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Influence of the pruning system on the fungal community of grapevine (*Vitis vinifera*)

Dissertationen aus dem Julius Kühn-Institut

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Bundesforschungsinstitut für Kulturpflanzen

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Influence of the pruning system on the fungal community of grapevine (*Vitis vinifera*)

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von

M. Sc. Christian Kraus
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2019

“Das schönste, was wir erleben können, ist das Geheimnisvolle.”

- Albert Einstein -

Inhalt

Vorbemerkung

Die hier vorliegende Dissertation wurde am Julius Kühn-Institut, Institut für Pflanzenschutz in Obst- und Weinbau, in Siebeldingen unter der Aufsicht von **Prof. Dr. Michael Fischer** in Kooperation mit **Prof. Dr. Ralf T. Vögele** von der Universität Hohenheim im Zeitraum von März 2015 bis Dezember 2018 durchgeführt. Teile dieser Arbeit wurden in internationalen, peer-reviewed Journals publiziert oder als mündlicher Vortrag oder Poster auf nationalen und internationalen Wissenschaftskonferenzen präsentiert. Die Publikationen und Präsentationen werden im Folgenden aufgelistet:

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Chapter ONE

Introduction

Viticulture and grapevine pruning systems

Over the last century grapevine (*Vitis vinifera*) has become one of the economically most important fruit crops in the world, due to the production of table grapes, dried fruits, juice and wine (FAO 2018). However, cultivation of this perennial plant crop goes along with multiple, labour-intensive cultivation practices: winter and summer pruning, wire positioning, plant protection, harvesting and more (Coombe & Dry, 1992). These numerous cultivation practices make viticulture one of the most time and labour intensive agrosystems. The grapevine production process and its associated costs are an important factor that determines competitiveness in an increasingly globalized market. For that reason, a strict minimization of the production cost is necessary to sustain in this highly competitive market.

Mechanization of several cultivation processes in the second half of the 20th century such as harvesting, summer pruning, or application of fungicides and pesticides could reduce the labour input enormously (Clingeleffer, 2013). On the other hand, processes with an immense manual effort, like winter pruning and wire positioning, require a high level of precision and thus could not be satisfactorily replaced by mechanical work. These two processes, winter pruning and wire positioning, are part of many grapevine training systems which exist in a broad variety around the world. Training systems were developed over centuries and are specific to each growing region in order to determine the most suitable vineyard practices for high quality grapevine production (Crespy, 2008). The most common training system for grapevine production in Germany is the vertical shoot positioning (VSP, Fig. 1 a) system, which is typical for cooler climate vineyards (Jackson, 1996). In this system, at dormancy, almost all grapevine canes from the preseason are detached with pruning shears

and removed from the trellis by hand. One or two canes with a defined number of buds remain. These discrete canes are positioned horizontally in the trellis and are origin for new shoots in the upcoming season. This intensive and time consuming procedure enables vine growers to keep control over the quantity and quality of the crop (Jackson, 1996). Nevertheless, increasing wages make this training system economically unsustainable and the establishment of a new, more cost effective training system is necesarry.

Fig. 1: Grapevine trained in VSP (a) and SMPH (b) at three leaves stadium.

As early as the 1970s, Australia and the eastern states of the USA had to face the problem of high production costs and the absence of skilled vineyard workers (May & Clingeleffer, 1977; Pollock *et al.*, 1977; Pool, 1995). Therefore, these regions have put strong efforts into a total mechanization of the grapevine production process, with particular focus on mechanical pruning. Over decades, several mechanized pruning systems were investigated, and some were adopted successfully into the production process (Sommer, 1995; Clingeleffer, 2013). By the end of the century, about 65% of Australian and around 50% of New York´s vineyards were mechanically pruned (Pool, 1995; Clingeleffer, 2000).

After 30 years of intensive trials, minimal pruning (MP) gained special attention due to its immense cost saving potential with constant productivity and crop quality in both warm and

cool regions (Clingeleffer & Possingham, 1986; Clingeleffer, 1993, 2005). Therefore, it was just a matter of time until other grapevine growing regions started their own trials with MP, trying to adapt it to the local conditions. In Europe, many surveys on MP were done during the 1990ies, however with conflicting and often dissatisfactory results (Carbonneau, 1991; Ollat *et al.*, 1993; Martinez de Toda & Sancha, 1998; Schultz *et al.*, 1999; Intrieri *et al.*, 2001). Susceptibility to weather and management practices, as well as low crop quality caused by bud overload were identified as primary disadvantages of this training system (Intrieri, 2013). Due to this and perhaps because of the traditional attitude of winegrowers towards grapevine cultivation MP never reached the acceptance like it did in Australia or in the Eastern USA.

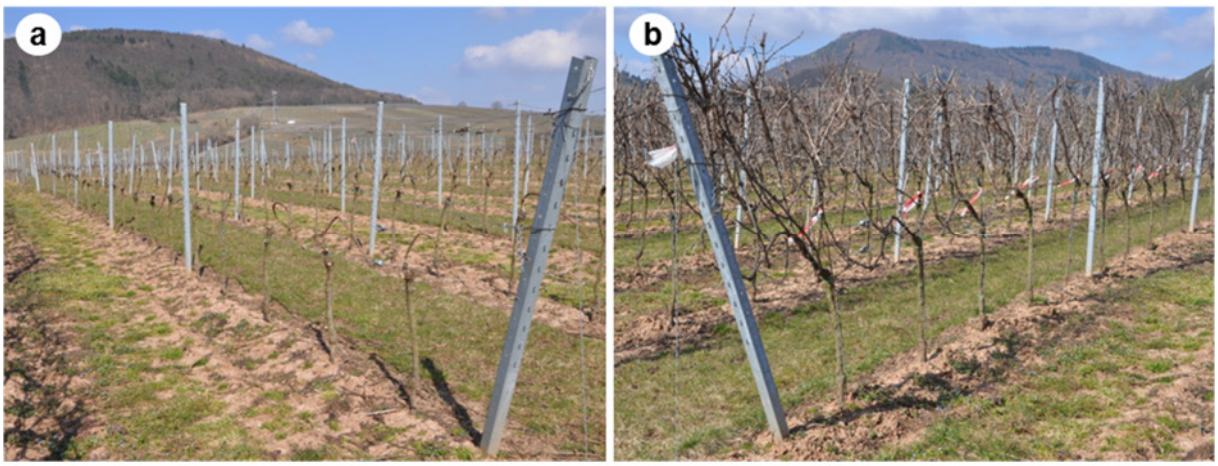

Fig. 2: VSP (a) and SMPH (b) trained grapevines after winter pruning.

Encouraged by the potential huge savings in production costs and increasing competitive pressure European scientists continuously worked on MP for further improvement and adaption. As a result, Intrieri and colleagues (2011, 2012) developed a training system called semi minimal pruned hedge (SMPH, Fig. 1 b) that should overcome the problems related to MP. The main concept of SMPH is a mechanical, rough winter pruning of formerly VSP trained vines to receive a stable vertical hedge wall of perennial canes (Fig. 2 b). Advantages of SMPH compared to MP are the simple and enhanced practicability on already established VSP vineyards without losing the benefits of minimal pruning, i.e., decreased production costs and high plant productivity (Walg, 2011a; 2015; Intrieri, 2013; Mend, 2013). Furthermore, by

use of mechanical yield reduction the quality of the grapes may achieve a similar level as traditionally treated vines (Intrieri *et al*., 2011; Walg, 2011b, 2012a, 2012b). The positive experiences with this system in several independent field trials in Germany initiated a change of thinking by grapevine growers and as a result SMPH is becoming more and more popular. For Rhineland-Palatinate it is estimated that currently about 3.000 to 3.500 ha of the vineyards are minimally, with the number constantly rising over the last years (pers. communication O. Walg, DLR Bad Kreuznach).

Plant physiology and morphology: VSP vs. SMPH

The grapevine training system has a striking effect on plant physiology and morphology. The most conspicuous differences between VSP and SMPH trained vines are the perennial canes in the trellis of SMPH grapevines (Fig. 2). The increased proportion of woody parts allows the plants to harbour more nutrient reserves (Sommer, 1995; Weyand & Schultz, 2006). Intensively pruned VSP vines have a defined number of around 15 buds per meter to achieve

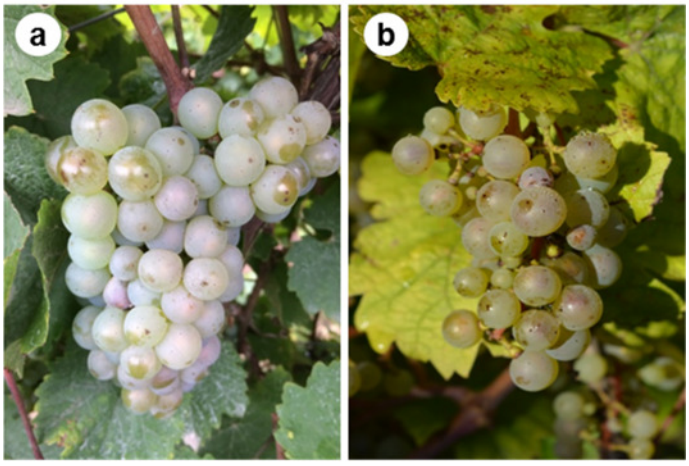

Fig. 3: Typical grape bunches of VSP (a) and SMPH (b) trained vines at harves

an optimal yield/quality ratio (Jackson, 1996). In contrast, the multiple canes of SMPH trained vines can be loaded with buds of more than 20 times the number of VSP vines (Intrieri *et al*., 2012; see Fig. 1). However, a physiologically self-regulating budburst results in a reduced budburst rate for SMPH of about 60% compared to 100% for VSP (Intrieri *et al*., 2011). Still,

the number of shoots per meter for SMPH can be 10 times higher than for VSP (Intrieri *et al*., 2011; Walg, 2012c, 2013). As a result, the SMPH trained vines carry more bunches and thus a higher yield per hectare can be achieved (Intrieri *et al*., 2011; Walg, 2011b; 2012a). Moreover, compared to the often densely packed VSP grape bunches SMPH bunches display a looser structure due to smaller and fewer berries (Fig. 3; Intrieri *et al*., 2011; Walg, 2013). The canopies of minimal pruned grapevines consist of numerous, small leaves, which develop a big and dense leaf wall (Fig. 4; Sommer *et al*., 1993; Intrieri *et al*., 2011). In contrast, the canopy of VSP plants consists of big but few leaves, creating a small, airy leaf wall. This intensive vegetative growth and the increased biomass of minimal pruned vines cause elevated water consumption and nutrient needs compared to VSP vines (Schmid & Schultz, 2000; Weyand & Schultz, 2006a, 2006b).

Fig. 4: Leaf canopy of VSP (left) and SMPH (right) trained grapevines at the phonological stage of pea size.

It is almost unexplored how and weather different training systems and the resulting morphological differences influence the fungal community of grapevine, but this question is of vital interest for vine growers with regard to phytopathogenic fungi and their control. Therefore, it is the foremost aim of this work to investigate the incidence of the major grapevine pathogens on the two training systems VSP and SMPH. The obtained results of this study should support grapevine growers by generating a plant protection regime which is adapted to the new training

system SMPH and thus contribute to a more sustainable and cost effective grapevine production.

Major fungal diseases in German viticulture

Botrytis Bunch Rot

Botrytis Bunch Rot (BR), also known as Grey Mould, is caused by *Botrytis cinerea*, a necrotrophic fungus which can infect more than 200 crop species throughout the world (Droby & Lichter, 2004; Williamson *et al*., 2007). In vineyards, this pathogen can induce blights and rots on all herbaceous tissues of the vine (Fig. 5; Wilcox *et al*., 2015a). However, the most frequent and problematic symptom is BR. Infected bunches not only lower the yield, but also have a negative effect on crop quality due to modified chemical compositions of diseased berries (Steel *et al*., 2013). This impact on grape quantity and quality means serious economic damage for wineries.

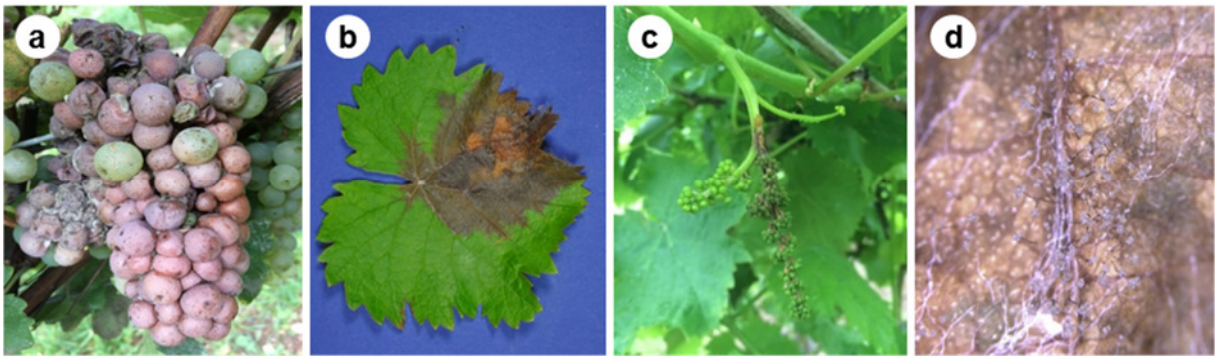

Fig. 5: Grapevine bunch (a), leaf (b) and inflorescence (c) infected by *B. cinerea*. Conidiophores of *B. cinerea* arising from a lesion (d).

Botrytis cinerea usually overwinters as sclerotia or mycelia in old grapevine parts like leaves, clusters or canes, which serve as primary inoculum in the subsequent season (Williamson *et al*., 2007). Under favourable conditions, i.e. moderate temperature, surface wetness, and changes in relative humidity, these overwintering structures start to produce conidia, which act as the main inoculum (Jarvis, 1962a; Wilcox *et al*., 2015a). Spreading of the conidia is primarily driven by wind, but also takes place by splashing water or by way of

animals, such as vertebrates or insects (Jarvis, 1962b, 1980a). After reaching an appropriate host tissue, conidia start to germinate and produce one or more germ tubes. At the apical end of the germ tubes, an appressorium-like structure is formed to penetrate the host surface by corrupting the epidermal cells with an arsenal of different degrading enzymes (van Kan, 2006; Choquer *et al*., 2007). After invading, *B. cinerea* kills the plant cells by secreting nonspecific phytotoxins, leading to maceration of the host tissue which the fungus can feed on (Collado *et al*., 2000). As a result, expanding necrotic lesions appear on the tissue. Under favourable climate conditions (as described below) *B. cinerea* readily sporulates and a typical grey fur develops on the necrotic lesions. On grapes, successful infection by conidia is achieved at saturated relative humidity (RH) after 15 h of temperatures between 15 °C and 20 °C (Jarvis, 1980b). A water film on the host tissue is however not necessary. Infections also occur under lower temperatures (3 °C) and a wet period of 72–84 h, demonstrating that a high relative humidity is more important for the epidemiology of *B. cinerea* than temperature (Nelson, 1951).

Under northern hemisphere conditions, BR mostly appears after rain in September and October. Rain, soil water status, plant rooting habit and excessive use of fertilizer are only a few of the factors that considerably influence the incidence of the disease (Jarvis, 1980c). Another factor is related to the ability of the berry skin to resist tearing and the most important risk factor for splitting is a tight bunch architecture. Caused by densely packed berries, a pressure builds up in the bunch during maturation until some berries begin to crack (Marois *et al*., 1986; Vail & Marois, 1991). Split berries or even small tears in the berry skin can then easily be infected by *B. cinerea*.

Besides the application of fungicides, several cultural practices help to control BR in vineyards. For example, removal of leaves in the bunch zone reduces the incidence and severity of BR significantly (English *et al*., 1993; Ashley *et al*., 2005). Additionally, the cultivation of grapevine cultivars that produce loose bunches structures decreases the susceptibility of the berries against *B. cinerea* infection (Vail & Marois, 1991).

Powdery Mildew

In the 1850s the ascomycete fungus *Erysiphe necator* was introduced from North America to Europe (Gadoury *et al*., 2012). It is the causal agent of grapevine Powdery Mildew (PM). After its appearance in Europe, this fungus produced massive losses in vineyards due to the high susceptibility of European grapevine, *V. vinifera*. Nowadays PM can be found in all grapevine growing regions around the world and an intensive use of fungicides is necessary to repress this pathogen (Halleen & Holz, 2001; Campbell, 2006; EUROSTAT, 2007).

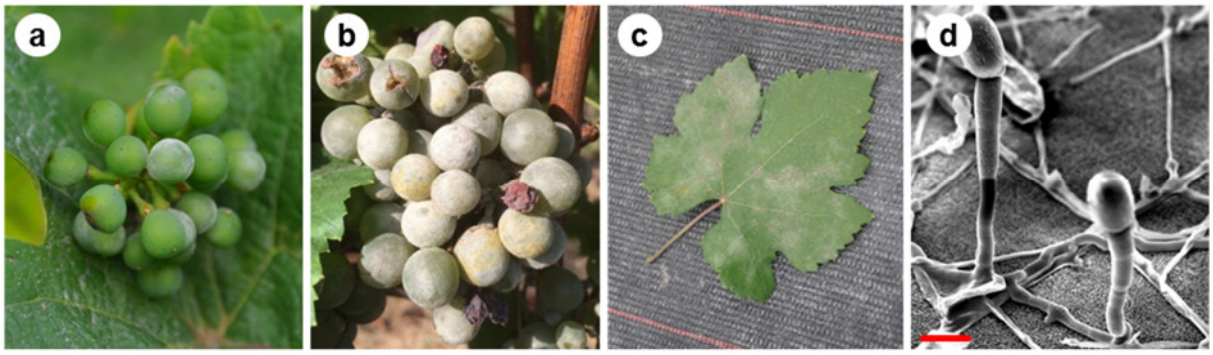

Fig. 6: Powdery Mildew on grapevine bunches at an early (a) and late (b) stage of infection. PM on grapevine leaf (c). Scanning electron microscopy picture of *E. necator* conidiophores formed on grapevine leaf (d). red bar = 25 µm.

Erysiphe necator is an obligate, biotrophic ectoparasite, which can infect all green parts of the vine (Fig. 6). A typical symptom of the disease is a whitish, powdery covering on the infected tissue, which originates from mycelium growth. Senescent, infected tissue sometimes appear yellowish or black due to chasmothecia, the teleomorphic state of the fungus. On berries, PM additionally may cause splitting of the skin which can lead to secondary infection and rot by opportunistic fungi and bacteria. Infested berries also often desiccate and mummify.

Distributed by wind or rain, conidiospores of this fungus which land on suitable host surface germinate and on the apical end of the germ tube an appressorium is formed to penetrate the epidermis (Gadoury *et al*., 2012; Wilcox *et al*., 2015b). Once the fungus has entered the intercellular room specialized structures called haustoria are generated, which penetrate host cells to absorb nutrients. After a successful establishment on the host surface, conidiophores with chains of asexual conidia are formed and the life cycles starts again. Chasmothecia formed

on leaves or shoots serve *E. necator* as overwintering structure. The optimum germination and growth of *E. necator* can be observed at temperatures between 20 and 27 °C, with RH around 84% (Delp, 1954; Fessler & Kassemeyer, 1995). RH above and below 84% both leads to a drastic reduction in germination of conidia. However, infection can still occur at RH between 40 and 100% (Carroll & Wilcox, 2003). Another abiotic factor that has a negative influence on PM severity is sunlight, in particular UV radiation (Austin, 2010).

Management of PM by cultural practices can be achieved by pruning and training systems such as leaf removal or an open leaf canopy. These practices enhance air movement in the bunch zone, fungicide deposition, and sunlight exposure of berries (Austin & Wilcox, 2011; Austin *et al*., 2011).

Downy Mildew

The causal agent of Downy Mildew (DM), *Plasmopara viticola*, is an obligate biotrophic fungus belonging to the Oomycetes. This pathogen, like *E. necator*, is native to North America and was introduced to Europe in the 1876 with fatal consequences for local viticulture, and years later in all vine growing regions worldwide (Farlow, 1876; Gessler *et al.*, 2011).

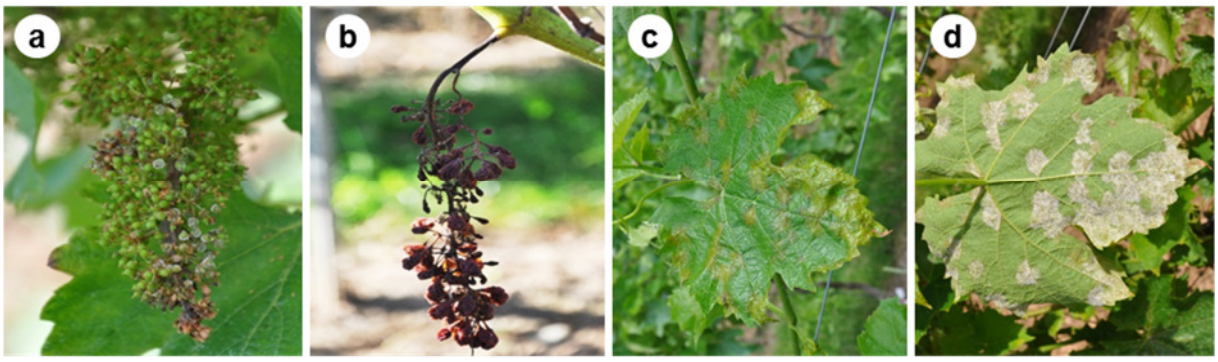

Fig. 7: DM infected grapevine inflorescence (a), bunch (b) and leaf, upper (c) and lower (d) site.

