both for infected and non-infected plants.

Within cultivars and within the applied infection-water combinations, the plants kept under "normal" water regime had gained significantly more weight than the "water stress" plants by the end of the trials, and this was evident both for "infected" and "non-infected" conditions (statistics of the latter not shown in Tab. 2). In general, weight increase was only very weak for all the plants under "water stress".

**Symptoms external:** None of the typical Esca-related symptoms such as leaf discoloration was developed during the cultivation period of 12 months. A reduced growth of shoots has been repeatedly reported from Esca plants in the field, but in the present trial this phenomenon apparently was not in relation to infection conditions, but could be more likely traced back to the simulated water stress (Tab. 1). A total of 7 plants (5 from 'Müller-Thurgau', 2 from 'Riesling') had died by the end of the trials, all of them linked to water stress conditions and with one exception all artificially infected.

For all plants the roots were visually examined after harvest. With one exception only (one plant of 'Müller-Thurgau': infected, "normal") a reduced formation of roots was evident in water stress-plants only, and here it was in some correlation with the "infected" or "non-infected" status. All in all, 15 plants exhibited a reduced formation of roots. Out of these, 10 plants (3 of 'Müller-Thurgau', 7 of 'Riesling') were within the combination "infected - water stress", and 5 (3 of 'Müller-Thurgau', 2 of 'Riesling') were within the combination "non-infected - water stress". A reduced root formation was evident for 4 out of the 7 died plants.

**Symptoms internal:** Internal symptoms of vines were checked in cross sections placed below the upper node (shown in Fig. 2 for 'Riesling'). Symptom severity was evaluated and numerically expressed according to the approximate number of affected vessels; mean values and standard deviations are summarized in Tab. 3.

After 12 months of cultivation, *i.e.*, appr. 10 mts. after infection, not a single vine out of 120 had remained fully unaffected at the sampling site. Symptoms also existed in control vines (non-infected, "normal" water regime). Except for died plants, rating was between 1.5 and 3.5. In particular, the following tendencies were noted: within both

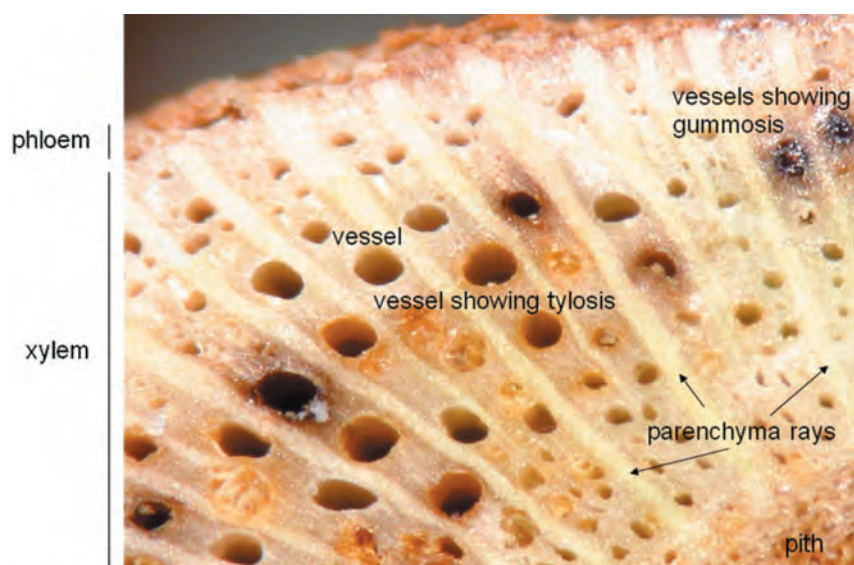


Fig. 2: Cross section of 'Riesling' after 12 mts cultivation (non-infected, "water stress").