Plasmopara viticola infects all green parts of the grapevine, in particular leaves, inflorescences, and young berries (Fig. 7; Wilcox *et al*., 2015c). On leaves, first symptoms appear as pale green or yellowish circles on the upper surface known as “oil spots”. These spots are delimited by leaf veins and often form a mosaic pattern. Also a white, fluffy coating

consisting of sporangiophores can occur on the lower leaf surface, directly under the "oil spots", when *P. viticola* starts to sporulate after moist nights. Also infected inflorescences/bunches may be covered with this white coating under humid conditions. Subsequently, parts of or even the entire inflorescence/bunch become necrotic and die. DM on young berries manifests as so-called brown rot or leather berries. However, berries become less susceptible with maturation and even impervious as their stomata evolve into lenticels (Kennelly *et al*., 2005).

Plasmopara viticola persists as oospores on fallen leaves on vineyard soil during winter (Hill, 1989). In late spring, when climate conditions are favourable with ground temperatures over 8 °C and high soil moisture, these overwintering structures begin to germinate and develop primary sporangia. These sporangia then release about 50 flagellated zoospores each. Deposited on green host tissue by splashing water droplets, zoospores start to move in the water film searching for stomata, the entering gates for the pathogen (Langcake & Lovell, 1980). After reaching stomata, zoospores drop their flagella, encyst and form an infection peg for penetration. The step from releasing zoospores to stomata penetration can take less than 90 min (Kiefer *et al*., 2002). After invasion of the intercellular space, the tip of the infection peg extends to a vesicle from which multiple hyphae arise, colonizing the host tissue. Then, the fungus develops haustoria to feed from plant cells without killing them (Unger *et al*., 2007). In a later phase of infection, when fungal mycelium is established to the extent that sporulation is possible, certain environmental conditions are necessary for development of sporangia and zoospores; a minimum of 98% RH, at least 4 h of darkness and temperatures over 13 °C are required (Blaeser, 1978; Blaeser & Weltzien, 1978, 1979).

Since the life cycle of *P. viticola* highly depends on rainfall and increased RH, cultural practices that promote rapid drying of the canopy, such as leaf removal in the bunch zone or training systems that produce small, well ventilated canopies, are recommended to reduce infection pressure and spread of the disease (Wilcox *et al*., 2015c).

Esca and grapevine trunk diseases

One of the most disruptive disease groups in viticulture are the so called grapevine trunk diseases (GTDs), which cover a variety of symptoms on foliage, berries and vascular tissue (Gramaje *et al*., 2018). Replacement of grapevines that suffered GTDs results in annual costs of about 1.5 billion $ US worldwide (Hofstetter *et al*., 2012).

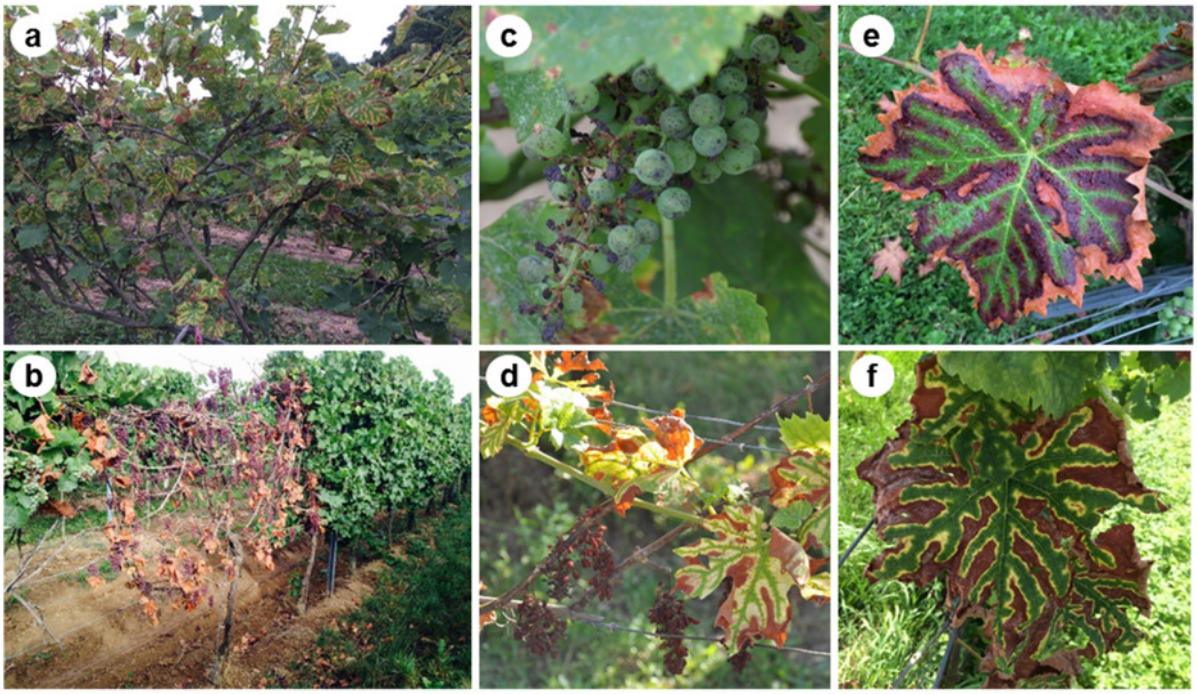

Fig. 8: Esca chronic (a) and apoplectic (b) on grapevine. Black measles on berries of an Esca affected vine (c). 'Tiger-stripe' leaf pattern, a typical symptom of Esca chronic, formed on grapevine leaves of a white (d, f) and red cultivar (e).

It is hypothesized that fungi enter the trunk mostly by pruning wounds, invade the xylem system and release symptom-inducing phytotoxins (Serra *et al.*, 2008; Andolfi *et al.*, 2011). The predominant diseases in this group are Esca, Eutypa Dieback and Botryosphaeria Dieback, each associated with one or several phytopathogenic fungi and symptoms (Bertsch *et al*., 2012). *Phaeomoniella chlamydospora*, *Phaeoacremonium* spp. and members of the basidiomyceteous genus *Fomitiporia* are related to the Esca diseases complex (Mugnai *et al.*, 1999; Larignon & Dubos, 2000; Crous & Gams, 2000; Fischer, 2002; Fischer & Kassemeyer, 2003; White *et al.*, 2011; Cloete *et al*., 2014). *Eutypa lata* and other members of the *Diatrypaceae* are associated with Eutypa Dieback, while fungi of the family *Botryosphaeriaceae* are linked to

Botryosphaeria Dieback (Trouillas *et al*., 2010, 2011; Úrbez-Torres, 2011; Luque *et al*., 2012; Úrbez-Torres *et al*., 2012; Mondello *et al*., 2013; Moyo *et al*., 2018).

In Europe, the most devastating GTD is Esca, a disease best known for its two external symptom types; chronic and apoplectic (Fig. 8; Mugnai *et al.*, 1999; Fontaine *et al*., 2016). Esca chronic comprises discoloration of leaves, forming the so called "tiger stripes" pattern, and/or withering of grape clusters. Sometimes spotting of berry skin ("black measles") appears. These symptoms can occur inconsistently over subsequent vegetation periods but end up lethal for the plant after some years (Surico, 2000). Grapevines with Esca apoplectic display a sudden wilting of leaves and grape clusters within a few days, affecting either the whole plant or discrete arms (Surico, 2009). This symptom type usually terminates the life of the vine or at least portions of it.

External symptoms, both chronic and apoplectic, begin to appear in early summer and newly symptomatic vines arise later throughout the summer (Surico *et al.*, 2000; Kuntzmann *et al.*, 2010; Lecomte *et al.*, 2012). However, the maximum peak of newly symptomatic vines is reached in mid-summer. Factors that influence occurrence of Esca related external symptoms are diverse. Among them are plant age, cultivar, grafted rootstock, grapevine microbiom and seasonal climate conditions (Marchi, 2001; Quaglia *et al.*, 2009; Bertsch *et al.*, 2012; Hofstetter *et al*., 2012; Bruez *et al.*, 2013; Kuntzmann *et al.*, 2013; Bruez *et al*., 2014; Bruez *et al.*, 2016).

No plant protection product is available on the market that successfully controls trunk diseases. At present, only phytosanitary and cultural practices can limit the spread of the diseases (Gramaje *et al*., 2018). Burning of potential inoculum material, like dead vines/arms or pruning residues, is one example to prevent new infections. Furthermore, it is recommended to reduce and protect pruning wounds (Fontaine *et al*., 2016). This can be achieved by grapevine training systems that produce less and smaller pruning wounds, e.g. minimal pruning (Gu *et al*., 2005; Lecomte *et al*., 2012; Travadon *et al*., 2016). Thereby, the risk of infection and damage to the vascular system can be decreased. Pruning wounds can be treated with a paste/liquid

containing a chemical or biocontrol agent, such as *Trichoderma*, to impede the pathogens from entering the vascular tissue (Sosnowski *et al*., 2008, 2013; Díaz & Latorre, 2013; Mutawila *et al*., 2015).

Aims of this work

Training vines in the noval SMPH system instead of the tradionall VSP system leads to significant cost savings in the production of grapes. That is why in the last decade SMPH became more popular by winegrowers. In fact, the number of SMPH vineyards is still increasing. However, information about some cultivation aspects of SMPH is marginal. One of those aspect deals with the incidendce of fungal grapevine diseases and their prevention. Therefore, it was the objective of this work to reveal whether and in which way the two training systems, i.e. intensive (VSP) and minimal (SMPH) pruning, impact the fungal grapevine community. Based on this general question, four different research areas emerged during this study, each resulting in an individual manuscript. As a common background, all four manuscripts as are indicated below address the incidence of the major fungal grapevine diseases in the two training systems and the related pathogens. However, the main focus was set on GTDs, in particular Esca, and the associated fungal pathogens. In the following, the single manuscript topics will be described in more detail.

Manuscript I:

"Effects of canopy architecture and microclimate on grapevine health in two training systems."

In the first manuscript, canopy architecture, microclimate and disease incidence of the grapevine training systems VSP and SMPH were compared. For this purpose, seven physiological properties were analysed for each training system: (1) the number and distributions of shoots and (2) inflorescences/bunches, (3) leaf area index, (4) average leaf size,

(5) canopy volume, (6) bunch architecture, and (7) berry skin characteristics. In addition, temperature and relative humidity in the canopy of two training systems were recorded, because of the high impact of the microclimate with regard to plant biocenosis. Finally, influence of the grapevine architecture and canopy microclimate on the incidence of the fungal grapevine diseases BR, PM and DM, as well as on the insect pest *Drosophila suzukii* was investigated.

The collected information shows how the plant morphology and canopy microclimate of VSP and SMPH trained vines differ from each other. Moreover, this study demonstrates to which degree these two factors have an effect on susceptibility of the two different training systems against fungal diseases, and an invasive insect pest. This may help grapevine growers to better estimate the threat of grapevine diseases for SMPH trained vineyards and thus adapt their plant protection management.

Manuscript II:

"Temporal development of the culturable, endophytic fungal community in healthy grapevine branches and occurrence of GTD-associated fungi."

This manuscript is focused on the endophytic fungal community in healthy grapevine branches. Since perennial branches represent the most outstanding feature of SMPH, the question emerged, whether these branches, compared to the annual branches of VSP trained vines, may have a positive or negative influence on plant vigour by means of occurrence of GTD-associated fungi. Therefore, the endophytic fungal community in grapevine branches of eight different age-groups between two months and eight years was analysed based on a cultural approach. In particular, the incidence of GTD-associated fungi in annual branches (one year and younger) of VSP trained vines and in perennial branches (two years and older) of SMPH vines were compared. Furthermore, it was of high interest to investigate the temporal development of the fungal community from young shoots to perennial branches to find out which fungi are primary settlers and which fungi are dominating in perennial branches.

This study serves not only to estimate the risk of GTD for SMPH trained grapevines, but also improves our knowledge about the development of the fungal community in grapevine wood. Besides, fungi playing a major role as pathogens should be surveyed both in their impact and incidence over time.

Manuscript III:

"Esca in German vineyards: Does the training system influence occurrence of foliar symptoms?"

The third manuscript deals with one of the most destructive GTDs worldwide, i.e. Esca. In a four year survey, incidence of foliar symptoms caused by this trunk disease was monitored in twelve vineyards of different age and cultivars, trained in either VSP or SMPH. The foremost objective of this work was to reveal whether the training system has a significant impact on the occurrence of external symptoms, both chronic and apoplectic. Furthermore, the influence of plant age, grapevine cultivar and precipitation on the visible disease outbreak was studied, as well as the appearance of newly affected vines over the season.

The results should give first evidence for a possible effect of the training system on the incidence of Esca and help to assess the susceptibility of intensive (VSP) and minimal (SMPH) pruned vines against the disease, which is of high importance for grapevine growers. Moreover, based on a continuous monitoring the findings of this study should enhance our basic knowledge about the epidemiology and impact of Esca in order to develop countermeasures and thus prevent further spreading.

Manuscript IV

"New species of *Phaeomoniellales* from a German vineyard and their potential threat to grapevine (*Vitis vinifera*) health."

The monitoring of airborne spores of the Esca associated fungi *Phaeomoniella chlamydospora* and its relatives, within the *Pheaomoniellales*, is the topic of the fourth

manuscript. For this work, spore traps based on sticky glass slides were closely attached to annual branches of VSP trained vines and perennial branches of SMPH trained vines in order to compare the incidence of *P. chlamydospora* spores between the training systems. In addition to spores of *P. chlamydospora,* other fungi of the order *Phaeomoniellales* were isolated from the spore traps, some of them known species, some of them so far undescribed. Since members of this order has gained significance as pathogens of economically important crop plants in the last years, it was one major aim of this study to describe these new species and to conduct a phylogenetic analysis. Furthermore, a pathogenicity test with potted plants was done to clarify whether the trapped fungi display the same pathogenicity against grapevine as one of the main causal agents of Esca, *P. chlamydospora*.

Introduction of new species into the *Phaeomoniellales* combined with a phylogenetic analysis of the order should provide new information about this relatively unknown, but important group of fungi both with respect to pathogenicity and geographic distribution. In addition, the study answers the question whether *P. chlamydospora* is the only member of the *Phaeomoniellales* that displays a significant pathogenicity against grapevine.

Chapter TWO

Manuscripts

Manuscript I

Effects of canopy architecture and microclimate on grapevine health in two training systems.

Kraus, C., Pennington, T., Herzog, K., Hecht, A., Fischer, M., Voegele, R.T., Hoffmann, C., Töpfer, T. & Kicherer, A. (2018). Vitis 57, 53–60.

Abstract

Semi minimal pruned hedge (SMPH) is a time and cost saving grapevine training system, which is becoming more and more popular in German viticulture. In this study we compared the canopy architecture and its effect on the microclimate of SMPH trained grapevines with those of plants trained in vertical shoot positioning (VSP). We detected a 3% points higher humidity and a 0.9 °C lower mean temperature within the complex canopy architecture of SMPH trained vines compared to VSP. Moreover, we investigated the influence of the differing microclimate, canopy and bunch architecture, as well as berry skin characteristics of the two training systems on the incidence of the major fungal grapevine diseases Downy Mildew, Powdery Mildew and Botrytis Bunch Rot, as well as on the occurrence and damage of the invasive insect pest *Drosophila suzukii*. We demonstrate that SMPH trained vines can be more susceptible to Downy Mildew and Powdery Mildew than VSP trained vines. The incidence of Botrytis Bunch Rot can be higher in the latter system, even if berry skin characteristics are the same in both training systems. We trapped a higher number of *D. suzukii* in SMPH canopies, however no increased berry damage was observed. Based on our results we recommend a more adapted plant protection regime for SMPH trained vines due to their higher susceptibility to the major fungal diseases. Furthermore, we propose a combination of SMPH and fungal resistant grapevine cultivars, e.g. 'Reberger', to achieve a more competitive, environmentally friendly and high quality grapevine production.

Keywords: Downy Mildew; *Drosophila suzukii*; PIWI; plant architecture; Powdery Mildew; training system; viticulture; *Vitis vinifera* ssp. *vinifera*

Introduction

Traditionally, grapevine in Germany is cultivated in the vertical shoot positioning (VSP) system, which is typical for cool climates. This type of grapevine training enables farmers to manage grape yield and quality by controlling the number of buds and their optimal distribution in the trellis (Jackson, 1996). However, the farmer has to undertake a time consuming winter pruning and wire positioning during the season, which causes high labor costs (Clingeleffer, 1993). In order to reduce the costs of manual labor, a novel training method called semi minimal pruned hedge (SMPH) was introduced. The mechanization of pruning, which is the basis of SMPH, in combination with the omission of wire positioning, reduces labor costs to a minimum (Clingeleffer, 1993). This makes SMPH a highly efficient and competitive grapevine production system, which is easily applicable by grapevine growers.

Cultivation of grapevines in SMPH affects plant physiology and as a consequence plant morphology. Compared to VSP, bunches of SMPH trained plants weigh less and have a more loose architecture, due to the fact that they consist of fewer and smaller berries (Intrieri *et al.*, 2011). Despite smaller bunches, the number of inflorescences and bunches per plant is elevated in minimal pruned grapevines and thus the yield per plant is enhanced in contrast to the traditional training system (Clingeleffer & Possingham, 1987; Wolf *et al*., 2003). The average leaf size in SMPH is smaller than in VSP vines (Sommer *et al.*, 1993), but the total leaf number per vine and hence the total leaf area (m^2/m of row) is higher if the grapevines are minimally pruned (Clingeleffer & Posingham, 1987; Schmid & Schultz, 2000; Intrieri *et al.*, 2001). We expect that these vast differences in canopy architecture between SMPH and VSP affect the grapevine microclimate. Because of the increased leaf volume SMPH canopies should show poor air movement and less light penetration. We therefore expect a lower temperature and a higher humidity in SMPH canopies than in the less voluminous VSP canopies.

European grapevine, *Vitis vinifera*, is threatened by several pests. Fungal diseases such as Downy Mildew (DM, caused by *Plasmopara viticola*), Powdery Mildew (PM, caused by *Erysiphe necator*) and Botrytis Bunch Rot (BR, caused by *Botrytis cinerea*) are the most destructive. Their development and spreading in the vineyard can be influenced by canopy management. Since disease progress of DM and BR is facilitated by a warm and moist climate, training systems which increase air movement and light penetration are beneficial for controlling these pathogens in the vineyard (Coombe & Dry, 1992). Canopy management during the season such as leaf removal in the bunch zone can additionally reduce wetness and improve light penetration, creating an environment which is less favorable for PM and BR (Gubler *et al.*, 1987; Austin & Wilcox, 2011). In addition, characteristics of the berry skin, e.g. thickness of the berry skin and of the cuticle, are described as further important traits influencing susceptibility against BR (Commenil *et al.*, 1997; Gabler *et al.*, 2003; Becker & Knoche 2012a, b; Herzog *et al.*, 2015).

We expect a higher incidence of DM in SMPH because of the elevated humidity and reduced light penetration in the canopy compared to VSP. Concerning *Botrytis* we assume a decreased rate of BR in SMPH panels as result of the loose bunch architecture. Furthermore we expect the incidence of PM to be elevated in SMPH panels, due to the more favorable microclimate and reduced light penetration (Gadoury *et al.*, 2012).

Drosophila suzukii (Matsumura, Diptera: *Drosophilidae*), also known as spotted wing drosophila (SWD) is a pest insect native to Asia which has recently spread to the Americas and Europe (Cini *et al*. 2012). In contrast to the common fruit fly *Drosophila melanogaster* which is attracted by overripe or rotten fruit, SWD prefers ripening or ripe red fruit. It may penetrate intact fruit skin with its serrated ovipositor and deposit eggs inside the fruit (Lee *et al.*, 2011). However, laboratory experiments revealed that artificially damaged berries are more attractive for the fly than intact ones (Ioriatti *et al.*, 2015; Jarausch *et al.*, 2017). SWD damage can be both direct through larval feeding and indirect, since oviposition leaves the fruit skin damaged

and susceptible to secondary pathogens such as bacteria and fungi (Ioriatti *et al.*, 2015). SWD has a wide range of host plants including blueberries, strawberries, cherries, and plums, as well as grapevine (Rouzes *et al.*, 2012; Bellamy *et al.*, 2013). Since SWD prefers humid conditions on a large as well as on a smaller scale (Hauser *et al.*, 2009; Tochen *et al.*, 2016) we hypothesize that more flies can be trapped in SMPH panels with their more voluminous canopy than in VSP. As a result of the higher density of SWD we expect a higher infestation rate of grapes in SMPH than in VSP trained grapevines. In addition to the microclimate, the characteristics of the grape skin might further influence the damage by SWD on berries. Thicker and more resilient skin might reduce the egg laying success of SWD females in SMPH trained grapevines (Ioratti *et al.*, 2015).

Materials and Methods

Plant material and cultivation practices: For this study the *Vitis vinifera* ssp. *vinifera* cultivars 'Chardonnay' (planted 2008) and 'Reberger' (planted 2001) were used. Vines were planted at the experimental vineyards of Geilweilerhof located at Siebeldingen, Germany (N 49°21.747, E 8°04.678). Since 2013, half of the rows of each cultivar were pruned mechanically and thereby converted to the SMPH system. Inter-row distance is 2 m and grapevine spacing is 1 m. For pest control plants were treated with an organic plant protection regime consisting of wettable sulphur (AgroStulln, Stulln, Germany), Funguran progress (Spiess-Urania, Hamburg, Germany) and Vitisan (Biofa, Münsingen, Germany). Pesticides were applied fortnightly, 12 times during the season. In 2017 conventional pesticides (Polyram WG, Enervin, Vivando; BASF SE, Ludwigshafen, Germany) were used in the first three plant protection applications, because of the severe plant damage caused by *P. viticola* and *E. necator* in the previous year. After flowering both panels, SMPH and VSP, were pruned mechanically.

All experiments and measurements were performed during the growing season 2016 and 2017. Phenological development of grapevines was determined using the BBCH scale according to Lorenz *et al.* (1995).

Canopy architecture: To compare the canopy architecture six main characters were chosen and analyzed for each cultivar and training system: (1) the number and distribution of shoots at bud burst (BBCH 10) was evaluated in four random 50 cm wide canopy sections, divided into five horizontal zones (Fig. S1 suppl. data); (2) based on this scheme the number and distribution of inflorescences/bunches at the phenological stages flowering (BBCH 65), pea size (BBCH 75) and veraison (BBCH 81) was determined; (3) for calculation of the leaf area index (LAI) all leaves from four random 50 cm wide canopy sections were removed and measured with a leaf area meter (Modell 3100 area meter, Li- COR, Lincoln, Nebrasca, USA); (4) fifty randomly selected leaves were measured to calculate the average leaf size; (5) the

canopy volume was calculated as the product of canopy height [m] x canopy width [m] x 10.000 m², divided by the inter-row distance [m] (Siegfried & Sacchelli, 2005). LAI, average leaf size, and canopy volume were also determined at flowering (BBCH 65), pea size (BBCH 75) and veraison (BBCH 81); (6) weight [g], length [cm] and width [cm] of ten randomly selected bunches were recorded as indicator for bunch architecture. Additionally, average berry number and size [mm] of 10 berries per bunch was measured. Bunch architecture was evaluated at ripening (BBCH 89). Data were analyzed using t tests in R (R Core Team, 2013).

Berry skin characteristics: Physical and morphological berry skin characteristics were determined of 'Reberger' (VSP and SMPH) at ripening stage (BBCH 89) and before harvest. First, impedance of the berry cuticle was measured at room temperature by using the I-Sensor from 30 berries per training system, 17% Brix and relative impedance Z_{rel} was calculated according to Herzog *et al*. (2015).

The TA.XT Texture analyzer (Stable Micro System, Godalming, Surrey, UK) was used to evaluate the penetration resistance of berries by mean of maximum break force [N] and skin break energy [mJ]. Settings were used according to Letaief *et al.* (2008). For each training system 50 berries were randomly harvested. Results were recorded with software Exponent Lite Express (Stable Micro System, Godalming, Surrey, UK) results were recorded.

The thickness of berry skin was measured using light microscopy in order to detect morphological differences between SMPH and VSP berries. Skin sections of 20 frozen berries were cut from the side and sliced into 6-8 µm thick discs with a cryomicrotome (Micro HM 525, Thermo Scientific, Waltham, Massachusetts, USA). 15 skin slices per berry were then fixed on a protein glycerol coated object plate and stained in an Astra Blue solution. Using Leica Application Suite 4.3 and a Leica DM 4000 B light microscope (Leica Microsystems GmbH, Wetzlar, Germany) under 100-fold magnification, the thickness of the berry skins was determined. All means were compared using t tests in R (R Core Team, 2013).

Microclimate: Temperature and relative humidity in the grapevine canopy were recorded with Tinytag Plus 2 data loggers (Gemini Data Logger Ltd, Chichester, UK). Three loggers per training system and variety were positioned 150 cm above ground in the canopy at random locations in the vineyard. Microclimate measurements were started when three leaves were visible (BBCH 13) and ended by the time of ripening (BBCH 89) with a recording interval of 1 h. For adjustment and reading of the loggers as well as for data evaluation the Tinytag Explorer Software (Gemini Data Logger Ltd) was used. Local climate data including mean temperature, total rainfall and leaf wetness were obtained from the institute DLR Rhineland-Palatinate (www.am.rlp.de). For statistical evaluation of the mean values a permutation test with the program R was performed (R Core Team, 2013).

Assessment of fungal diseases: Monitoring of fungal grapevine diseases was done according to the European and Mediterranean Plant Protection Organization (EPPO) guidelines: *Plasmopara viticola* (PP 1/31(3)), *Erysiphe necator* (PP 1/4(4)), *Botrytis cinerea* (PP 1/17(3)). For each training system and variety 100 grape bunches were screened and rated for disease symptoms of the particular fungal pathogen. The score ranged from 0% (no symptoms) to 100% (symptoms on the whole bunch) with a scaling interval of 10%. Additionally, a scoring of 5% was added to the ranking for the assessment of minimal symptoms. With this method we determined both incidence rate and level. For statistical evaluation Fisher's exact test for the incidence rate and Kruskal-Wallis test for the incidence level was performed with the program R (R Core Team, 2013).

Spotted wing drosophila (SWD): Trap design and evaluation: During the season the occurrence of SWD in the two training systems was evaluated from BBCH 83 to BBCH 89 in the variety 'Reberger'. SWD appears almost exclusively on red varieties, which is why the white 'Chardonnay' variety was not sampled for this experiment (Saguez *et al.*, 2013). Three traps per training system were randomly distributed in the canopy and analyzed weekly for four weeks. A trap consisted of a 500 mL clear plastic drinking vessel with lid. The vessel was manipulated

in the upper third by affixing a red tape and drilling 15 holes with a diameter of 1 mm into it. As trapping liquid we used 100 mL of a 1:1 mixture of water and unfiltered cider vinegar plus a drop of wetting agent (Tween® 20, Sigma-Aldrich, Munich, Germany). *Drosophila suzukii* flies were counted through a stereomicroscope (Zeiss, Jena, Germany). Statistical analysis was done using t test in R (R Core team, 2013).

SWD: Berry infestation rate: Between BBCH 83 to 89, 50 intact berries per training system were collected weekly from random vines and different bunches within the 'Reberger' variety to evaluate the infestation rate. Oviposition of SWD was observed under a stereomicroscope (Zeiss) and the number of eggs per 50 berries was counted. Data was analyzed using t tests in R (R Core Team, 2013).

Results

Canopy architecture

In Tab. 1 all investigated characteristics of the canopy architecture for 2016 (a) and 2017 (b) are listed (the complete Table including a comparison of the different trellis zones can be seen in Tab. S1, suppl. data). The number of shoots per 0.5 m was significantly higher in SMPH panels than in VSP panels in both years: Eight to 15 times for 'Chardonnay' and six to eleven times for 'Reberger'. We also noticed differences in shoot distribution between the two training systems. While the majority of the shoots were found in the upper zones (Tab. S1, 3-5) in the SMPH trellis, shoots in the VSP trellis were almost completely restricted to the lower zones (Tab. S1, 1-2) and virtually equally distributed.

A similar result was observed for the number of inflorescences/bunches per 0.5 m. In 2016 and 2017 at BBCH 65 the total amount of inflorescences in the 'Chardonnay' field was two to four times higher for SMPH compared to VSP and three to six times higher in the 'Reberger' field. The majority of SMPH inflorescences/bunches were located in the higher zones (Tab. S1, 4 and 5). In the VSP panel the inflorescences/bunches were most frequently found in zone two and three.

For 'Chardonnay' the LAI and the canopy volume were at least 1.5 times higher in the SMPH than in the VSP panel during the complete seasons of both experimental years, except for BBCH 75 in 2017 (Tab. 1). In the 'Reberger' variety both parameters showed significant differences between the training systems the entire season of 2017. In 'Chardonnay', leaves of VSP plants were 1.5 times bigger than leaves from SMPH plants at all phenological stages. For 'Reberger' this was only the case at BBCH 75 in 2016 and at BBCH 65 in 2017.

Regarding bunch architecture, all investigated characteristics, weight, length, width, number of berries and mean berry size, were significantly higher for VSP bunches compared to SMPH bunches in both years and cultivars, except for 'Reberger' in 2016. For 'Chardonnay' the

differences in bunch architecture between the two training systems are clearer in 2017 than in the previous year.

Tab. 1: Canopy architecture characteristics of the two grapevine varieties 'Chardonnay' and 'Reberger' as a function of training system, 2016 (a) and 2017 (b). T test; *p<0.05, **p<0.001.

a)

2016		Chardonnay			Reberger		
	BBCH	SMPH	VSP		SMPH	VSP	
Number of shoots [per 0.5m]	10	68.3 ± 9.6	8.3 ± 1.3	**	44.4 ± 6.9	6.5 ± 1.8	**
Number of	65	40.0 ± 5.9	9.0 ± 0.8	**	30.0 ± 7.9	9.3 ± 3.1	*
inflorescences/bunches	75	19.0 ± 7.7	9.5 ± 3.7	n.s.	25.0 ± 11.5	7.8 ± 3.1	n.s.
[per 0.5m]	81	11.5 ± 2.4	7.5 ± 3.7	n.s.	10.3 ± 3.8	9.5 ± 5.8	n.s.
LAI	65	4.7 ± 0.7	1.0 ± 0.1	**	2.6 ± 1.0	1.2 ± 0.1	n.s.
	75	2.8 ± 0.2	1.7 ± 0.3	*	2.6 ± 0.5	1.9 ± 0.4	n.s.
	81	3.1 ± 0.3	1.8 ± 0.4	*	3.2 ± 0.5	1.9 ± 0.6	*
Average leaf size [cm²]	65	65.9 ± 6.6	106.7 ± 11.7	*	87.3 ± 4.2	107.4 ± 36.6	n.s.
	75	59.3 ± 3.1	85.0 ± 10.2	*	70.9 ± 4.6	119.5 ± 13.7	**
	81	51.2 ± 3.9	74.4 ± 5.8	**	76.0 ± 13.6	99.5 ± 2.6	n.s.
Canopy volume [m³]	65	47046.0 ± 6770.8	9945.5 ± 1122.6	**	25884.4 ± 10246.0	12094.6 ± 728.5	n.s.
	75	28041.6 ± 2350.2	17234.5 ± 3139.9	*	26322.2 ± 4797.8	18604.0 ± 3796.0	n.s.
	81	31120.9 ± 2787.9	17986.3 ± 3139.9	**	31812.4 ± 4608.5	19209.8 ± 5902.6	*
Bunch weight [g]	89	96.5 ± 37.3	152.3 ± 40.0	*	126.7 ± 27.1	182.6 ± 69.0	*
Bunch length [cm]	89	11.2 ± 2.0	12.9 ± 1.1	*	11.3 ± 2.5	13.3 ± 3.3	n.s.
Bunch width [cm]	89	6.9 ± 1.3	8.7 ± 1.8	*	8.7 ± 1.9	9.6 ± 1.1	n.s.
Berry number per bunch	89	78.1 ± 26.9	110.1 ± 27.5	*	71.3 ± 17.1	85.6 ± 24.1	n.s.
Ø berry size [mm]	89	12.0 ± 1.0	12.4 ± 1.0	*	14.2 ± 1.2	14.2 ± 1.3	n.s.

Tab. 1 continued

b)

2017		Chardonnay			Reberger		
	BBCH	SMPH	VSP		SMPH	VSP	
Number of shoots [per 0.5m]	10	150.0 ± 14.4	10.0 ± 1.2	**	101.0 ± 11.3	9.0 ± 0.8	**
Number of	65	18.5 ± 6.5	9.8 ± 2.2	n.s.	52.0 ± 15.4	8.0 ± 2.1	*
inflorescences/bunches	75	19.0 ± 7.5	9.0 ± 0.8	*	18.0 ± 8.0	7.8 ± 3.0	n.s.
[per 0.5m]	81	12.3 ± 4.5	9.8 ± 2.2	n.s.	27.5 ± 6.4	7.0 ± 1.6	n.s.
LAI	65	3.5 ± 0.4	1.1 ± 0.3	**	3.7 ± 0.3	0.8 ± 0.1	**
	75	3.0 ± 0.3	2.6 ± 0.5	n.s.	2.6 ± 0.5	1.5 ± 0.5	*
	81	3.5 ± 0.7	2.1 ± 0.4	*	4.0 ± 0.6	2.1 ± 0.2	*
Average leaf size [cm²]	65	60.4 ± 5.7	105.3 ± 6.7	**	68.1 ± 10.6	98.8 ± 8.4	*
	75	60.7 ± 6.9	76.2 ± 8.7	*	79.7 ± 16.2	95.0 ± 9.8	n.s.
	81	63.4 ± 3.6	96.8 ± 5.6	**	84.0 ± 10.2	96.9 ± 7.7	n.s.
Canopy volume [m³]	65	35039.0 ± 4414.3	10726.4 ± 2860.0	**	36713.1 ± 3465.7	8288.9 ± 1196.5	**
	75	30205.5 ± 3148.3	25861.5 ± 4969.6	n.s.	26167.3 ± 5174.9	15326.1 ± 5108.7	*
	81	34957.6 ± 6964.7	20611.1 ± 4041.3	*	40488.8 ± 6456.3	20934.6 ± 1851.5	*
Bunch weight [g]	89	76.2 ± 34.8	163.7 ± 33.0	**	101.4 ± 35.7	257.7 ± 83.5	**
Bunch length [cm]	89	8.7 ± 2.6	12.0 ± 1.4	*	10.9 ± 2.3	14.7 ± 2.2	*
Bunch width [cm]	89	6.9 ± 1.7	8.9 ± 1.6	*	6.9 ± 1.7	8.9 ± 1.6	*
Berry number per bunch	89	57.8 ± 23.7	125.1 ± 26.8	**	56.0 ± 20.5	134.1 ± 45.5	**
Ø berry size [mm]	89	12.2 ± 1.0	13.2 ± 1.3	**	12.8 ± 2.0	15.3 ± 1.7	**

Berry skin characteristics

No significant differences were detected between the two training systems in terms of investigated berry skin characteristics, *i.e.* impedance of the cuticle, maximum break force, skin break energy and berry skin thickness (Tab. S2, suppl. data).

Microclimate

The evaluation of microclimate as a function of the training system showed significant differences between SMPH and VSP, predominantly in the 'Chardonnay' field in 2016 (Tab. 2 a). In the first year of the study, increased leaf wetness was measured in BBCH 13-71 and 83-89 due to rainfall and morning dew, respectively. At this time the relative humidity in the SMPH canopy was significantly higher and the temperature lower compared to VSP canopies. This was also the case for 'Reberger', but only at BBCH 13-71. However, in 2017 (Tab. 2 b), when the leaf wetness only reached a maximum of 29% in spite of intense rainfall, minor differences in the canopy microclimate between the two training systems were noted.

A more detailed look on the canopy microclimate during the course of the day revealed that the relative humidity in the SMPH canopy is up to 20% points higher compared to VSP after a rain event, while the temperature in the two training systems may differ by up to 3 °C (Fig. 1 b, 2nd d, 12:00 h). Similar results could also be observed during morning dew (Fig. 1 a and b, 3rd d, 7:00 h). These observations were made in both trial vineyards, 'Chardonnay' and 'Reberger'.

Tab. 2: Local and micro climate during different phenological stages in the trial fields 'Chardonnay' and 'Reberger' as a function of grapevine training system, 2016 (a) and 2017 (b). Except for 'total rainfall' all parameters are mean values. Permutation test; *$p<0.05$, **$p<0.001$.

a)
2016

	local climate				Chardonnay			Reberger		
BBCH	Temperature [°C]	total rainfall [mm]	leaf wetness [%]	Microclimate	SMPH	VSP		SMPH	VSP	
13 - 71	17.4	131.4	49.1	Relative humidity [%]	83.4 ± 17.5	80.5 ± 19.7	**	83.3 ± 16.7	80.0 ± 20.4	**
				Temperature [°C]	18.2 ± 5.3	18.7 ± 5.7	*	17.9 ± 4.8	18.8 ± 6.0	**
71 - 83	19.8	63.1	28.8	Relative humidity [%]	75.4 ± 19.6	72.7 ± 20.2	*	74.7 ± 74.7	74.3 ± 19.9	n.s.
				Temperature [°C]	20.7 ± 5.8	20.9 ± 5.0	n.s.	20.4 ± 5.5	20.5 ± 5.6	n.s.
83 - 89	18.3	32.6	44.6	Relative humidity [%]	77.9 ± 18.8	74.8 ± 20.5	**	75.2 ± 21.0	75.4 ± 21.0	n.s.
				Temperature [°C]	18.8 ± 6.1	19.3 ± 5.5	*	19.1 ± 7.0	18.9 ± 6.7	n.s.

b)
2017

	local climate				Chardonnay			Reberger		
BBCH	Temperature [°C]	total rainfall [mm]	leaf wetness [%]	Microclimate	SMPH	VSP		SMPH	VSP	
13 - 71	18.8	74.8	19.7	Relative humidity [%]	69.6 ± 21.1	67.7 ± 22.4	n.s.	68.9 ± 21.4	68.1 ± 20.5	n.s.
				Temperature [°C]	19.6 ± 6.8	20.2 ± 7.2	n.s.	19.5 ± 6.6	20.4 ± 7.3	*
71 - 83	20.7	65.6	23.0	Relative humidity [%]	73.1 ± 20.4	71.7 ± 20.4	n.s.	72.9 ± 20.1	73.6 ± 19.7	n.s.
				Temperature [°C]	21.3 ± 5.9	21.5 ± 5.9	n.s.	21.1 ± 5.6	21.5 ± 5.9	n.s.
83 - 89	17.1	145.2	29.0	Relative humidity [%]	78.7 ± 18.1	76.8 ± 18.8	*	77.3 ± 18.3	78.1 ± 17.9	n.s.
				Temperature [°C]	19.0 ± 4.9	19.3 ± 5.2	n.s.	19.1 ± 4.8	19.5 ± 5.1	n.s.

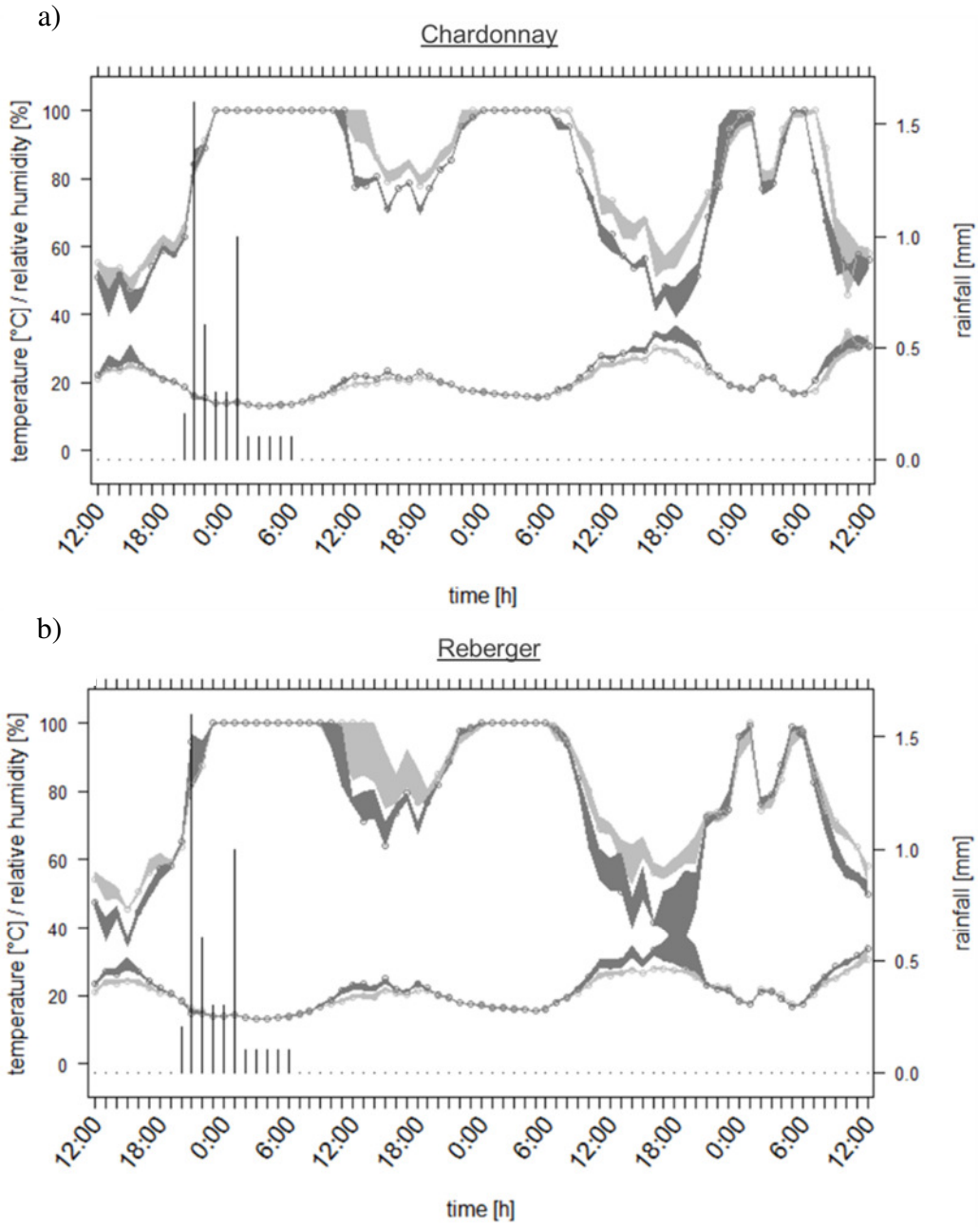

Fig. 1: 72 h recording section of the microclimate data from 20th to 23th of June 2016 in the two trial fields 'Chardonnay' (a) and 'Reberger' (b) as a function of grapevine training system SMPH (bright) and VSP (dark). Relative humidity is shown in the upper lines, temperature in the lower lines. Black columns represent rainfall [mm].