Table 3

'Müller-Thurgau' and 'Riesling': numerical evaluation of wood symptoms in the end (12 mts) of trials

Cultivar		Müller-Thurgau	Riesling
Growth conditions	water	Symptom severity	Symptom severity
infection		(No. of dead plants by end of trial)	(No. of dead plants by end of trial)
no	"normal"	2.05 ± 0.44 a <sup>1</sup>	2.2 ± 0.48 a
(n = 10)	(n = 10)	(0)	(0)
no	"stress"	2.45 ± 0.98 a	2.25 ± 0.35 a
(n = 10)	(n = 10)	(1)	(0)
yes	"normal"	2.98 ± 0.30 b	3.0 ± 0.0 b
(n = 20)	(n = 20)	(0)	(0)
yes	"stress"	3.43 ± 0.83 c	3.2 ± 0.62 b
(n = 20)	(n = 20)	(4)	(2)

<sup>1</sup> Comparison of all water-infection combinations within cultivars; LSD ( $\alpha = 0.05$ ). Means followed by the same letter are not significantly different ( $P > 0.05$ ) from control (infection "no", water "normal").

cultivars, rating was better under "normal" water regime, both for infected and non-infected conditions, and it was better in non-infected than in infected variants. With two exceptions, "non-infected – normal" against "non-infected – stress", all recorded discrepancies were statistically significant. In general, rating was 3 and worse for infected vines, *i.e.*,  $\geq 5\%$  of the xylem vessels were found to be affected by plugs and/or gummosis. For non-infected vines, rating was between appr. 2 and 2.5, *i.e.*, between  $\sim 2\%$  and  $\sim 3\%$  of the vessels were symptomatic.

Arrangement of symptomatic vessels within sections was variable; in case of a more massive infestation mostly ring-like or more or less symmetrical half-moon structures were evident. Gummosis, *i.e.*, extrusion of dark droplets out of affected vessels, seems to be in some relation to the overall wetness of vines. Usually, watering of the material for several hours often resulted in the appearance of such droplets.

Random samples were also checked for longitudinal distribution of symptoms. Specific discolorations always could be recovered in some distance from the pruning wound, in this way confirming the success of the chosen infection protocol. After all, the pathogen is able to spread against the water flux inside the vascular cylinder. Still, symptom severity was distinctly less pronounced in the middle sections of the vines.

## Discussion

In recent years a number of studies have been published dealing with the possible impact of abiotic factors on the symptom expression of fungal grapevine trunk diseases. Water stress has been repeatedly quoted as one of the more important issues (for instance, see LECOMTE *et al.* 2009, LUQUE *et al.* 2010, SOSNOWSKI *et al.* 2011, VAN NIEKERK *et al.* 2011). As a novelty, the present Esca-related study uses the combination of artificial inoculation with conidia and a numerical evaluation of symptoms. The tendencies recorded after 12 mts of cultivation, *i.e.*, 10 mts. after treatment of pruning wounds, were in accordance for both cultivars and may be summarized as follows (except for two particular

combinations within "internal wood symptoms" all statements are statistically supported):

- Shoot length: a faster growth occurred under "normal" water regime, a slower growth under "water stress"; this applies both to "infected" and "non-infected" conditions.
- Weight: in general, 'Riesling' cuttings were lighter than 'Müller-Thurgau' cuttings. A distinct increase in weight was observed under "normal" water regime, while increase was weak or essentially non-existing under "water stress"; this applies both to "infected" and "non-infected" conditions.
- External and internal symptoms: no symptoms were observed on the leaves. In general, lesser internal symptoms were observed in non-infected plants, and this applies both to "normal" and "water stress" regime.
- Numerical evaluation of internal wood symptoms: a better rating was obtained under "normal" water regime, and this applies both to "infected" and "non-infected" conditions. *Vice versa*, rating was better for non-infected plants, and this was applicable to "normal" and "water stress" conditions.
- Losses: plant losses were higher under "water stress" when compared to "normal" (7 out of 60 = 11.7 % against 0 out of 60 = 0 %); losses were more evident under "infected" than under "non-infected" conditions (6 out of 40 = 15 % against 1 out of 20 = 5 %).