Fungal diseases

At the first DM assessment in 2016, when young fruits begin to swell (BBCH 71), 25% of the VSP and 48% of the SMPH bunches in the 'Chardonnay' trial field were infected with *P. viticola* (Tab. 3). The mean incidence level for SMPH reached 19.3% and was significantly higher compared to VSP with 2.9%. At beginning of veraison, 92% of the SMPH bunches and almost all examined VSP bunches showed DM symptoms. The incidence level was 43.3% for SMPH and 37.7% for VSP. No significant differences between the two training system could be observed at the second DM assessment.

Tab. 3: Assessment results for the fungal grapevine diseases Downy Mildew, Powdery Mildew and Botrytis Bunch Rot as a function of grapevine training system, 2016 (a) and 2017 (b). Statistical analysis was done with Fisher´s exact test for the incidence rate and Kruskal-Wallis test for the incidence level; *p<0.05, **p<0.001.

a)			Chardonnay			Reberger		
2016	BBCH	Incidence	SMPH	VSP		SMPH	VSP	
Downy Mildew	71	rate [%]	48	25	**	48	28	**
	71	level [%]	19.3 ± 26.7	2.9 ± 6.4	**	13.3 ± 22.4	3.8 ± 8.2	**
	83	rate [%]	92	99	n.s.	96	100	n.s.
	83	level [%]	43.3 ± 37.6	37.7 ± 27.2	n.s.	46.5 ± 35.8	45.2 ± 33.3	n.s.
Powdery Mildew	83	rate [%]	78	76	n.s.	0	0	n.s.
	83	level [%]	22.2 ± 23.0	25.8 ± 27.7	n.s.	0 ± 0	0 ± 0	n.s.
	85	rate [%]	97	100	n.s.	0	0	n.s.
	85	level [%]	63.3 ± 29.0	62.1 ± 31.4	n.s.	0 ± 0	0 ± 0	n.s.
Botrytis Bunch Rot	89	rate [%]	3	5	n.s.	0	0	n.s.
	89	level [%]	0.2 ± 1.2	0.6 ± 3.0	n.s.	0 ± 0	0 ± 0	n.s.

b)			Chardonnay			Reberger		
2017	BBCH	Incidence	SMPH	VSP		SMPH	VSP	
Downy Mildew	71	rate [%]	0	0	n.s.	0	0	n.s.
	71	level [%]	0 ± 0	0 ± 0	n.s.	0 ± 0	0 ± 0	n.s.
	83	rate [%]	0	0	n.s.	0	0	n.s.
	83	level [%]	0 ± 0	0 ± 0	n.s.	0 ± 0	0 ± 0	n.s.
Powdery Mildew	83	rate [%]	77	48	**	3	2	n.s.
	83	level [%]	18.2 ± 23.0	6.4 ± 12.0	**	0.3 ± 2.1	0.3 ± 2.1	n.s.
	85	rate [%]	98	94	n.s.	0	0	n.s.
	85	level [%]	47.4 ± 27.9	36.5 ± 29.3	*	0 ± 0	0 ± 0	n.s.
Botrytis Bunch Rot	89	rate [%]	11	42	**	6	11	n.s.
	89	level [%]	1.8 ± 8.0	8.7 ± 14.4	**	0.4 ± 1.5	2.6 ± 10.5	n.s.

Similar results were observed in the 'Reberger' field. In the beginning of the DM infection process, at BBCH 71, 48% of the SMPH and 28% of the VSP bunches showed symptoms. With incidence levels of 13.3% for SMPH and 3.8% for VSP, a significant difference between the training systems could be noticed at this point of plant development. At

BBCH 83 an increase of infection pressure was evident in both experimental panels, which led to disease symptom in 96% of the sampled SMPH and 100% of the VSP bunches. In the SMPH panel the mean infection level was 46.5% and for VSP 45.2%, resulting in no significant differences between the training systems. The assessment results for DM in 2017 revealed no infection in either of the both experimental fields during the whole season.

In the 'Chardonnay' trial field in 2016 78% SMPH and 76% VSP bunches were infected with PM at the beginning of ripening (BBCH 83). The mean incidence level was 22.2% for SMPH and 25.8% for VSP. At BBCH stage 85 when the berries started to soften, 97% of the SMPH bunches and all tested VSP bunches showed symptoms of *E. necator*. Additionally, an increase in the infection level occurred. Both training systems had a similar mean value of about 62.5%. In the subsequent year 29% more infected bunches were found in the SMPH panel than in the VSP at BBCH 83. The incidence level was three times higher for infected SMPH bunches compared to the VSP bunches. At the second PM assessment the number of infected bunches was almost equal in both training systems. However, the incidence level was still significantly higher in the SMPH panel.

During the whole season 2016 and 2017 no or only minimal PM symptoms could be observed in the trial field of the grapevine cultivar 'Reberger'. For 2016 no severe *Botrytis* infection could be noticed in either of the both grapevine cultivars until ripening (BBCH 89). Only 3% of the SMPH and 5% of the VSP bunches in the 'Chardonnay' field showed slight symptoms of BR and all examined 'Reberger' bunches were free of BR. In 2017 significant differences between the two training systems regarding BR infection could be noticed, at least in the 'Chardonnay' field. Here, 42% of the monitored VSP and 11% of the SMPH bunches showed BR symptoms. The incidence level was almost five times higher in the VSP panel compared to the SMPH. Also in the 'Reberger' field BR symptoms could be observed, but the incidence rate as well as the incidence level was quite low with no significant differences between both training systems.

Drosophila suzukii

In both seasons the mean number of *D. suzukii* flies was up to two times higher in SMPH compared to VSP trained grapevine (Tab. 4). However, only in 2017 this difference is significant. Despite this striking difference we did not observe a higher number of eggs on intact SMPH grape berries. In both panels the number of detected eggs on grapevine berries was marginal over the two seasons and no differences between the training systems were observed.

Tab. 4: Number of trapped SWD flies in SMPH and VSP trained 'Reberger' vineyards (mean value, n=12 traps). Counted *D. suzukii* eggs on 50 randomly selected SMPH and VSP berries with intact skin (mean value, n=4 runs). Results from 2016 (a) and 2017 (b) are shown. T test; *$p<0.05$, **$p<0.001$.

a)

2016	SMPH	VSP	
mean no. of trapped *D. suzukii* flies	31.3 ± 18.7	20.5 ± 20.2	n.s.
mean number of eggs per 50 berries	2.3 ± 3.3	0.5 ± 1.0	n.s.

b)

2017	SMPH	VSP	
mean no. of trapped *D. suzukii* flies	10.3 ± 6.6	4.8 ± 2.6	*
mean number of eggs per 50 berries	0.8 ± 1.0	1.0 ± 1.2	n.s.

Discussion

The aim of this study was to compare two training systems, SMPH and VSP, with regard to canopy architecture, berry skin characteristics, microclimate and the influence of those factors on incidence of common fungal grapevine pathogens as well as the damage caused by the invasive insect pest *Drosophila suzukii* in German viticulture. We found that the large amount of leaves produced by SMPH trained plants create a bigger and denser canopy structure than VSP trained plants, even if the leaves of the latter are larger in size. SMPH bunches showed a looser structure than VSP bunches, due to a smaller architecture, a reduced number of berries and smaller sized fruits. These findings are in line with other studies, which compared the morphology of minimal and intensely pruned grapevines (Clingeleffer & Possingham, 1987; Sommer *et al.*, 1993; Schmid & Schultz, 2000; Wolf *et al.*, 2003; Intrieri *et al.*, 2011). The analyses of canopy architecture in the different trellis zones demonstrate that the plant vigor in the SMPH system is mainly located in the upper zones (3-5), while in the VSP system it is restricted to the lower zones (1-2), perhaps because of the apical dominance (Jackson, 1996).

These differences in plant morphology between the two training system have a clear effect on the microclimate in the canopy. SMPH canopies dry much slower and need several hours longer after rain or morning dew to achieve a similar humidity level as VSP canopies. Local weather increased leaf wetness and lead to a higher relative humidity, but lower temperature in SMPH canopies compared to VSP canopies. This is clearer for 'Chardonnay' than for 'Reberger', probably because of the hillside location of the latter. The dense leaf structure of the SMPH plants prevents sunlight from reaching the inside of the canopy, thus the moisture takes longer to evaporate. Additionally, air movement is reduced, which also impedes the canopy from drying.

Under certain climate conditions with high leaf wetness, as observed in 2016, we found SMPH trained grapevines more susceptible to DM than VSP trained, for 'Chardonnay' and

'Reberger'. However, in the second assessment no significant differences between the two training systems were found. This is probably caused by a considerable decline of SMPH inflorescences/bunches caused by DM, which are not included in the assessment made at BBCH 83. *Plasmopara viticola*, the causal agent of DM, needs an environment rich in moisture for successful infection and spreading (Blaeser & Weltzien, 1978, 1979). It is possible that the slower drying of the SMPH canopies provides an extended time frame for *P. viticola* to successfully infect grapevine tissue after rain or morning dew.

SMPH bunches were more sensitive to PM infection than VSP bunches in 'Chardonnay' in 2017. The results of Austin *et al.* (2011) demonstrate that training systems showing a high light penetration in the fruit zone are less susceptible to PM, due to sunlight exposure of the pathogen and improved pesticide deposition. According to this assumption, VSP should be the more robust training system, since the SMPH bunches are more often located within the dense leaf canopy. This was only the case in 2017. In the previous year no differences in PM incidence between the two training systems could be observed. Since the infection pressure of DM and PM reached an extraordinary high level in 2016, the first three plant protection applications in 2017 were performed with conventional pesticides to achieve a profound cleaning effect of the plants against the pathogens. It is possible that the use of these pesticides maintained a better protection shield for VSP trained vines than minimal pruned vines, due to the enhanced accessibility of the VSP bunches.

Because of the *E. necator* resistance gene *Ren3* located in the genome of the grapevine variety 'Reberger' no or very few PM symptoms could be observed during the study of this work (Zendler *et al.*, 2017).

In this study, differences between SMPH and VSP regarding their susceptibility against BR could only be noticed in the 'Chardonnay' field in 2017. Bunches from minimal pruned vines with their loose bunch structure were less susceptible to BR compared to the densely packed bunches from cane pruned vines, which tend to burst and open the gates for BR

infection, which is in consensus with Ashley *et al.* 2005. Also Emmett *et al.* (1995) reported that the bunch architecture of minimally pruned vines is usually characterized by a smaller and less compact structure, which promotes robustness against BR. However, in 2016 no differences between the training systems could be noted. An explanation for these inconsistent results could be the influence of DM on bunch architecture in 2016. In this year the heavy DM epidemic demolished many berries in the trial fields, creating loose bunch structures in both training systems, SMPH and VSP.

As expected we found significantly more SWD in the SMPH trained than in the VSP trained panels, but the difference was only significant in 2017. SWD prefers shady and humid microhabitats, even within a single plant species (Diepenbrockand & Burrack, 2017). In this experiment, a high number of captured SWD did not correspond with a high incidence of SWD damage to the grapes. It appears that *D. suzukii* uses the grapevine as a habitat, but does not necessarily use grapes as a substrate for oviposition. Despite their wide host range, grapevine does not seem to be a preferred host for SWD. In laboratory studies only very few eggs were laid on grape berries and those eggs had very slow developmental rates as well as a low survivorship to the adult stage (Maiguashca *et al.*, 2010; Lee *et al.*, 2011; Bellamy *et al.*, 2013; Poyet *et al.*, 2015; Jarausch *et al.*, 2017). The small numbers of eggs that we could find in both trial years on grape berries confirms that grapevine appears to be a low quality host for SWD. The resistance of fruit skin to penetration has been previously discussed as a factor driving oviposition in SWD (Lee *at al.*, 2011; Burrack *et al.*, 2013). Ioratti *et al.* (2015) reported that oviposition by *D. suzukii* increases with decreasing penetration force. Since the two training systems did not influence the grape skin characteristics significantly, we cannot directly confirm their results.

In conclusion, SMPH trained grapevines were more susceptible to DM and PM compared to VSP trained vines, possibly due to differences in canopy microclimate. The incidence of BR in contrast was higher for VSP vines showing a more compact bunch

architecture. Regarding SWD, a higher activity was noticed in SMPH canopies. However, the number of damaged berries was the same in both training system. Because of the higher susceptibility of SMPH against the two major fungal grapevine diseases a plant protection regime specifically adapted to this new training system should be established. The benefit of fungus resistant cultivars such as 'Reberger' will be particularly high in SMPH vines, enabling winegrowers to combine advantages of SMPH with the economic and environmental benefits of reduced fungicide applications.

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References

Ashley, R., Clingeleffer, P., Emmett, R. & Dry, P. (2005) Effects of canopy and irrigation management on Shiraz production, quality and disease development in Sunraysia region. In: D. Oag, K. DeGaris, S. Partridge, C. Dundon, M. Francis, R. S. Johnstone, R. Hamilton (Eds): Finishing the job: Optimal ripening of Cabernet Sauvignon and Shiraz, Proc. ASVO Seminar, Mildura Arts Centre, Mildura, Victoria, 21 July 2006. Aust. Soc. Vitic. Oenol. Adelaide. 36–40.

Austin, C.N. & Wilcox, W.F. (2011) Effects of fruitzone leaf removal, training systems, and irrigation on the development of Grapevine Powdery Mildew. Am. J. Enol. Vitic. 62, 193–198.

Becker, T. & Knoche, M. (2012a) Deposition, strain, and microcracking of the cuticle in developing 'Riesling' grape berries. Vitis 51, 1–6.

Becker, T. & Knoche, M. (2012b) Water induces microcracks in the grape berry cuticle. Vitis 51, 141–142.

Bellamy, D.E., Sisterson, M.S. & Walse, S.S. (2013) Quantifying host potentials: indexing postharvest fresh fruits for spotted wing drosophila, *Drosophila suzukii*. PLoS One 8, e61227.

Blaeser, M. & Weltzien, H.C. (1978) Die Bedeutung von Sporangienbildung, -ausbreitung und -keimung für die Epidemiebildung von *Plasmopara viticola*. J. Plant. Dis. Protect. 85, 155–161.

Blaeser, M. & Weltzien, H.C. (1979) Epidemiological studies of *Plasmopara viticola* for improving determination of spraying dates. J. Plant. Dis. Protect. 86, 489–498.

Burrack, H.J., Fernandez G.E., Spivey, T. & Kraus D.A. (2013) Variation in selection and utilization of host crops in the field and laboratory by *Drosophila suzukii* Matsumara (Diptera: *Drosophilidae*), an invasive frugivore. Pest. Manag. Sci. 69, 1173–1180.

Cini, A., Ioriatti, C. & Anfora, G. (2012) A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. Bull. Insect. 65, 149–160.

Clingeleffer, P.R. (1993) Development of management systems for low cost, high quality wine production and vigour control in cool climate Australian vineyards. Wein-Wissenschaft 48, 130–134.

Clingeleffer, P.R. & Possingham, J.V. (1987) The role of minimal pruning of cordon trained vines (MPCT) in canopy management and its adoption in Australian viticulture. Aust. Grapegrow. Winemak. 280, 7–11.

Commenil, P., Brunet, L. & Audran, J.C. (1997) The development of the grape berry cuticle in relation to susceptibility to bunch rot disease. J. Exp. Bot. 48, 1599–1607.

Coombe, B.G. & Dry, P.R. (1992) Viticulture, vol. 2, Practices, Aust. Indust. Publ. PTY LTD, Adelaide, 232–246.

Diepenbrock, L.M. & Burrack, H.J. (2017) Variation of withincrop microhabitat use by *Drosophila suzukii* (Diptera: *Drosophilidae*) in blackberry. J. Appl. Entomol. 141, 1–7.

Emmett, R.W., Clingeleffer, P.R., Wicks, T.J., Nair, N.G., Hall, B., Hart, K.M., Clarke, K. & Somers, T. (1995) Influence of canopy architecture on disease development. In 'Canopy Management: Proceedings of a seminar organized by the Australian society of viticulture and oenology, Inc. and held on 18. August, 1994 at Mildura, Victoria', pp. 22–23. Eds. P. Hayes, Australian society of viticulture and oenology, Adelaide.

Gabler, F.M., Smilanick, J.L., Mansour, M., Ramming, D.W. & Mackey, B.E. (2003) Correlations of morphological, anatomical, and chemical features of grape berries with resistance to *Botrytis cinerea*. Phytopathology 93, 1263–1273.

Gadoury, D.M., Cadle-Davidson, L., Wilcox, W.F., Dry, I.B., Seem, R.C. & Milgroom, M.G. (2012) Grapevine powdery mildew (*Erysiphe necator*): a fascinating system for the study of the biology, ecology and epidemiology of an obligate biotroph. Mol. Plant Pathol. 13, 1–16.

Gubler, W.D., Marois, J.J., Bledsoe, A.M. & Bettiga, L.J. (1987) Control of Botrytis Bunch Rot of grape with canopy management. Plant Dis. 71, 599–601.

Hauser, M., Gaimari, S. & Damus, M. (2009) *Drosophila suzukii* new to North America. Fly Times 43, 12–15.

Herzog, K., Wind, R. & Töpfer, R. (2015) Impedance of the grape berry cuticle as a novel phenotypic trait to estimate resistance to *Botrytis cinerea*. Sensors 15, 12498–12512.

Intrieri, C., Poni, S., Lia, G. & Del Campo, M.G. (2001) Vine performance and leaf physiology of conventionally and minimally pruned Sangiovese grapevines. Vitis 40, 123–130.

Intrieri, C., Filippetti, I., Allegro, G., Valentini, G., Pastore, C. & Colucci, E. (2011) The semi-minimal-pruned hedge: A novel mechanized grapevine training system. Am. J. Enol. Vitic. 62, 312–318.

Ioriatti, C., Walton, V., Dalton, D., Anfora, G., Grassi, A., Maistri, S. & Mazzoni, V. (2015) *Drosophila suzukii* (Diptera: *Drosophilidae*) and its potential impact to wine grapes during harvest in two cool climate wine grape production regions. J. Econ. Entomol. 108, 1148–1155.

Jackson, D. (1996) Pruning and Training. Monographs in Cool Climate Viticulture – 1 Wellington, N.Z. Daphne Brasell Associates Ltd in association with Lincoln University Press.

Jarausch, B., Müller, T., Gramm, T. & Hoffmann, C. (2017) Comparative evaluation of insecticide efficacy tests against *Drosophila suzukii* on grape berries in laboratory, semi-field and field trials. Vitis 56, 133–140.

Lee, J.C., Bruck, D.J., Curry, H., Edwards, D.L., Haviland, D.R., Van Steenwyk, R. & Yorgey, B. (2011) The susceptibility of small fruits and cherries to the spotted wing drosophila, *Drosophila suzukii*. Pest. Manag. Sci. 67, 1358–1367.

Letaief, H., Rolle, L., Zeppa, G. & Gerbi, V. (2008) Assessment of grape skin hardness by a puncture test. J. Sci. Food Agric. 88, 1567–1575.

Lorenz, D.H., Eichhorn, K.W., Bleiholder, H., Klose, R., Meier, U. & Weber, E. (1995) Growth stages of the grapevine: Phenological growth stages of the grapevine (*Vitis vinifera* L.

ssp. *vinifera*) – Codes and descriptions according to the extended BBCH scale. Aust. J. Grape Wine Res. 1, 100–103.

Maiguashca, F., Ferguson, H., Bahder, B., Brooks, T., O'Neal, S. & Walsh, D. (2010) SWD ovipositing on grapes in laboratory, partial maggot survival inconclusive. Washington State University Extension, Spotted Wing Drosophila Grape Update, 28 August. http://ipm.wsu.edu/small/pdf/NoChoiceSWDonGrapesAug28.pdf (accessed 18th May 2017).

Poyet, M., Le Roux, V., Gibert, P., Meirland, A., Prévost, G., Eslin, P. & Chabrerie, O. (2015) The wide potential trophic niche of the asiatic fruit fly *Drosophila suzukii*: The key of its invasion success in temperate Europe? PLoS One 10, e0142785.

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/ (accessed 18th May 2017).

Rouzes, R., Delbac, L., Ravidat, M.L. & Thiéry, D. (2012) First occurrence of *Drosophila suzukii* in the Sauternes vineyards. OENO One 46, 145–147.

Saguez, J., Lasnier, J. & Vincent, C., (2013) First record of *Drosophila suzukii* in Quebec vineyards. OENO One 47, 69–72.

Schmid, J. & Schultz, H.R. (2000) Influence of two training systems and irrigation on water consumption of grapevines in the field. Acta Hort. 537, 587–595.

Siegfried, W. & Sacchelli, M. (2005) Blattflächenbezogene Dosierung von Pflanzenschutzmitteln im Rebanbau. Schweiz. Z. Obst–Weinbau, 4, 13–16.

Sommer, K.J., Clingeleffer, P.R. & Ollat, N. (1993) Effects of minimal pruning on grapevine canopy development, physiology and cropping level in both cool and warm climates. Wein-Wiss. 48, 135–139.

Tochen, S., Woltz, J. M., Dalton, D.T., Lee, J.C., Wiman, N.G. & Walton, V.M. (2016) Humidity affects populations of *Drosophila suzukii* (Diptera: *Drosophilidae*) in blueberry. J. Appl. Entomol. 140, 47–57.

Wolf, T.K., Dry, P.R., Iland, P.G., Botting, D., Dick, J., Kennedy, U. & Ristic, R. (2003) Response of Shiraz grapevines to five different training systems in the Barossa Valley. Aust. J. Grape Wine Res. 9, 82–95.

Zendler, D., Schneider, P., Töpfer, R. & Zyprian, E. (2017) Fine mapping of *Ren3* reveals two loci mediating hypersensitive response against *Erysiphe necator* in grapevine. Euphytica 213, 68.

Supplemental data to manuscript

Effects of canopy architecture and microclimate on grapevine health in two training systems.

Kraus, C., Pennington, T., Herzog, K., Hecht, A., Fischer, M., Voegele, R.T., Hoffmann, C., Töpfer, T. & Kicherer, A. (2018). Vitis 57, 53–60.

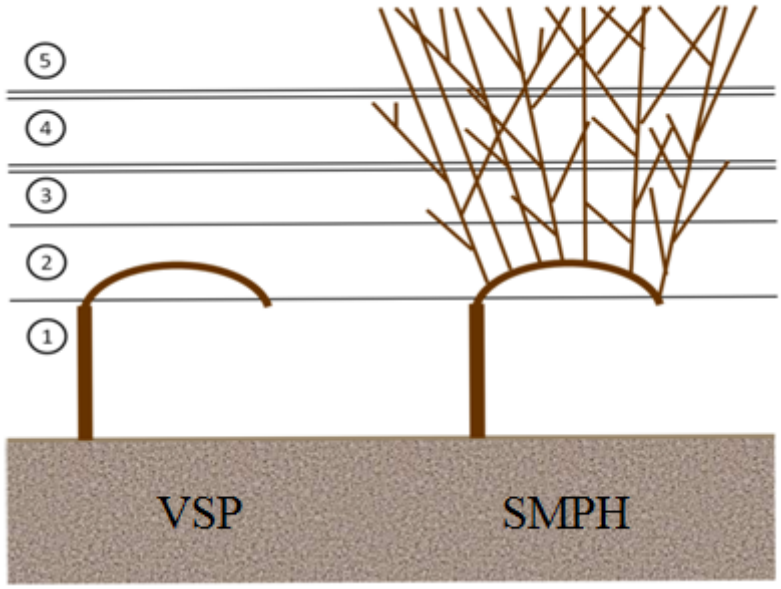

Fig. S1: Schematic illustration of the pruning systems VSP (left) and SMPH (right) during dormancy (BBCH 00). Numbers indicate horizontal zones in the trellis, which were used to study canopy architecture.

Tab. S1: Canopy architecture characteristics of the two grapevine varieties 'Chardonnay' and 'Reberger' as a function of training system, 2016 (a) and 2017 (b). Additionally, a comparison of the different trellis zones is shown. T test; *$p<0.05$, **$p<0.001$.

a)

2016			Chardonnay			Reberger		
	Zone	BBCH	SMPH	VSP		SMPH	VSP	
Number of shoots [per 0.5m]	1	10	2.6 ± 2.0	2.4 ± 1.3		3.1 ± 3.9	3.3 ± 1.3	
	2	10	5.8 ± 1.9	5.1 ± 1.7		5.1 ± 3.8	3.3 ± 0.5	
	3	10	20.0 ± 1.5	0.8 ± 0.3		9.1 ± 4.5	0.0 ± 0.0	
	4	10	13.6 ± 4.3	0.0 ± 0.0		10.5 ± 4.4	0.0 ± 0.0	
	5	10	26.3 ± 5.5	0.0 ± 0.0		16.5 ± 2.0	0.0 ± 0.0	
	Average total	10	68.3 ± 9.6	8.3 ± 1.3	**	44.4 ± 6.9	6.5 ± 1.8	**
Number of inflorescences/ bunches [per 0.5m]	1	65	0.0 ± 0.0	0.0 ± 0.0		2.0 ± 1.6	0.3 ±.0.5	
	2	65	3.0 ± 0.8	3.5 ± 0.6		4.3 ± 3.6	8.8 ± 2.9	
	3	65	7.0 ± 3.4	5.3 ± 1.3		2.3 ± 2.9	0.3 ± 0.5	
	4	65	11.5 ± 9.5	0.3 ± 0.5		5.8 ± 6.3	0.0 ± 0.0	
	5	65	18.5 ± 9.7	0.0 ± 0.0		15.8 ± 8.1	0.0 ± 0.0	
	Average total	65	40.0 ± 5.9	9.0 ± 0.8	**	30.0 ± 7.9	9.3 ± 3.1	*
	1	75	1.5 ± 1.3	0.0 ± 0.0		0.5 ± 1.0	1.3 ± 1.5	
	2	75	1.0 ± 1.4	3.8 ± 1.5		7.8 ± 4.2	6.5 ± 2.4	
	3	75	5.3 ± 4.2	5.5 ± 2.6		4.0 ± 2.2	0.0 ± 0.0	
	4	75	7.3 ± 4.2	0.3 ± 0.5		5.5 ± 3.0	0.0 ± 0.0	
	5	75	4.0 ± 2.4	0.0 ± 0.0		7.3 ±.4.9	0.0 ± 0.0	
	Average total	75	19.0 ± 7.7	9.5 ± 3.7	n.s.	25.0 ± 11.5	7.8 ± 3.1	n.s.
	1	81	0.3 ± 0.5	0.3 ± 0.5		1.0 ± 0.8	1.5 ± 2.4	
	2	81	1.8 ± 1.0	2.0 ± 1.4		2.5 ± 1.9	8.0 ± 3.7	
	3	81	5.8 ± 3.5	5.3 ± 2.8		1.3 ± 1.0	0.0 ± 0.0	
	4	81	2.8 ± 1.0	0.0 ± 0.0		3.0 ± 2.2	0.0 ± 0.0	
	5	81	1.0 ± 1.4	0.0 ± 0.0		2.5 ± 1.3	0.0 ± 0.0	
	Average total	81	11.5 ± 2.4	7.5 ± 3.7	n.s.	10.3 ± 3.8	9.5 ± 5.8	n.s.
LAI		65	4.7 ± 0.7	1.0 ± 0.1	**	2.6 ± 1.0	1.2 ± 0.1	n.s.
		75	2.8 ± 0.2	1.7 ± 0.3	*	2.6 ± 0.5	1.9 ± 0.4	n.s.
		81	3.1 ± 0.3	1.8 ± 0.4	*	3.2 ± 0.5	1.9 ± 0.6	*
Average leaf size [cm²]		65	65.9 ± 6.6	106.7 ± 11.7	*	87.3 ± 4.2	107.4 ± 36.6	n.s.
		75	59.3 ± 3.1	85.0 ± 10.2	*	70.9 ± 4.6	119.5 ± 13.7	**
		81	51.2 ± 3.9	74.4 ± 5.8	**	76.0 ± 13.6	99.5 ± 2.6	n.s.
Canopy volume [m³]		65	47046.0 ± 6770.8	9945.5 ± 1122.6	**	25884.4 ± 10246.0	12094.6 ± 728.5	n.s.
		75	28041.6 ± 2350.2	17234.5 ± 3139.9	*	26322.2 ± 4797.8	18604.0 ± 3796.0	n.s.
		81	31120.9 ± 2787.9	17986.3 ± 3139.9	**	31812.4 ± 4608.5	19209.8 ± 5902.6	*
Bunch weight [g]		89	96.5 ± 37.3	152.3 ± 40.0	*	126.7 ± 27.1	182.6 ± 69.0	*
Bunch length [cm]		89	11.2 ± 2.0	12.9 ± 1.1	*	11.3 ± 2.5	13.3 ± 3.3	n.s.
Bunch width [cm]		89	6.9 ± 1.3	8.7 ± 1.8	*	8.7 ± 1.9	9.6 ± 1.1	n.s.
Berry number per bunch		89	78.1 ± 26.9	110.1 ± 27.5	*	71.3 ± 17.1	85.6 ± 24.1	n.s.
Ø berry size [mm]		89	12.0 ± 1.0	12.4 ± 1.0	*	14.2 ± 1.2	14.2 ± 1.3	n.s.

Tab. S1 continued

b)

2017			Chardonnay			Reberger		
	Zone	BBCH	SMPH	VSP		SMPH	VSP	
Number of shoots [per 0.5m]	1	10	5.8 ± 2.5	1.0 ± 0.8		7.3 ± 3.8	5.8 ± 0.5	
	2	10	16.0 ± 6.1	8.0 ± 1.4		14.8 ± 5.1	3.3 ± 1.0	
	3	10	40.3 ± 7.4	1.0 ± 1.2		16.5 ± 5.2	0.0 ± 0.0	
	4	10	49.3 ± 4.6	0.0 ± 0.0		26.8 ± 18.9	0.0 ± 0.0	
	5	10	38.5 ± 16.1	0.0 ± 0.0		35.3 ± 21.4	0.0 ± 0.0	
	Average total	10	150.0 ± 14.4	10.0 ± 1.2	**	101.0 ± 11.3	9 ± 0.8	**
Number of inflorescences/ bunches [per 0.5m]	1	65	0.0 ± 0.0	0.0 ± 0.0		3.3 ± 3.3	0.5 ± 0.6	
	2	65	1.5 ± 1.3	3.3 ± 1.5		12.0 ± 6.7	7.0 ± 2.2	
	3	65	2.8 ± 1.3	6.5 ± 1.9		6.0 ± 5.4	0.0 ± 0.0	
	4	65	6.5 ± 3.8	0.0 ± 0.0		7.0 ± 0.8	0.0 ± 0.0	
	5	65	7.8 ± 5.0	0.0 ± 0.0		23.3 ± 9.7	0.0 ± 0.0	
	Average total	65	18.5 ± 6.5	9.8 ± 2.2	n.s.	52.0 ± 15.4	8.0 ± 2.1	*
	1	75	1.0 ± 2.0	0.0 ± 0.0		0.3 ± 0.5	1.8 ± 1.5	
	2	75	1.3 ± 1.0	6.3 ± 1.7		3.8 ± 3.3	6.0 ± 1.6	
	3	75	5.5 ± 2.4	2.8 ± 2.2		3.8 ± 2.6	0.0 ± 0.0	
	4	75	7.0 ± 5.7	0.0 ± 0.0		4.8 ± 3.8	0.0 ± 0.0	
	5	75	4.3 ± 2.9	0.0 ± 0.0		5.5 ± 1.0	0.0 ± 0.0	
	Average total	75	19.0 ± 7.5	9.0 ± 0.8	*	18.0 ± 8.0	7.8 ± 3.0	n.s.
	1	81	0.5 ± 0.6	0.3 ± 0.5		1.8 ± 1.5	1.8 ± 1.7	
	2	81	0.5 ± 1.0	3.3 ± 0.5		7.5 ± 0.6	5.3 ± 2.6	
	3	81	2.0 ± 2.2	6.3 ± 2.6		2.0 ± 0.8	0.0 ± 0.0	
	4	81	4.5 ± 3.1	0.0 ± 0.0		10.5 ± 4.7	0.0 ± 0.0	
	5	81	4.8 ± 2.2	0.0 ± 0.0		5.8 ± 3.5	0.0 ± 0.0	
	Average total	81	12.3 ± 4.5	9.8 ± 2.2	n.s.	27.5 ± 6.4	7.0 ± 1.6	n.s.
LAI		65	3.5 ± 0.4	1.1 ± 0.3	**	3.7 ± 0.3	0.8 ± 0.1	**
		75	3.0 ± 0.3	2.6 ± 0.5	n.s.	2.6 ± 0.5	1.5 ± 0.5	*
		81	3.5 ± 0.7	2.1 ± 0.4	*	4.0 ± 0.6	2.1 ± 0.2	*
Average leaf size [cm²]		65	60.4 ± 5.7	105.3 ± 6.7	**	68.1 ± 10.6	98.8 ± 8.4	*
		75	60.7 ± 6.9	76.2 ± 8.7	*	79.7 ± 16.2	95.0 ± 9.8	n.s.
		81	63.4 ± 3.6	96.8 ± 5.6	**	84.0 ± 10.2	96.9 ± 7.7	n.s.
Canopy volume [m³]		65	35039.0 ± 4414.3	10726.4 ± 2860.0	**	36713.1 ± 3465.7	8288.9 ± 1196.5	**
		75	30205.5 ± 3148.3	25861.5 ± 4969.6	n.s.	26167.3 ± 5174.9	15326.1 ± 5108.7	*
		81	34957.6 ± 6964.7	20611.1 ± 4041.3	*	40488.8 ± 6456.3	20934.6 ± 1851.5	*
Bunch weight [g]		89	76.2 ± 34.8	163.7 ± 33.0	**	101.4 ± 35.7	257.7 ± 83.5	**
Bunch length [cm]		89	8.7 ± 2.6	12.0 ± 1.4	*	10.9 ± 2.3	14.7 ± 2.2	*
Bunch width [cm]		89	6.9 ± 1.7	8.9 ± 1.6	*	6.9 ± 1.7	8.9 ± 1.6	*
Berry number per bunch		89	57.8 ± 23.7	125.1 ± 26.8	**	56.0 ± 20.5	134.1 ± 45.5	**
Ø berry size [mm]		89	12.2 ± 1.0	13.2 ± 1.3	**	12.8 ± 2.0	15.3 ± 1.7	**

Tab. S2: Skin characteristics from SMPH and VSP trained grapevine berries obtained from the I-Sensor (n=30 berries), TA.XT Texture (n=50 berries) and microscopy analysis (n=20 berries), 2016 (a) and 2017 (b). T test; *p<0.05, **p<0.001.

a)

2016	SMPH	VSP	
relative impedance Z_{rel}	810.1 ± 130.2	823.9 ± 122.5	n.s.
maximum break force [N]	0.9 ± 0.2	0.9 ± 0.2	n.s.
skin break energy [mJ]	0.5 ± 0.1	0.5 ± 0.1	n.s.
skin thickness [µm]	90.1 ± 11.6	85.6 ± 11.5	n.s.

b)

2017	SMPH	VSP	
relative impedance Z_{rel}	642.6 ± 92.1	626.7 ± 97.7	n.s.
maximum break force [N]	0.8 ± 0.2	0.8 ± 0.2	n.s.
skin break energy [mJ]	0.5 ± 0.3	0.5 ± 0.2	n.s.
skin thickness [µm]	85.0 ± 4.2	87.1 ± 4.2	n.s.

Manuscript II

Temporal development of the culturable, endophytic fungal community in healthy grapevine branches and occurrence of GTD-associated fungi.

Kraus, C., Voegele, R.T. & Fischer, M. (2018). Microb. Ecol. DOI: https://doi.org/10.1007/s00248-018-1280-3 (accessed 4th December 2018).

Abstract

Endophytic fungi play an important role in the life of grapevine, either as beneficial microorganisms or as pathogens. Many surveys concerning the fungal grapevine community have been conducted. Nevertheless, exactly how the fungal community arises within the plant and develops from young shoots to mature vines is still unknown. Therefore, it was the aim of this study to investigate the early development of endophytic fungal communities in healthy grapevine branches from 2 months to 8 years old. More than 3800 fungi belonging to 86 operational taxonomic units (OTUs) were isolated from wood samples and assigned to eight age groups. The community composition within the age groups changed and significant differences between young (≤ 1 year) and old (> 1 year) branches were found. The former were primarily dominated by ubiquitous, fast-growing fungi like *Alternaria* spp., *Aureobasidium pullulans*, *Cladosporium* spp., or *Epicoccum nigrum*, while communities of perennial branches additionally harbored many grapevine trunk disease (GTD)-associated fungi such as *Diplodia seriata* or *Eutypa lata*. This work gives an insight into the early development of fungal communities in grapevine, the nature and composition of primary settlers and core communities, as well as the emergence of GTD-associated fungi in perennial wood. This information may help grapevine growers to better estimate the risk in relation to the applied training system, producing mainly old branches or young shoots.

Keywords: Endophytic fungi; Fungal community; Grapevine wood; GTD; Training system; Vitis vinifera

Introduction

Microorganisms like fungi, which inhabit plants, can exert beneficial or detrimental effects on their host. Understanding the mycoflora and their interaction with the host can help to improve growth and protection of plants (Berg *et al*., 2016). Grapevine, which is one of the economically most important fruit crops in the world, also harbors various microorganisms and numerous studies have been conducted in order to reveal the communities of different plant organs and their function (Pinto & Gomes, 2016; Kernaghan *et al*., 2017). Special focus was given on the microbiome in woody tissue of grapevine, due to the problems arising from grapevine trunk diseases (GTDs) (Casieri *et al*., 2009; González & Tello, 2011; Hofstetter *et al*., 2012; Bruez *et al*., 2014, 2016; Fontaine *et al*., 2016; Travadon *et al*., 2016; Gramaje *et al*., 2018). This group of diseases, of which Esca, Eutypa, and Botryosphaeria dieback are the most common and destructive, covers a variety of symptoms on foliage, berries, and vascular tissue. These symptoms are most frequently observed in older vineyards that are over 7 years old and are linked to symptom-inducing phytotoxins released by associated fungi located in the trunk of the vine (Mugnai *et al*., 1999; Andolfi *et al*., 2011; Bertsch *et al*., 2012; Fontaine *et al*., 2016). Since the onset of GTD research, many fungi have been associated with these diseases due to their isolation from symptomatic trunk tissue. The most frequently isolated GTD fungi are *Phaeomoniella chlamydospora*, *Phaeoacremonium* spp., and *Fomitiporia* spp. for Esca (Mugnai *et al*., 1999; Crous & Gams, 2000; Fischer, 2002; Fischer & Kassemeyer, 2003; Cloete *et al*., 2014), *Eutypa lata* and other species of the Diatrypaceae for Eutypa dieback (Trouillas *et al*., 2010; Trouillas *et al*., 2011; Luque *et al*., 2012; Moyo *et al*., 2018), and several members of Botryosphaeriaceae for Botryosphaeria dieback (Úrbez-Torres, 2011; Úrbez-Torres *et al*., 2012; Mondello *et al*., 2013). For all the mentioned fungi, virulence has been confirmed, except for the basidiomycetes associated with Esca (Gramaje *et al*., 2018). Interestingly, for Esca, it has been shown that fungi associated with the disease also appear in externally healthy vines

and that the fungal community in these trunks hardly differs from those of Esca-affected vines (Hofstetter *et al*., 2012; Bruez *et al*., 2014). These results led to a controversial discussion about the role of these fungi in the disease process.

Besides phytopathogenic, also phytoprotective fungi can be found in grapevine wood. The most common and extensively studied phytoprotective fungi are *Trichoderma* spp. With its antagonistic ability, this endophytic ascomycete is basically able to parasitize GTD-associated fungi and thus may protect grapevines from infection, which was already demonstrated in the field and in nurseries (John *et al*., 2005, 2008; Halleen & Fourie, 2016; Pertot *et al*., 2016). *Trichoderma* spp. also occur naturally in grapevine and several species were isolated from wood samples (Casieri *et al*., 2009; González & Tello, 2011; Bruez *et al*., 2014, 2016). Some other fungi isolated from pruning wounds, e.g., *Fusarium lateritium*, *Cladosporium herbarum*, and *Alternaria alternata*, showed an effective control of *E. lata* probably through competition (Munkvold & Marois, 1993). These studies demonstrate that grapevine wood is a highly competitive habitat, with phytopathogenic fungi on the one side and beneficial, potentially protective fungi on the other side (Casieri *et al*., 2009; González & Tello, 2011; Hofstetter *et al*., 2012; Pancher *et al*., 2012; Bruez *et al*., 2014, 2016; Travadon *et al*., 2016).

Although many studies were undertaken to reveal the fungal communities of grapevine wood, little is known about the changes in the composition of these communities from young shoots to perennial branches as it occurs for vines converted from vertical shoot positioning (VSP) to semi minimal pruned hedge (SMPH) (Casieri *et al*., 2009; González & Tello, 2011; Hofstetter *et al*., 2012; Pancher *et al*., 2012; Bruez *et al*., 2014, 2016; Travadon *et al*., 2016). As the branches take up a major part of the plant vascular system after conversion, it was of high interest to investigate the development of the fungal community with regard to the occurrence of GTD-associated fungi. For sampling, apparently asymptomatic grapevines were chosen given that GTD-associated fungi appear in both healthy and diseased vines and that the

fungal community in the woody tissue does not differ no matter in which condition the grapevine is (Hofstetter *et al*., 2012; Bruez *et al*., 2014, 2016). Comparative analysis of the species composition was based on cultured isolates and should show (1) which fungi are primary settlers, (2) which fungi dominate mature wood tissue, (3) how fungal diversity develops over time, and (4) how the occurrence of GTD-associated fungi develops. For young grapevine branches, we expected a low fungal diversity and a community dominated by ubiquitous, fast-growing fungi, e.g., *Alternaria* spp., *Aureobasidium pullulans*, and *Cladosporium* spp. With increasing age, an increase in fungal diversity and a dominance of GTD-associated fungi was suspected, as well as non-pathogenic fungi with antagonistic potential.

Materials and Methods

Plant material and sample processing: Branches from *Vitis vinifera* variety 'Riesling' cultivated in the region of Rhineland-Palatinate, Germany, were randomly picked from asymptomatic vines during the years 2016 and 2017 (Tab. 1). Branches that are 2, 6, and 10 months old were obtained from VSP-trained grapevines. One year and older branches (2 to 8 years old) were taken from SMPH-trained grapevines, where the branches, in contrast to the VSP system, remain in the trellis after winter pruning. For each time point, 20 branches were collected and taken to the lab for processing. All branches were divided into three sections, i.e. basal, central, and apical. A 10-cm-long segment of each section was cut from the branches and debarked using a scalpel. These branch segments were further processed under sterile conditions under the hood. Debarked wood was surface sterilized by flaming and cut in small pieces with a disinfected pruning scissor. Wood pieces of about 5 × 5 × 5 mm size were put on malt-yeast-agar (MYA; malt extract 20 g/L; yeast extract 1 g/L; agar 20 g/L; chloramphenicol 2.5 µg/mL) plates. Eight wood pieces per branch segment were distributed over two MYA plates. This resulted in a sample size of 480 wood pieces per time point.

Tab. 1: Location, year of planting, training system, and year of conversion to SMPH of the vineyards consulted for branch sampling.