Basically, the above statements rely on a successful and reproducible infection protocol. *Pch* is thought to invade vines by way of conidia, and main entrance is through pruning wounds (for instance, see LARIGNON and DUBOS 2000). As with other wood-inhabiting fungi the number of conidia necessary for successful infection under field conditions is not known (see EDMAN and GUSTAFSSON 2003, for some basic information on basidiomycetes). The conidial suspension used in this trial was  $1 \times 10^4$  conidia·mL<sup>-1</sup>. 40  $\mu$ L were applied per pruning wound corresponding to a total of approx. 400 conidia and this amount proved to be sufficient for successful infection. A similar approach was used by ROLSHAUSEN *et al.* (2010) in a field study based on *Pch* and other trunk disease pathogens, and here a quan-

tity of ~ 1000 spores was used per treatment. Instead of conidia, mycelium has been repeatedly used in the past, *via* inoculated tooth picks (MUGNAI *et al.* 1999) or *via* agar plugs (URBEZ-TORRES *et al.* 2009); in both assays, sampled canes were artificially wounded with cork borers.

All cuttings had been visually examined in the beginning of the experiments and were found to be without symptoms. After 12 months of cultivation, not a single out of these 120 plants had remained symptomless. Statistically significant, symptoms were more apparent in infected plants. The origin of symptoms in untreated control plants is unknown, but three possibilities may be taken into consideration: i) some/all of the plants are pre-infected with *Pch*, with the symptoms becoming more clearly visible after some incubation time only; ii) some/all of the plants became infected by airborne conidia of *Pch* during the cultivation period in the greenhouse, and/or iii) infection originates from infected soil. The noted distribution of symptoms both in transections and longitudinal sections is not discriminative between these suggestions.

The correlation between symptomatic vessels and physical presence of the pathogen remains an open question. The numerical evaluation of symptoms as is introduced in this study is based on the number and approximate percentage of visually affected xylem vessels. In addition, random samples were examined by re-isolation tests. As a drawback an accurate sampling of single vessels was not possible; in this way, re-isolation results usually refer to complete cross sections. Mycelial isolates were derived from 12 out of 31 sampled vines, *i.e.*, re-isolation rate was appr. 40 %. This number points to an indistinct spatial correlation only between symptom expression and pathogens.

Formation of tylosis may be in reaction to infection processes by pathogens such as fungi or bacteria, and in shoots of grapevine they are also a response to pruning treatment (SUN *et al.* 2006). Since nothing is known about the particular degree of tylosis formation after infection with *Pch*, no related statement is offered in this study. At least, inclusion of tylosis affected vessels would result in slightly higher infestation rates than the ones indicated in Tab. 3.

The categories 1-5, applied for numerical evaluation of symptoms in the wood are basically arbitrary and do not allow definite conclusions with respect to outer appearance and future perspectives of the plants. Besides, the numbers are referable only to a limited area within the plant. In the present study they are used to circumscribe a specific condition that exists a few cm apart from the original infection site. Still, the numerical system allows a semiquantitative comparison between different experimental assays, and for example might be useful in fungicide testing. No limitations exist regarding duration of experiments or sampling area in the wood.

With the data available, no statements are possible about the relation between symptom expression and cultivar; with this in mind, the low number of used cultivars is one of the deficits of the presented experiments.

The importance of water supply to young vines in the greenhouse has been clearly demonstrated in this study; growth characteristics, expression of wood symptoms and,

probably, survival rate of plants were found to be influenced by this particular factor. While a correlation was observed between infection with *Pch* and symptom incidence in the wood (see also LUQUE *et al.* 2010; however, *Eutypa lata* and *Neofusicoccum parvum* are used as pathogens in their study), no statement is possible concerning the impact of the pathogen on phenomena such as leaf water potential and stomatal conductance. This relies on future studies and might represent another step in understanding the relation between abiotic and biotic stress factors.