Branch sample	Location	Year of planting	Training system	Year of conversion to SMPH
2 months	49°13'11.5"N 8°02'34.4"E	2008	VSP	- -
6 months	49°13'11.5"N 8°02'34.4"E	2008	VSP	- -
10 months	49°13'11.5"N 8°02'34.4"E	2008	VSP	- -
1 year	49°14'14.6"N 8°05'53.2"E	1989	SMPH	2016
2 years	49°13'56.6"N 8°07'37.1"E	1983	SMPH	2015
3 years	49°13'11.5"N 8°02'33.4"E	2008	SMPH	2013
5 years	49°25'06.8"N 8°13'35.5"E	1999	SMPH	2011
8 years	49°51'01.6"N 7°50'41.2"E	1990	SMPH	2008

A cultural approach for fungal isolation and identification was chosen since all fungi of interest (GTD-associated) are cultivable. Also, the chosen method enables an exact classification of the isolated fungi and a high sample size.

Fungal identification: For fungal identification, colonies growing from wood pieces on MYA plates were first subcultured on a separate plate and afterwards identified by morphological and molecular analyses. For the latter, fungal DNA was extracted according to Tillett and Neilan (2000) from pure cultures growing for 2 weeks on MYA plates at 20 °C. Quality and quantity of the DNA were measured using a spectrophotometer (Nanodrop 2000c, Thermo Scientific, Waltham, USA). Polymerase chain reactions (PCR) were performed to amplify selected genomic regions. For a first classification, the internal transcribed spacer (ITS) region was chosen, using the primer pair ITS5 and ITS4 (Tab. 2; White *et al*., 1990). In some cases, β-tubulin (primers: BT2A and BT2B; Glass & Donaldson, 1995) and elongation factor 1-α (primers: EF1-728F and EF1-986R; Carbone & Kohn, 1999) were used for a more precise identification. The PCR reaction mix was prepared according to the KAPAHiFi™ Hot Start Polymerase user manual (PEQLAB Biotechnologie GmbH, Erlangen, Germany). PCR reactions were conducted in a SimpliAmp™ Thermal Cycler (Applied Biosystems, Darmstadt, Germany) as follows: 95 °C initial denaturation (5 min, 1 cycle), 98 °C denaturation (20 s, 35 cycles), 58 °C annealing (15 s, 35 cycles), 72 °C extension (20 s, 35 cycles), 72 °C final extension (1 min, 1 cycle), and 4 °C holding. The same PCR protocol was used for all three primer pairs. Quantity and quality of the amplified DNA were checked by gel electrophoresis. Amplicons were purified with the QIAquick PCR Purification Kit (Qiagen, Hilden, Germany) and sequenced with an ABI Prism 3130XL DNA sequencer, using one of the primers used for PCR amplification. For species identification, the BLASTn search option of the NCBI Database was used. A sequence similarity of ≥ 97% is considered as a sufficient threshold value for taxonomic identification and was used in this study (Taylor & Houston, 2011).

Tab. 2: Primer used in this study.

Primer	Sequence (5' - 3')
ITS5	GGAAGTAAAAGTCGTAACAAGG
ITS4	TCCTCCGCTTATTGATATGC
BT2A	GGTAACCAAATCGGTGCTGCTTTC
BT2B	ACCCTCAGTGTAGTGACCCTTGGC
EF1-728F	CATCGAGAAGTTCGAGAAGG
EF1-986R	TACTTGAAGGAACCCTTACC

Statistical analyses: All statistical analyses and plots were run with the programs R and RStudio 1.1.383 (R Core Team, 2013). The R packages "vegan" (Version 2.2-4; Oksanen *et al*., 2018) and "agricolae" (Version 1.2-8; de Mendiburu, 2017) were used for analysis of diversity and ecological communities. OTU accumulation curves for each branch age were plotted to investigate the relationship between the number of operational taxonomic units (OTUs) and the sampling size. Additionally, species richness (number of species isolated) and abundance (number of isolated strains) were calculated for each branch sample. Based on these values, mean Shannon (H'), Simpsons (D_1), and Pielou's evenness index (J') indices were calculated and compared between age groups using Welch test with p values corrected by Holms (H', D_1) or ANOVA (J').

A nonmetric multidimensional scaling (nMDS) analysis was employed to visualize the development of endophytic fungal communities in grapevine branches with increasing age. For this analysis, OTUs appearing less than five times and communities with only one fungal species were rejected in order to not overrate rare fungal species or communities with only one fungal species, respectively. With these settings, a matrix with the 15 most abundant fungal species was created, based on the Bray-Curtis dissimilarity. For each community group, an ellipse with a confidence interval of 95% was added into the nMDS plots to arrange communities.

To identify significant differences between the single branch ages, a comparative ANOSIM and a PermMANOVA were conducted. P values of both analysis were Holm

corrected. In addition to the *p* values of the two statistical tests, the *R* value of the ANOSIM analysis was consulted to describe the magnitude of difference between two groups.

Results

Isolated fungi

From a total of 3.840 wood samples, 3.829 individual fungal isolates were obtained and assigned to 86 OTUs (Fig. 1). From these, 64 were identified to the species level. 3.776 out of 3.829 isolates (98.6%) belong to the division Ascomycota, 34 (0.9%) to the Basidiomycota, and 19 (0.5%) belong to an unknown taxa. The Dothideomycetes (3.196 out of 3.829 isolates, 83.5%) were the most dominant subdivision followed by the Sordariomycetes (402, 10.5%), Leotiomycetes (143, 3.7%), Eurotiomycetes (35, 0.9%), Agaricomycetes (34, 0.9%), and an unknown taxa (19, 0.5%). The five most frequently isolated OTUs were *Alternaria* spp. (1.372 out of 3.829, 35.8%), *A. pullulans* (752, 19.6%), *Diplodia seriata* (376, 9.8%), *Cladosporium* spp. (357, 9.3%), and *Epicoccum nigrum* (258, 6.7%). Based on the literature, 18 of the 86 OTUs (Fig. 1, marked in red) are considered to be associated with GTDs. Of these, *D. seriata* was the most common (376 out of 3.829 isolates, 9.8%), followed by *Diaporthe ampelina* (123, 3.2%) and *E. lata* (65, 1.7%). Also, many antagonistic fungi (2.749 out of 3.829 isolates, 71.8%, Fig. 1, marked in green) were found, among them *Alternaria* spp., *A. pullulans*, *Cladosporium* spp., *E. nigrum*, and three species of *Trichoderma* (10, 0.3%). *Alternaria* spp., *A. pullulans*, *Botrytis cinerea*, *D. seriata*, *Cladosporium* spp., and *E. nigrum* were present at all investigated branch ages. *Penicillium* spp. and the possible GTD pathogen *Truncatella angustata* were found in almost all age groups but were absent from 1- and 2-year and 10-month-old branches. The GTD-associated fungi *Cadophora luteo- olivacea*, *P. chlamydospora*, and *E. lata* were found exclusively in perennial branches of 1 year and older, while species of *Diaporthe* first emerged in 10-month-old branches.

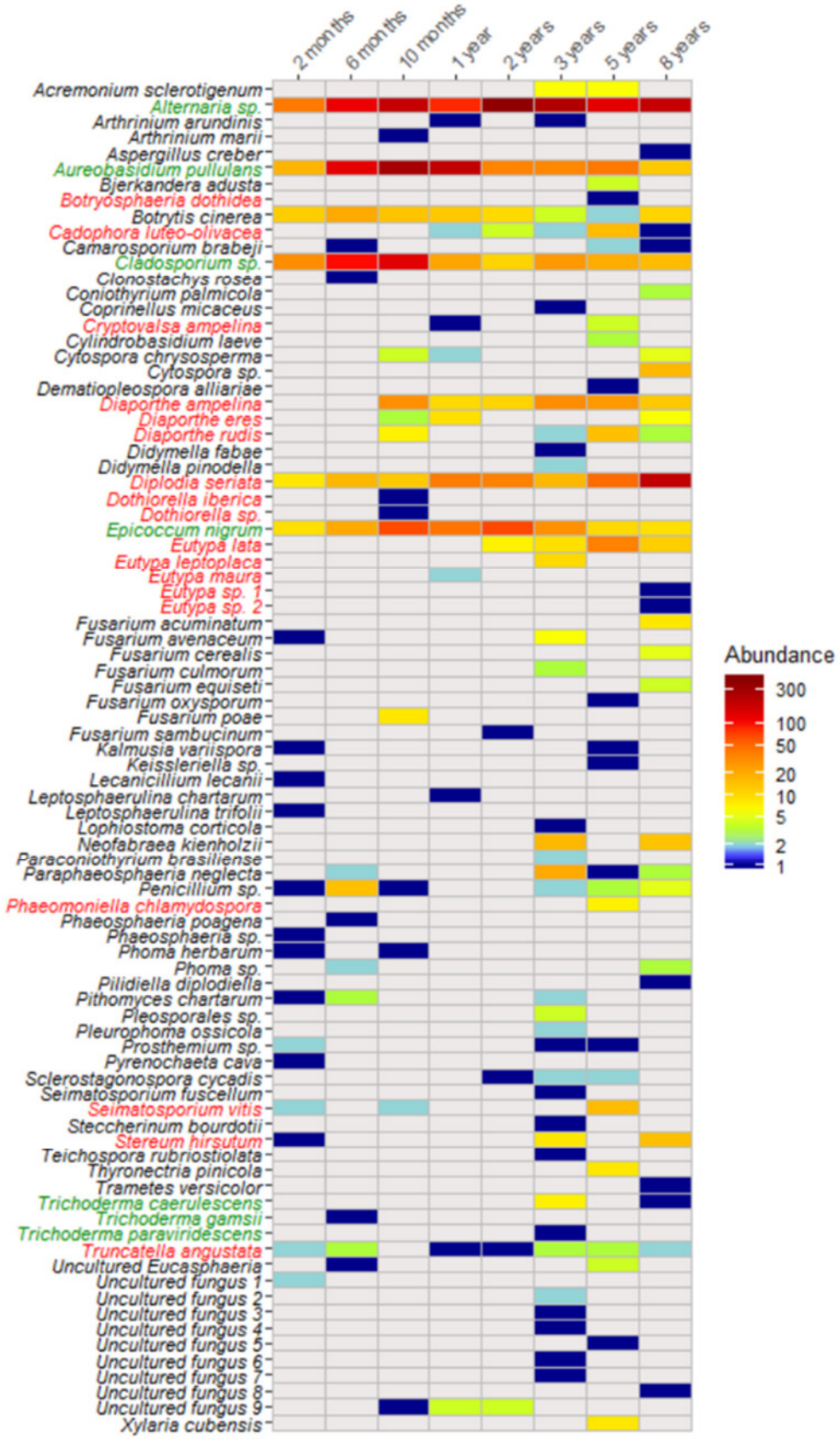

Fig. 1: Fungi isolated from grapevine branches and their abundance in the respective branch age illustrated by a heat map. Fungi discussed as related to GTDs are marked in red and those with antagonistic abilities in green. Color bar is log10 transformed.

OTU abundance and accumulation curve

Concerning the general abundance of the OTUs, about half of the taxa were found to be singletons or doubletons, representing only 1.4% of total isolates (Fig. 2), whereas the five most common OTUs (5.8% of taxa) represent 81.4%. The OTU accumulation curves produced for each branch age only commenced to saturate, indicating that more samples would probably reveal more OTUs (Fig. 3). Moreover, in samples of 3-, 5-, and 8-year-old branches, the number of OTUs was higher compared to the younger branches.

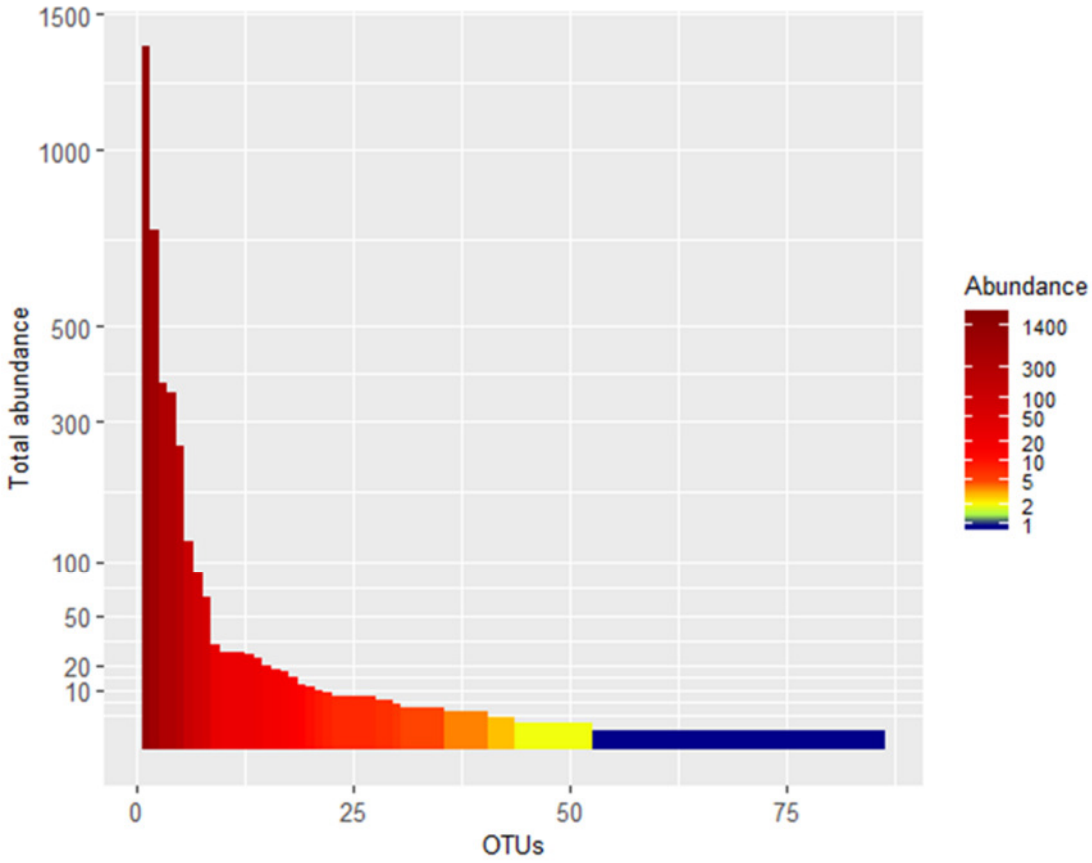

Fig. 2: Distribution of the obtained OTUs by mean of their total abundance. Color bar and y-axis are log10 transformed. Singletons are shown in blue, doubletons in yellow.

Fungal diversity

The number of obtained fungal taxa was 1.5–3.1 times higher for 3- to 8-year-old branches (31–40 OTUs) than in younger age groups (13–20 OTUs; Tab. 3). In contrast, the abundance of isolated fungi was similar for all ages (425–567 isolates), except for 2- and 10-

Tab. 3: Fungal diversity within branch age-groups. Species richness and abundance are expressed as total number of species and isolated strains per 20 branches. Diversity values (Shannon, Simpson and Pielou´s) correspond to mean numbers per 20 branches.

	Species richness	Abundance	Mean Shannon index (H')	Mean Simpson index (D_1)	Pielou´s Evenness (J')
2 months	20	137	0.96^{ab}	0.52^{bc}	0.88^{a}
6 months	16	431	1.28^{ab}	0.65^{ab}	0.86^{ab}
10 months	18	758	1.36^{a}	0.69^{a}	0.82^{abc}
1 year	16	437	1.20^{a}	0.62^{abc}	0.77^{bc}
2 years	13	563	0.93^{b}	0.49^{c}	0.66^{d}
3 years	40	511	1.36^{a}	0.65^{ab}	0.75^{c}
5 years	31	425	1.24^{ab}	0.64^{ab}	0.80^{abc}
8 years	31	567	1.23^{a}	0.64^{ab}	0.77^{bc}

month-old branches, with 137 and 758 isolates, respectively. Significant differences in diversity (Shannon H', Simpsons D_1, and Pielou's evenness index J') between two age groups are rare and no age group distinguished significantly from all other groups – except for Pielou's evenness index J' for 2-year-old branches. Here, the evenness ($J' = 0.66$) of the community was lower than in all other age groups.

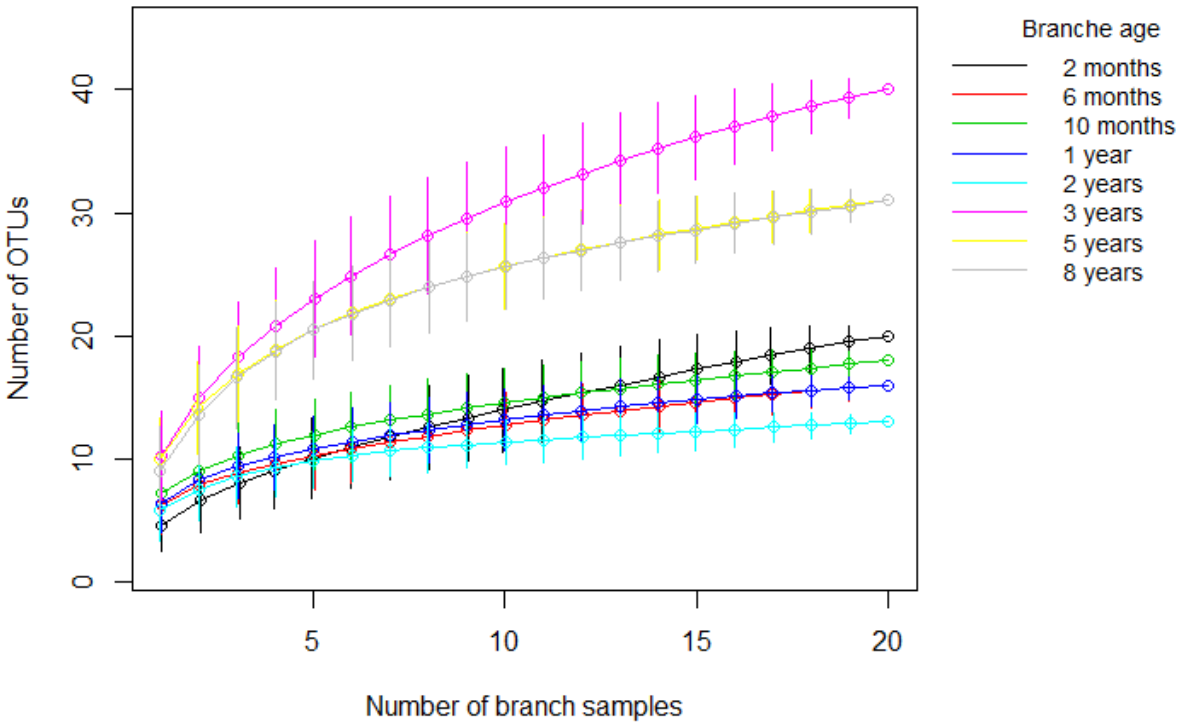

Fig. 3: OTUs accumulation curves of the eight analyzed branch ages showing the relationship between number of OTUs and sample size.

Comparative analysis of community composition

For a comparison of fungal communities, a nMDS biplot based on community dissimilarities was used (Fig. 4). An arrangement of communities by mean branch age resulted in an accumulation of communities in the center of the biplot, mainly comprising communities derived from 2-month to 1-year-old branches (Fig. 4 a). In addition, the ellipses of the corresponding branch ages showed an overlap. Also some part of the ellipses from 2- and 3-year-old branches agreed with this “core” community, but more than half were located outside. Communities of the 5-year-old branches were widely distributed over the plot and form a large ellipse, embedding almost all communities of every branch age. No fungal community of 8-year-old branches could be found within the core communities. Additionally, compared to the 5-year communities, 8-year communities were more close to each other, resulting in a smaller ellipse. Grouping the communities in the categories annual and perennial wood revealed a partial congruence between the two groups (Fig. 4 b). However, the majority of perennial communities showed dissimilarity when compared to the annual group.

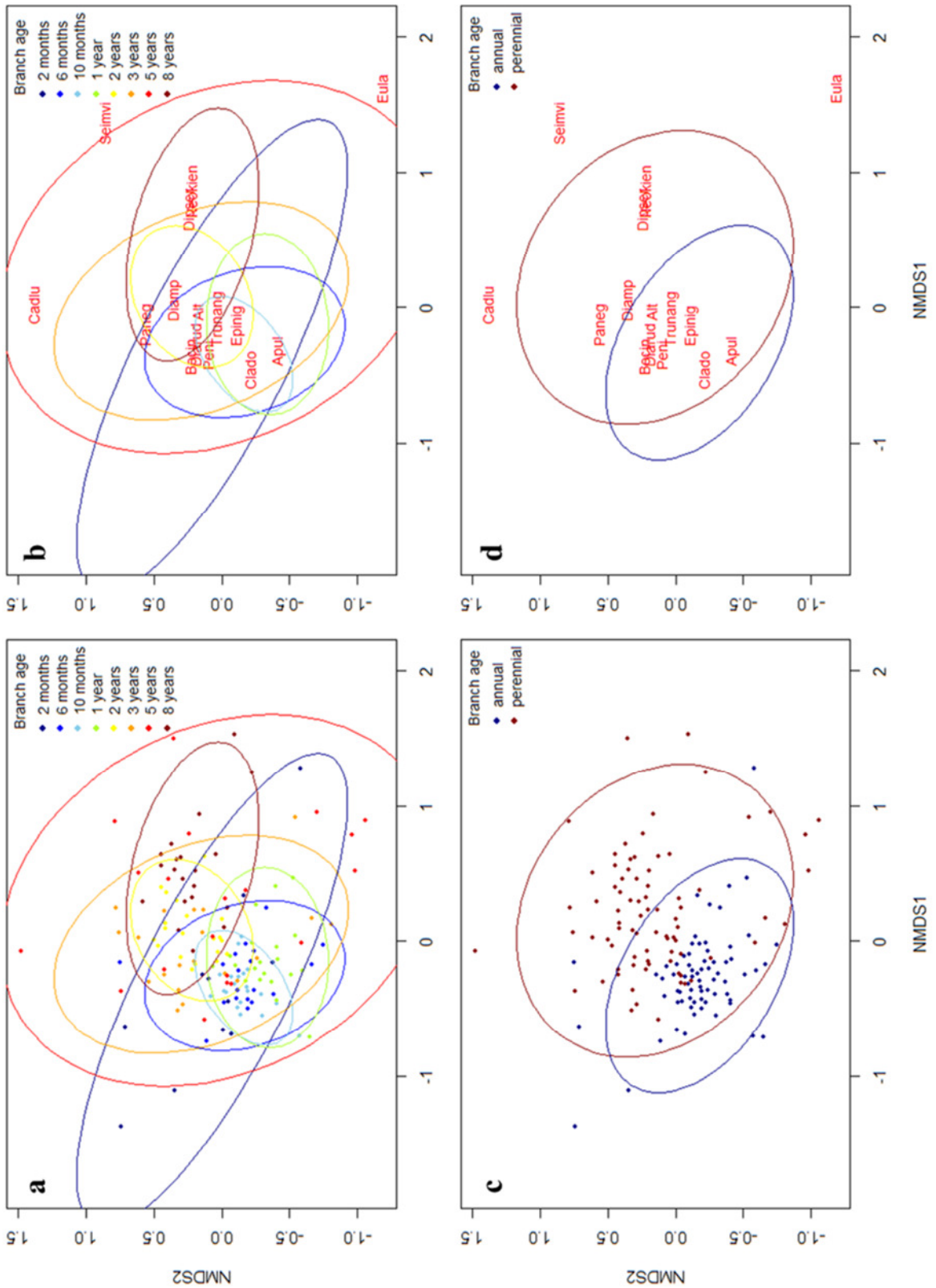
a
b
c
d
Branch age
2 months
6 months
10 months
1 year
2 years
3 years
5 years
8 years
annual
perennial
Cadlu
Seimvi
Eula
Paneg
Diamp
Clado
Epinig
Apul
NMDS1
NMDS2

Fig. 4: Nonmetric multidimensional scaling (nMDS) biplots of fungal communities grouped by (a and b) branch age 2 month to 8 year old, and (c and d) by annual (< 2 year) and perennial (2–8 year) branches. Shown is the position of the single communities by means of dissimilarities (a and c), and the position of fifteen most abundant fungal species based on co-occurrence in multiple samples (b and d). Alt = *Alternaria* spp., Apul = *Aureobasidium pullulans*, Bocin = *Botrytis cinerea*, Cadlu = *Cadophora luteo-olivacea*, Clado = *Cladosporium* spp., Diamp = *Diaporthe ampelina*, Diarud = *Diaporthe rudis*, Dipser = *Dilpodia seriata*, Epnig = *Epicoccum nigrum*, Eula = *Eutypa lata*, Neokien = *Neofabrea kienholzii*, Paneg = *Paraphaeosphaeria neglecta*, Peni = *Penicillium* spp., Seimvi = *Seimatosporium vitis*, Trunang = *Truncatella angustata*.

Branches of some age groups (2, 6, and 10 months; 3 years) were sampled from grapevines of identical age and from the same location. The community composition was found to differ between these age groups, especially when comparing 3-year branches with 6- and 10-month branches. One-year-old branches, sampled from grapevines of a different age and location, expressed similarities to 6- and 10-month branches.

Seven fungal species (*Alternaria* spp., *B. cinerea*, *D. ampelina*, *Diaporthe rudis*, *E. nigrum*, *T. angustata*, *Penicillium* spp.) formed the core community for all branch ages (Fig. 4 c, d). They can be found in branches of almost all age groups. The two taxa *Cladosporium* spp. and *A. pullulans* were also part of this core community but were more characteristic for annual communities. On the other hand, *C. luteo- olivacea*, *D. seriata*, *E. lata*, *Neofabrea kienholzii*, *Paraphaeosphaeria neglecta*, and *Seimatosporium vitis* were more common in perennial communities.

Tab. 4: Comparative ANOSIM analysis of fungal communities from grapevine branches of different age. *p* values (Holm corrected) for significance (bottom left) and R values (top right) for factor strength are shown. Combinations with R values > 0.500 (green) are considered as different, 0.250–500 (blue) as different with some overlaps and <0.250 (red) as similar.

	2 months	6 months	10 months	1 year	2 years	3 years	5 years	8 years
2 months	-- --	0.281	0.670	0.530	0.684	0.326	0.16	0.728
6 months	0.028	-- --	0.208	0.239	0.657	0.362	0.245	0.705
10 months	0.028	0.028	-- --	0.286	0.711	0.478	0.505	0.885
1 year	0.028	0.028	0.028	-- --	0.567	0.416	0.405	0.814
2 years	0.028	0.028	0.028	0.028	-- --	0.149	0.369	0.582
3 years	0.028	0.028	0.028	0.028	0.028	-- --	0.124	0.443
5 years	0.034	0.028	0.028	0.028	0.028	0.028	-- --	0.284
8 years	0.028	0.028	0.028	0.028	0.028	0.028	0.028	-- --

Pairwise analysis of similarities (ANOSIM) (Tab. 4) and analysis of similarities (PermANOVA) (Tab. 5) tests led to significant differences ($p < 0.05$) between all compared branch groups. Nevertheless, the *R* value of the ANOSIM analysis is more reliable, since it describes the degree of (dis-)similarity between two groups. According to these values, if comparing one of the age groups with all other groups, at least two were similar or showed overlap to the output group. Only the 8-year group shared no match with another group. Furthermore, 3-year branches were similar to the 2- and 5- year groups but differed in some parts from other groups.

Tab. 5: Comparative PermANOVA analysis of fungal communities from grapevine branches of different ages. *p* values (Holm corrected) for significance level are shown.

	2 months	6 months	10 months	1 year	2 years	3 years	5 years	8 years
2 months	-- --							
6 months	0.028	-- --						
10 months	0.028	0.028	-- --					
1 year	0.028	0.028	0.028	-- --				
2 years	0.028	0.028	0.028	0.028	-- --			
3 years	0.028	0.028	0.028	0.028	0.028	-- --		
5 years	0.028	0.028	0.028	0.028	0.028	0.028	-- --	
8 years	0.028	0.028	0.028	0.028	0.028	0.028	0.028	-- --

Discussion

The aim of this work was to investigate the early development of the fungal endophytic community in healthy grapevine branches of different age (2 months to 8 years). A comparative analysis of the community composition revealed that grapevine wood harbors a core community of fungal species, which appears almost age-independent. This group consists of *Alternaria* spp., *A. pullulans*, *B. cinerea*, *Cladosporium* spp., *Diaporthe* spp., *E. nigrum*, *Penicillium* spp., and *T. angustata*. They appear from the beginning of community development in annual branches with high abundance. With aging of the wood, other fungi, like *Neofabraea kienholzii* and *P. neglecta*, join the community or become more abundant. Among them are GTD-associated pathogens also, most strikingly *C. luteo- olivacea*, *D. seriata*, and *E. lata*. There is also a group of fungi which includes the singleton and doubleton OTUs. This large group of rare fungi encompasses half of the identified taxa in this study and prevents a full characterization of the fungal communities, as reflected in the unsaturated OTU accumulation curves. In other surveys, this high diversity of uncommon endophytes in grapevine also led to no clear plateau phase of the accumulation curve (Hofstetter *et al*., 2012; Bruez *et al*., 2016).

Even though our main interest was in the relation between branch age and endophytic fungal community, the experimental set up made it necessary to collect some branch samples (1, 2, 5, and 8 years) from plants at different locations and year of planting. Here, the nMDS analysis showed some similarities in community composition between 1-year branches on one side and 6- and 10-month branches on the other side. In contrast, branch samples of different age but taken from grapevines of the same age group and location showed differences in the community composition. For instance, this was demonstrated for 3-year-old branches compared to 6 and 10-month branches. With this background, we assume that, at least for this work, the impact of plant age and location on the fungal community in grapevine branches are low.

In 3-year and older branches, about double the number of species could be isolated compared to younger branches. However, the investigated diversity indices revealed only significant differences between the 2-year group and 10-month group, as well as the 2-year group and 3-year group, probably because of the low number of species isolated from 2-year-old branches. This marginal or almost lacking dynamic in fungal diversity is in line with the work of Bruez and colleagues (2014, 2016), who could not find differences in diversity irrespective of grapevine health status (Esca leaf-symptomatic or asymptomatic), age, or season.

Alternaria spp., *A. pullulans*, *D. seriata*, *Cladosporium* spp., *E. nigrum*, and *B. cinerea* were isolated from all age groups. Except for *B. cinerea*, these were also the most frequently recovered fungi in this survey. *Alternaria* spp., *A. pullulans*, *Cladosporium* spp., and *E. nigrum* are also frequently mentioned in other publications, dealing with community analysis of grapevine wood (Hofstetter *et al*., 2012; Pancher *et al*., 2012). However, in the work of Bruez *et al*. (2014), who investigated the fungal community of 10-year-old grapevine wood, these fungi were shown to be less important and hardly appeared in 42- and 58-year-old wood in a subsequent study (Bruez *et al*., 2016). It is therefore possible that these fungi mainly act as primary settlers and with time get replaced by other fungi during aging of the particular tissue. This would mean that *Alternaria* spp., *A. pullulans*, and *Cladosporium* spp., which showed antagonistic abilities against *E. lata* due to rapid colonization of pruning wounds, cannot compete against other fungi in mature wood and thus would not be suitable Biological Control Agents (BCAs) against GTD-associated pathogens (Munkvold & Marois, 1993).

Another promising BCA, *F. lateritium* (John *et al*., 2005; Munkvold & Marois, 1993), could not be isolated in our study. However, seven other species of the genus *Fusarium*, namely *Fusarium acuminatum*, *Fusarium avenaceum*, *Fusarium cerealis*, *Fusarium culmorum*, *Fusarium equiseti*, *Fusarium oxysporum*, and *Fusarium poae*, were identified. This observed diversity of *Fusarium* species seems to be common for grapevine wood. For example,

Hofstetter *et al.* (2012) could isolate eight different species of *Fusarium* from mature grapevine wood and nursery plants. Essentially, all publications working on grapevine communities in wood reported on the appearance of *Fusarium* spp., with more or less abundant occurrence (Casieri *et al*., 2009; González & Tello, 2011; Pancher *et al*., 2012; Bruez *et al*., 2014; Travadon *et al*., 2016).

During this work, three species of *Trichoderma* (*Trichoderma caerulescens*, *Trichoderma gamsii*, and *Trichoderma paraviridescens*) were isolated, mostly from perennial wood samples and with low frequency. As with *Fusarium*, the diversity of *Trichoderma* spp. in grapevine can be very high. In grapevine of ≥ 42 years of age, more than five species of *Trichoderma* were found and in sum this genus was the most abundant species within the fungal community (Bruez *et al*., 2016). Two further authors reported on a high occurrence of *Trichoderma* spp. in grapevine wood of older plants (González & Tello, 2011; Bruez *et al*., 2014). However, our marginal findings of *Trichoderma* species are in agreement with the study of Hofstetter *et al*. (2012) based on 15- to 30-year-old grapevines in Switzerland.

Úrbez-Torres (2011) proposed the name Botryosphaeria dieback to summarize all grapevine trunk disease symptoms caused by species of the family Botryosphaeriaceae. Among these, *D. seriata* is one of the most frequently isolated species from grapevine-growing regions worldwide (Bertsch *et al*., 2012) and in the present study even turned out to be the most dominant GTD-associated fungus. *D. seriata* could be isolated from all eight age groups, with an increased abundance in perennial wood. This high occurrence of *D. seriata*, especially in older wood, is in line with earlier observations (González & Tello, 2011; Hofstetter *et al*., 2012; Bruez *et al*., 2014). In contrast, analysis of 42- and 58- year-old grapevine wood revealed only sporadic incidence of this pathogen (Bruez *et al*., 2016), indicating that in higher aged grapevines, *D. seriata* becomes repressed by highly competitive fungi.

D. ampelina (anamorph: *Phomopsis viticola*) was the second most dominant GTD pathogen in our analysis. This fungus can infect all green parts and causes variable symptoms,

including Phomopsis dieback (Úrbez-Torres *et al*., 2013; Fontaine *et al*., 2016). The genus *Diaporthe* is highly diverse on *V. vinifera*. However, *D. ampelina* seems to be the most abundant and shows the highest pathogenicity on grapevine (Baumgartner *et al*., 2013; Lawrence *et al*., 2015). Beside *D. ampelina*, also *Diaporthe eres*, and *D. rudis* were isolated in this work. It seems that *Diaporthe* spp. need several months after bud burst before they become established in the community, since all species were only found in branches of 10 months and older.

Regarding *Eutypa* dieback, six species of the Diatrypaceae family were found in this work, of which *E. lata* was the most prominent. All isolates were obtained from 1-year and older grapevine wood, indicating that these fungi prefer perennial wood. This assumption is supported by Casieri *et al.* (2009) and Hofstetter *et al.* (2012), who could isolate no or only few *E. lata* from grapevine nurseries. On the other hand, in older plants, *D. lata* shows a higher abundance (Hofstetter *et al*., 2012; Bruez *et al*., 2016). In general, the diversity of *Diatrypaceae* spp. associated with *Eutypa* dieback is increasing worldwide (Trouillas *et al*., 2010; Trouillas *et al*., 2011; Luque *et al*., 2012; Moyo *et al*., 2018). Our findings are the first of *Eutypa maura* on grapevine worldwide and the first of *Eutypa leptoplaca* and *Cryptovalsa ampelina* in Germany.

The role of *C. luteo-olivacea* as a possible agent of GTD was emphasized in many studies. This cosmopolitan pathogen is associated with young grapevine decline (Petri disease) but also with wood decay of grapevine in North America (Gramaje *et al*., 2011; Travadon *et al*., 2015; Fontaine *et al*., 2016). In our study, *C. luteo-olivacea* appeared rarely in 1-year- old and older branches, whereas in other publications, the isolation rate was very high in grapevine nurseries and planting material (Casieri *et al*., 2009; Hofstetter *et al*., 2012). With this background, it appears that *C. luteo-olivacea* plays a significant role as a pathogen in young and a minor in old vineyards. Nevertheless, this fungus in interaction with other fungi is also able to induce GTD-related symptoms in mature grapevine.

Recently, *S. vitis* was recovered from grapevine plants in Hungary expressing GTD symptoms and it has been shown that this fungus can produce lesions in grapevine wood under artificial conditions (Váczy, 2017). This fungus of the Sporocadaceae was first described by Senanayake *et al*. in 2015 on dead stems of grapevine. Also reports from Californian vineyards are known (Lawrence & Travadon, 2018). Our finding is the first report for Germany.

In contrast to *S. vitis*, *T. angustata*, another newly introduced GTD-associated fungus, appears in every survey done on fungal grapevine wood communities, however, in low abundance (Casieri *et al*., 2009; González & Tello, 2011; Pancher *et al*., 2012; Arzanlou *et al*., 2013; Bruez *et al*., 2014, 2016; Maharachchikumbura *et al*., 2016). Also, in the present study, *T. angustata* could be found in nearly all branch ages; however, the frequency was low throughout. Given that *T. angustata* is ubiquitous in grapevine wood, irrespective of plant age, health status, or geographic location, we assume that this fungus takes a passive part in the core community of grapevine wood.

With our background of inspecting healthy wood tissue only, it is not surprising that we could not isolate prominent Esca-associated fungi such as *Fomitiporia mediterranea* or *Phaeoacremonium* spp. The number of *P. chlamydospora* isolates was also quite low. This is in good agreement with the study of Bruez *et al*. (2014), where *Phaeomoniella* and *Phaeoacremonium* spp. were only found sporadically in unaffected wood of 10-year-old grapevines, whereas with increasing age, these fungi take a more prominent role in the endophytic community (Bruez *et al*., 2016). It seems reasonable to assume that irrespective of the pruning system applied, typical Esca pathogens do not play a major role in healthy grapevine wood of 10 years and younger.

In conclusion, from the beginning of bud burst, niches in grapevine branches become colonized very fast by a diverse set of primary settlers, which also form the core community of grapevine branches. Communities in perennial branch of 2 years and older are dominated by a group of late settlers, among them are numerous GTD-associated fungi. For grapevine pruning

systems, which produce a high amount of perennial branches, such as SMPH, this implies an increased threat of GTDs compared to systems like VSP, from which perennial branches are removed every season. On the other hand, it has been shown that minimal pruned vines have a lower incidence of Esca, their trunks express less necrotic wood and are associated with fewer virulent GTDs compared to intensive pruned vines (spur pruning) (Travadon *et al*., 2016).

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References

Andolfi, A., Mugnai, L., Luque, J., Surico, G., Cimmino, A. & Evidente, A. (2011) Phytotoxins produced by fungi associated with grapevine trunk diseases. Toxins 3, 1569–1605.

Arzanlou, M., Narmani, A., Moshari, S., Khodaei, S. & Babai-Ahari, A. (2013) *Truncatella angustata* associated with grapevine trunk disease in northern Iran. Arch. Phytopathol Plant Protec. 46, 1168–1181.

Baumgartner, K., Fujiyoshi, P.T., Travadon, R., Castlebury, L.A.,Wilcox, W.F. & Rolshausen, P.E. (2013) Characterization of species of *Diaporthe* from wood cankers of grape in eastern North American vineyards. Plant Dis. 97, 912–920.

Berg, G., Rybakova, D., Grube, M. & Köberl, M. (2016) The plant microbiome explored: implications for experimental botany. J. Exp. Bot. 67, 995–1002

Bertsch, C., Ramírez-Suero, M., Magnin-Robert, M., Larignon, P., Chong, J., Abou-Mansour, E., Spagnolo, A., Clément, C. & Fontaine, F. (2012) Grapevine trunk diseases: complex and still poorly understood. Plant Pathol. 62, 243–265.

Bruez, E., Vallance, J., Gerbore, J., Lecomte, P., Da Costa, J.-P., Guérin-Dubrana, L. & Rey, P. (2014) Analyses of the temporal dynamics of fungal communities colonizing the healthy wood tissues of esca leaf-symptomatic and asymptomatic vines. PLoS One 9:e95928.

Bruez, E., Baumgartner, K., Bastien, S., Travadon, R., Guérin-Dubrana, L. & Rey, P. (2016) Various fungal communities colonise the functional wood tissues of old grapevines externally free from grapevine trunk disease symptoms. Aust. J. Grape Wine Res. 22, 288–295.

Carbone, I. & Kohn, L.M. (1999) Amethod for designing primer sets for speciation studies in filamentous ascomycetes. Mycologia 91, 553– 258.

Casieri, L., Hofstetter, V., Viret, O. & Gindro, K. (2009) Fungal communities living in the wood of different cultivars of young *Vitis vinifera* plants. Phytopathol. Mediterr. 48, 73–83.

Cloete, M., Fischer, M., Mostert, L. & Halleen, F. (2014) A novel *Fomitiporia* species associated with esca on grapevine in South Africa. Mycolog. Progr. 13, 303–311.

Crous, P.W. & Gams, W. (2000) *Phaeomoniella chlamydospora gen. et comb. nov*., a causal organism of Petri grapevine decline and esca. Phytopathol. Mediterr. 39, 112–118.

Fischer, M. (2002) A new wood-decaying basidiomycete species associated with esca of grapevine: *Fomitiporia mediterranea* (*Hymenochaetales*). Mycol. Progr. 1, 315–324.

Fischer, M. & Kassemeyer, H.H. (2003) Fungi associated with esca disease of grapevine in Germany. Vitis 42, 109–116

Fontaine, F., Gramaje, D., Armengol, J., Smart, R., Nagy, Z.A., Borgo, M., Rego, C. & Corio-Costet, M.-F. (2016) Grapevine trunk diseases. A review. OIV Publications, Paris. http://www.oiv.int/public/medias/4650/trunk-diseases-oiv-2016.pdf (Accessed 28th Feb 2018).

Glass, L.N. & Donaldson, G.C. (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous Ascomycetes. Appl. Environ. Microb. 61, 1323–1330.

Gramaje, D., Mostert, L. & Armengol, J. (2011) Characterization of *Cadophora luteo-olivacea* and *C. melinii* isolates obtained from grapevines and environmental samples from grapevine nurseries in Spain. Phytopathol. Mediterr. 50, S112–S126.

Gramaje, D., Úrbez-Torres, J.R. & Sosnowski, M.R. (2018) Managing grapevine trunk diseases with respect to etiology and epidemiology: current strategies and future prospects. Plant Dis. 102, 12–39.

González, V. & Tello, M.L. (2011) The endophytic mycota associated with *Vitis vinifera* in central Spain. Fungal Divers. 47, 29–42.

Hallen, F. & Fourie, P.H. (2016) An integrated strategy for the proactive management of grapevine trunk disease pathogen infections in grapevine nurseries. S. Afr. J. Enol. Vitic. 37, 104–114.

Hofstetter, V., Buyck, B., Croll, D., Viret, O., Couloux, A. & Gindro, G. (2012) What if esca disease of grapevine were not a fungal disease? Fungal Divers. 54, 51–67.

John, S.,Wicks, T.J., Hunt, J.S., Lorimer, M.F., Oakey, H. & Scott, E.S. (2005) Protection of grapevine pruning wounds from infection by *Eutypa lata* using *Trichoderma harzianum* and *Fusarium lateritium*. Australas. Plant Pathol. 34, 569–575.

John, S.,Wicks, T.J., Hunt, J.S. & Scott, E.S. (2008) Colonisation of grapevine wood by *Trichoderma harzianum* and *Eutypa lata*. Aust. J. Grape Wine Res. 14:18–24.