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### References

- ABOU-MANSUR, E.; COUCHÉ, E.; TABACCHI, R.; 2004: Do fungal naphthalenones have a role in the development of esca symptoms? *Phytopathol. Mediterr.* **43**, 75-82.
- ANDOLFI, A.; MUGNAI, L.; LUGUE, J.; SURICO, G.; CIMMINO, A.; EVIDENTE, A.; 2011: Phytotoxins produced by fungi associated with grapevine trunk diseases. *Toxins* **3**, 1569-1605.
- AROCA, A.; RAPOSO, R.; 2007: PCR-based strategy to detect and identify species of *Phaeoacremonium* causing grapevine diseases. *Appl. Environ. Microbiol.* **73**, 2911-2918.
- CROUS, P. W.; GAMS, W.; WINGFIELD, M. J.; VAN WYK, P. S.; 1996: *Phaeoacremonium* gen. nov. associated with wilt and decline diseases of woody hosts and human infections. *Mycologia* **88**, 786-796.
- CROUS, P. W.; GAMS, W.; 2000: *Phaeoconiella chlamydospora* gen. et comb. nov., a causal organism of Petri grapevine decline and esca. *Phytopathol. Mediterr.* **39**, 112-118.
- DI MARCO, S.; MAZZULLO, A.; CALZARANO, F.; CESARI, A.; 2000: The control of esca: status and perspectives. *Phytopathol. Mediterr.* **39**, 232-240.
- DI MARCO, D.; OSTI, F.; 2008: Foliar symptom expression of wood decay in *Actinidia deliciosa* in relation to environmental factors. *Plant Dis.* **92**, 1150-1157.
- EDMAN, M.; GUSTAFSSON, M.; 2003: Wood-disk traps provide a robust method for studying spore dispersal of wood-decaying basidiomycetes. *Mycologia* **95**, 553-556.
- EDWARDS, J.; PASCOE, I. G.; SALIB, S.; 2007: Impairment of grapevine xylem function by *Phaeoconiella chlamydospora* infection is due to more than physical blockage of vessels with "goo". *Phytopathol. Mediterr.* **46**, 87-90.
- ESAU, K.; 1960: *Plant Anatomy*, 3<sup>rd</sup> ed. New York, London, John Wiley and Sons.
- ESSAKHI, S.; MUGNAI, L.; CROUS, P. W.; GROENEWALD, J. Z.; SURICO, G.; 2008: Molecular and phenotypic characterization of novel *Phaeoacremonium* species isolated from esca diseased grapevines. *Persoonia* **21**, 119-134.
- EVIDENTE, A.; SPARAPANO, L.; ANDOLFI, A.; BRUNO, G.; 2000: Two naphthalenone pentaketides from liquid cultures of *Phaeoacremonium aleophilum*, a fungus associated with esca of grapevine. *Phytopathol. Mediterr.* **39**, 162-168.
- FISCHER, M.; 2002: A new wood-decaying basidiomycete species associated with esca of grapevine: *Fomitiporia mediterranea* (Hymenochaetales). *Mycol. Progr.* **1**, 314-324.
- FISCHER, M.; 2003: Esca jetzt auch in Junganlagen? *Der Badische Winzer* **11**, 26-27.
- FISCHER, M.; 2009: Esca im Freiland. Erfahrungen mit weinbaulichen Maßnahmen. *Obstbau Weinbau* **46**, 280-282.

- FISCHER, M.; KASSEMAYER, H.-H.; 2003: Fungi associated with Esca disease of grapevine in Germany. *Vitis* **42**, 109-116.
- GRAMAJE, D.; ALANIZ, S.; PÉREZ-SIERRA, A.; ABAD-CAMPOS, P.; GARCÍA-JIMÉNEZ, J.; ARMENGOL, J.; 2007: First report of *Phaeoacremonium mortioniae* causing Petri disease of grapevine in Spain. *Plant Dis.* **91**, 1206.
- KUNTZMANN, P.; VILLAUME, S.; LARIGNON, P.; BERTSCH, C.; 2010: Esca, BDA and Eutypiosis: foliar symptoms, trunk lesions and fungi observed in diseased vinestocks in two vineyards in Alsace. *Vitis* **49**, 71-76.
- LARIGNON, P.; DUBOS, B.; 1997: Fungi associated with Esca disease in grapevine. *Eur. J. Plant Pathol.* **103**, 147-157.
- LARIGNON, P.; DUBOS, B.; 2000: Preliminary studies on the biology of *Phaeoacremonium*. *Phytopathol. Mediterr.* **39**, 184-189.
- LECOMTE, P.; LOUVET, G.; GOUTOULY, J. P.; CORIO-COSTET, M. F.; REY, P.; GUÉRIN-DUBRANA, L.; 2009: Impact of biotic and abiotic factors on plant susceptibility to Esca-vine trunk disease. IOBC/WPRS – OILB/SROP “Integrated protection and production in viticulture”. Staufen, Germany 1<sup>st</sup>-4<sup>th</sup> November, 2009. Abstracts.
- LORENA, T.; CALAMASSI, R.; MORI, B.; MUGNAL, L.; SURICO, G.; 2001: *Phaeoacremonium chlamydospora* – grapevine interaction: histochemical reactions to fungal infection. *Phytopathol. Mediterr.* **40**, 400-406.
- LUQUE, J.; MARTOS, S.; GARCIA-FIGUERES, F.; 2010: Effects of water stress and inoculation with *Eutypa lata* and *Neofusicoccum parvum* on young grapevine plants. 7<sup>th</sup> Int. Workshop on Grapevine Trunk Diseases, Santa Cruz, Chile, 17.-21. January, 2010. Abstracts.
- MACE, M. E.; BELL, A. A.; BECKMAN, C. H.; 1981: Fungal wilt diseases of plants. London, New York, Academic Press.
- MORTON, L.; 1995: Mystery diseases hit young vines. *Wines vines* **76**, 46-47.
- MOSTERT, L.; GROENEWALD, J. Z.; SUMMERBELL, R. C.; GAMS, W.; CROUS, P. W.; 2006: Taxonomy and pathology of *Togninia* (Diaporthales) and its *Phaeoacremonium anamorphs*. *Studies Mycol.* **54**, 1-115.
- MUGNAL, L.; GRANITI, A.; SURICO, G.; 1999: Esca (Black Measles) and Brown Wood-Streaking: Two old and elusive diseases of grapevines. *Plant Dis.* **83**, 404-418.
- PETRI, L.; 1912: Osservazioni sopra le alterazioni del legno della vite in seguito a ferrite. *La Stazioni Sperimentali Agrarie Italiane* **45**, 510-547.
- ROLSHAUSEN, P. E.; URBEZ-TORRES, J. R.; ROONEY-LATHAM, S.; ESKALEN, A.; SMITH, R. J.; GUBLER, W. D.; 2010: Evaluation of pruning wound susceptibility and protection against fungi associated with grapevine trunk diseases. *Am. J. Enol. Vitic.* **61**, 113-119.
- SOSNOWSKI, M.; LUQUE, J.; LOSCHIAVO, A.; MARTOS, S.; GARCIA-FIGUERES, F.; WICKS, T.; SCOTT, E.; 2011: Studies on the effect of water and temperature stress in grapevines inoculated with *Eutypa lata*. *Phytopathol. Mediterr.* **50**, 127-138.
- SURICO, G.; 2001: Towards commonly agreed answers to some basic questions on esca. *Phytopathol. Mediterr.* **40**, 487-490.
- SUN, Q.; ROST, T. L.; MATTHEWS, M. A.; 2006: Pruning-induced tylose development in stems of current-year shoots of *Vitis vinifera* (Vitaceae). *Am. J. Bot.* **93**, 1567-1576.
- TABACCHI, R.; FKYERAT, A.; POLIART, C.; DUBIN, G. M.; 2000: Phytotoxins from fungi of esca of grapevine. *Phytopathol. Mediterr.* **39**, 156-161.
- URBEZ-TORRES, J. R.; ADAMS, P.; KAMAS, J.; GUBLER, W. D.; 2009: Identification, incidence, and pathogenicity of fungal species associated with grapevine dieback in Texas. *Am. J. Enol. Vitic.* **60**, 497-507.
- VAN NIEKERK, J.; STREVER, A. E.; DU TOIT, G.; HALLEEN, F.; FOURIE, P. H.; 2011: Influence of water stress on Botryosphaeriaceae disease expression in grapevines. 2011. *Phytopathol. Mediterr.* **50**, 151-165.

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