Kernaghan, G., Mayerhofer, M. & Griffin, A. (2017) Fungal endophytes of wild and hybrid *Vitis* leaves and their potential for vineyard biocontrol. Can. J. Microbiol. 63, 583–595.

Lawrence, D.P., Travadon, R. & Baumgartner, K. (2015) Diversity of *Diaporthe* species associated with wood cankers of fruit and nut crops in northern California. Mycologia 107, 926–940.

Lawrence, D.P. & Travadon R. (2018) Novel *Seimatosporium* species from grapevine in northern California and their interactions with fungal pathogens involved in the trunk-disease complex. Plant Dis. 102, 1082–1092.

Luque, J., Garcia-Figueres, F., Legorburu, F.J., Muruamendiaraz, A., Armengol, J. & Trouillas, F.P. (2012) Species of Diatrypaceae associated with grapevine trunk diseases in Eastern Spain. Phytopathol. Mediterr. 51, 528–540.

Maharachchikumbura, S.S.N., Larignon, P., Hyde, K.D., Al-Sadi, A.M. & Liu, Z.Y. (2016) Characterization of *Neopestalotiopsis*, *Pestalotiopsis* and *Truncatella* species associated with grapevine trunk diseases in France. Phytopathol. Mediterr. 55, 380–390.

de Mendiburu F (2017) Agricolae: statistical procedures for agricultural research. R package version 1.2-8. https://CRAN.Rproject.org/package=agricolae. Accessed 29th Oct 2018.

Mondello, V., Lo Piccolo, S., Conigliaro, G., Alfonzo, A., Torta, L. & Burruano, S. (2013) First report of *Neofusiccoccum vitifusiforme* and presence of other Botryosphaeriaceae species associated with Botryosphaeria dieback of grapevine in Sicily (Italy). Phytopathol. Mediterr. 52, 388–396.

Moyo, P., Mostert, L., Spies, C., Damm, U. & Hallen, F. (2018) Diversity of Diatrypaceae species associated with dieback of grapevines in South Africa, with the description of *Eutypa cremea sp. nov*. Plant Dis. 102, 220–230.

Mugnai, 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.

Munkvold, G.P. & Marois, J.J. (1993) Efficacy of natural epiphytes and colonizers of grapevine pruning wounds for biological control of Eutypa dieback. Phytopathology 83, 624–629.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O'Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E. & Wagner, H. (2018) Vegan: community ecology package. R package version 2.5-1. https://CRAN.Rproject.org/package=vegan. Accessed 29th Oct 2018.

Pancher, M., Ceol, M., Corneo, P.E., Longa, C.M.O., Yousaf, S., Pertot, I. & Campisano, A. (2012) Fungal endophytic communities in grapevines (*Vitis vinifera L.*) respond to crop management. Appl. Environ. Microbiol. 78, 4308–4317.

Pertot, I., Prodorutti, D., Colombini, A. & Pasini, L. (2016) *Trichoderma atroviride* SC1 prevents *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* infection of grapevine plants during the grafting process in nurseries. BioControl 61, 257–267.

Pinto, C. & Gomes, A.C. (2016) *Vitis vinifera* microbiome: from basic research to technological development. BioControl 61, 243–256.

R Core Team (2013) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/ (accessed 18th May 2017).

Senanayake, I.C., Maharachchikumbura, S.S.N., Hyde, K.D., Bhat, J.D., Jones, E.B.G., McKenzie, E.H.C., Dai, D.Q., Dagaranagama, D.A., Dayarathne, M.C., Goonasekara, I.D., Konta, S., Li, W.J., Shang, Q.J., Stadler, M., Wijayawardene, N.N., Xiao, Y.P., Norphanphoun, C., Li, Q., Liu, X.Z., Bahkali, A.H., Kang, J.C., Wang, Y., Wen, T.C., Wendt, L., Xu, J.C. & Camporesi, E. (2015) Towards unraveling relationships in Xylariomycetidae (Sordariomycetes). Fungal Divers. 73, 73–144.

Taylor, D.L. & Houston, S. (2011) A bioinformatics pipeline for sequence-based analyses of fungal biodiversity. Methodes Mol. Biol. 722, 141–155

Tillett, D. & Neilan, B.A. (2000) Xanthogenate nucleic acid isolation from cultured and environmental cyanobacteria. J. Phycol. 36, 251–258.

Travadon, R., Lawrence, D.P., Rooney-Latham, S., Gubler, W.D.,Wilcox, W.F., Rolshausen, P.E. & Baumgartner, K. (2015) *Cadophora* species associated with wood-decay of grapevine in North America. Fungal Biol. 119, 53–66.

Travadon, R., Lecomte, P., Diarra, B., Lawrence, D.P., Renault, D., Ojeda, H., Rey, P. & Baumgartner, K. (2016) Grapevine pruning system and cultivars influence the diversity of wood-colonizing fungi. Fungal Ecol. 24, 82–93.

Trouillas, F., Úrbez-Torres, J.R. & Gubler, W.D. (2010) Diversity of diatrypaceous fungi associated with grapevine canker diseases in California. Mycologia 102, 319–336.

Trouillas, F.P., Pitt, W.M., Sosnowski, M.R., Huang, R., Peduto, F., Loschiavo, A., Savocchia, S., Scott, E.S. & Gubler, W.D. (2011) Taxonomy and DNA phylogeny of Diatrypaceae associated with *Vitis vinifera* and other woody plants in Australia. Fungal Divers. 49, 203–223.

Úrbez-Torres, J.R. (2011) The status of Botryosphaeriaceae species infecting grapevines. Phytopathol. Mediterr. 50(Supplement), S5–S45.

Úrbez-Torres, J.R., Peduto, F., Striegler, R.K., Urrea-Romero, K.E., Rupe, J.C., Cartwright, R.D. & Gubler, W.D. (2012) Characterization of fungal pathogens associatedwith grapevine trunk diseases in Arkansas and Missouri. Fungal Divers. 52, 169–189.

Úrbez-Torres, J.R., Peduto, F., Smith, R.J. & Gubler, W.D. (2013) Phomopsis dieback: a grapevine trunk disease caused by *Phomopsis viticola* in California. Plant Dis. 97, 1571–1579.

Váczy, K.Z. (2017) First report of *Seimatosporium vitis* associated with grapevine trunk disease symptoms in Hungary. Plant Dis. 101, 253.

White, T.J., Bruns, T., Lee, S. & Taylor, J.W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ, White TJ (eds) PCR protocols: a guide to methods and applications, pp. 315–322. Academic Press, Inc., New York.

Manuscript III

Esca in German vineyards: Does the training system influence occurrence of foliar symptoms

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Abstract

Esca is one of the most destructive grapevine trunk diseases (GTDs) worldwide. Several factors, such as plant age, grapevine cultivar, or pattern of precipitation have been identified as possible driving forces of the disease. In the present study, a four years monitoring of Esca foliar symptoms in vineyards located in three areas in Rhineland-Palatinate was conducted. Vineyards of different age and planted with different cultivars, both traditional and fungus-resistant, were chosen. All vineyards were subdivided into minimally and intensively pruned sections. The following aspects were investigated; i) the occurrence of external Esca foliar symptoms over the season; ii) a possible influence of cultivar, plant age and precipitation on symptom development; iii) a possible impact of the training system on incidence of foliar symptoms. In summary, all parameters were shown to have at least some influence on symptoms incidence, even though some of the results were inconsistent over the period of monitoring. Concerning the influence of the training system, in 2015 no differences between minimally pruned (1.8%) and intensively pruned (1.9%) vines were found. However, in the following year minimally pruned vines (6.9%) expressed significantly more symptoms than intensively pruned vines (4.9%). In the years 2017 and 2018 the opposite was the case. 2.6% and 2.4%, respectively, of the minimally pruned vines showed foliar symptoms, while for the intensively pruned vines the mean values were 4.5% and 3.6%, respectively. Our study represents the first systematic Esca related data collection in German vineyards over an extended period of time. Our data should help to better understand the relation between

incidence of external foliar symptoms in the course of the year and possibly influencing parameters, both biotic and abiotic.

Keywords: esca, grapevine trunk diseases, intensive pruning, minimal pruning, monitoring, training systems, *Vitis vinifera*

Introduction

Grapevine is one of the economically most important fruit crops in the world due to the production of wine, table grapes and dried fruits (FAO, 2018). Its cultivation involves a broad range of plant protection measures, since a wide range of pathogenic microorganisms such as viruses, bacteria and fungi are threatening the health of this perennial plant (Armijo *et al*., 2016; Pertot *et al*., 2017). Grapevine trunk diseases (GTDs), caused by a set of phytopathogenic fungi, ensemble a variety of symptoms visible on the foliage, the berries and in the vascular tissue (Mugnai *et al*., 1999; Bertsch *et al*., 2012; Fontaine *et al*., 2016). Within GTDs the Esca complex is one of the most striking diseases, causing massive losses in viticulture (Gramaje *et al.*, 2018). The worldwide economic cost for the replacement of dead grapevines is estimated to be more than 1.5 billion dollars per year (Vasquez *et al.,* 2007; Romanazzi *et al*., 2009; Hofstetter *et al*., 2012; Lecomte *et al.*, 2012). It is assumed that the pathogens are invading the plant mostly by pruning wounds; they colonize the vascular tissue and, presumably by release of phytotoxins, induce the typical symptoms (Serra *et al.*, 2008; Andolfi *et al.*, 2011). In this context *Phaeomoniella chlamydospora*, *Phaeoacremonium* spp., as well as members of the basidiomyceteous genus *Fomitiporia* are the most frequently found fungi in Esca affected plants (Mugnai *et al.*, 1999; Larignon & Dubos, 2000; Crous & Gams, 2000; Fischer, 2002; Fischer & Kassemeyer, 2003; White *et al.*, 2011; Cloete *et al*., 2014). Other authors assume that alterations in water supply caused by occlusions, disruption of the sap flow or other disturbances may be the reason for the symptoms (Lecomte *et al.*, 2012; Pouzoulet *et al*., 2014, 2017).

As for external symptoms, two forms of Esca are acknowledged, i.e. chronic and apoplectic (Mugnai *et al.*, 1999). The former is characterized by discoloration of the leaves, forming the so called “tiger stripes” pattern, spotting of the berry skin (“black measles”) and/or withering of grape clusters. Chronic symptoms can occur inconsistently over subsequent years,

i.e. a short-term lethal outcome may not necessarily occur. The apoplectic form however usually terminates the life of the plant (Surico, 2009). Here the entire vine or discrete arms suddenly wilt and within a few days lose all leaves and grape clusters. Both forms begin to appear in early summer and reach the maximum number of newly symptomatic vines in midsummer (Surico *et al.*, 2000; Kuntzmann *et al.*, 2010; Lecomte *et al.*, 2012).

Incidences of Esca may vary from year to year and seem to be linked to multiple factors. Among them are plant age, grapevine cultivar and climatic condition (Surico *et al.*, 2000; Marchi, 2001; Quaglia *et al.*, 2009; Bertsch *et al.*, 2012; Bruez *et al.*, 2013; Kuntzmann *et al.*, 2013; Fontaine *et al*., 2016; Gramaje *et al.*, 2018). For plant age it was demonstrated that over the years the risk for external Esca symptoms can increase drastically (Surico *et al.*, 2006). First symptoms can be observed after only a few years (Díaz & Latorre, 2013; Fontaine *et al.* 2016). Later the number of affected vines elevates until saturation. Regarding the influence of the cultivar, it was reported that some cultivars are more susceptible to Esca than others. In Italian vineyards with the cultivars ‘Cabernet Sauvignon’, ‘Grechetto’ or ‘Sangiovese’ incidence of Esca was much higher than in ‘Chardonnay’ or ‘Merlot’ containing vineyards (Marchi, 2001; Quaglia *et al.*, 2009). The scion-rootstock combination also seems to affect the development of symptoms (Murolo & Romanazzi, 2014). Another factor lies in the local climate, in particular in the amount of rainfall: in Italian vineyards, Surico *et al*. (2000) observed a higher incidence rate of Esca in years with an increased level of rainfall in June and July. This observation was supported by Marchi *et al*. (2006), who surveyed vineyards in Italy over a period of five years and found a negative correlation between rainfall in early summer and the percentage of vines with “hidden Esca” (externally asymptomatic in the given year, but symptomatic in at least one previous year). Besides the three factors mentioned above further aspects, e.g. the grapevine microbiom, can contribute to the expression of Esca (Hofstetter *et al*., 2012; Bruez *et al*., 2014; Bruez *et al.*, 2016). Many of the Esca influencing factors are yet poorly understood and some might even be still unknown.

In German vineyards, Esca is the most destructive GTD and within the last two decades its relevance has constantly increased. More recent information about the prevalence of Esca in Germany and its influence factors is however sparse (Fischer & Kassemeyer, 2003; Fischer, 2006). During a four years survey we therefore systematically investigated the appearance of external Esca symptoms over the year in twelve different vineyards from three different regions of Rhineland-Palatinate. A more accurate insight should be gained into the current status of Esca in Germany and some of the elements which are thought to drive the disease. Special emphasis was on the possible contributions of the training system to disease incidence. In this respect we compared minimally (semi minimal pruned hedge, SMPH) and intensively pruned (vertical shoot positioning, VSP) vineyards. SMPH vines show numerous perennial branches in the trellis and develop more buds, shoots, inflorescences and leaves; eventually they form a dense canopy with large leaf area (Clingeleffer, 1984; Intrieri *et al.*, 2001, 2011; Kraus *et al.*, 2018). In addition, we have monitored the impact of grapevine variety and age on Esca development, as well as the temporal occurrence of the symptoms during the season. The findings of this study should help to better understand the complex relation between a variety of environmental parameters, both biotic and abiotic, and visible outbreaks of Esca.

Materials and Methods

Vineyards: Twelve vineyards located in three regions in the vine growing area of Rhineland-Palatinate, Germany, are monitored (Tab. 1). At the location Siebeldingen (A – F) six fields were planted with the cultivars ‘Villaris’, ‘Felicia’, ‘Gf-884-58-988’, ‘Reberger’, ‘Chardonnay’ and ‘Riesling’ (the first four representing fungi resistant cultivars). Four vineyards were located near Bad Kreuznach (G – J), and were planted with the cultivars ‘Silvaner’, ‘Müller-Thurgau’, ‘Pinot Noir’ and ‘Riesling’. Two additional vineyards, ‘Dornfelder’ and ‘Acolon’, near Hahnheim (K – L) were included in the surveys made of 2017 and 2018 to compensate the loss of the ‘Silvaner’ vineyard (A) after 2017. All vineyards were subdivided to approximately equal parts between minimally (SMPH) and intensively pruned (VSP) vines. Furthermore, the vineyards differ in berry color, root stock, age and conversion to SMPH (see Table 1). The vineyards located in Bad Kreuznach were older (year of planting 1982 – 1990) compared to those located in Siebeldingen and Hahnheim (year of planting 2001 – 2008). In all vineyards symptomatic or dead vines were not removed or replaced during this study.

Tab. 1: Vineyards selected for Esca monitoring.

	Cultivar	Berry color	Root stock	Year of planting	Training system	no. of plants	Conversion to SMPH	Location
A	Chardonnay	white	SO4	2008	VSP SMPH	200 200	2013	49°13'11.3"N 8°02'31.5"E
B	Riesling	white	SO4	2008	VSP SMPH	200 200	2013	49°13'11.5"N 8°02'34.5"E
C	Villaris	white	SO4	2001	VSP SMPH	433 448	2013	49°13'13.0"N 8°02'43.1"E
D	Felicia	white	SO4	2001	VSP SMPH	463 461	2013	49°13'13.3"N 8°02'42.7"E
E	Gf-884-58-988	red	SO4	2001	VSP SMPH	217 298	2013	49°13'14.0"N 8°02'42.5"E
F	Reberger	red	SO4	2001	VSP SMPH	477 420	2013	49°13'14.6"N 8°02'42.5"E
G	Riesling	white	356 Fin (B)	1990	VSP SMPH	324 322	2008	49°51'01.9"N 7°50'39.9"E
H	Pinot noir	red	M1	1988	VSP SMPH	341 349	2008	49°51'22.8"N 7°50'21.4"E
I	Silvaner	white	SO4	1982	VSP SMPH	318 345	2008	49°51'21.8"N 7°49'58.8"E
J	Müller-Thurgau	white	SO4	1984	VSP SMPH	316 357	2008	49°51'40.3"N 7°50'35.7"E
K	Dornfelder	red	5BB	2003	VSP SMPH	2130 2266	2013	49°52'15.3"N 8°14'03.3"E
L	Acolon	red	SO4	2003	VSP SMPH	755 807	2013	49°52'15.3"N 8°14'03.3"E

Monitoring: Between 2015 and 2018 all vineyards were monitored for Esca related foliar symptoms, both chronic ("tiger stripes") and apoplectic (Fig. 1). Monitoring was performed every two weeks, from calendar week (CW) 28 through CW 36. Symptomatic plants were marked in a field map made for each vineyard. In addition, missing and dead plants were also noted in the map.

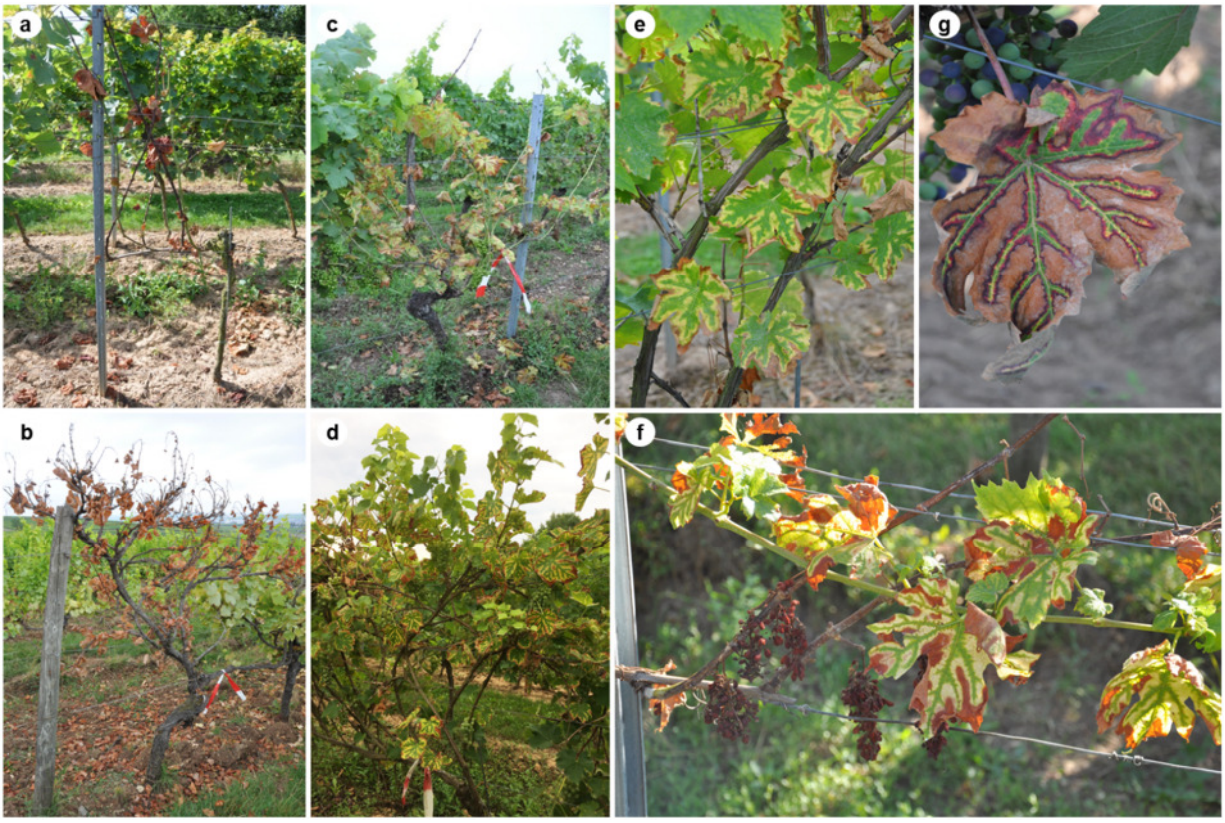

Fig. 1: VSP (a, c) and SMPH (b, d) trained grapevines expressing external Esca symptoms, apoplectic (a, b) and chronic (c, d). Grapevine leaves with "tiger stripes", a typical symptom for Esca chronic diseased plants (e – g).

Climatic conditions: Since climatic conditions, in particular rainfall, have an influence on the expression of Esca foliar symptoms, various climatic factors such as temperature, precipitation, and relative humidity were assessed (Tab. 2) (Surico *et al*., 2000; Marchi *et al*., 2006). Weather data were provided by the DLR Rhineland-Palatinate (www.dlr.rlp.de).

Statistics: For statistical analysis and plot drawing the packages 'lme4' (Bates *et al*., 2015), 'lattice' (Deepayan, 2008), 'multcomp' (Hothorn *et al*., 2008) and 'ggplot2' (Wickham, 2009) from the program RStudio Version 1.1.383 (RStudio Team, 2016) were used. A

generalized linear mixed model (GLMM) was applied to analyze the impact of the training system on the incidence of Esca.

Tab. 2: Climate conditions in Siebeldingen, Bad Kreuznach and Hahnheim April – August, 2015 – 2018: mean temperature, precipitation and relative humidity.

		Temperature Ø [°C]				Precipitation ∑ [mm]				Relative humidity Ø [%]			
		2015	2016	2017	2018	2015	2016	2017	2018	2015	2016	2017	2018
Siebeldingen	Apr	11.2	9.2	9.7	13.8	27.5	58.8	3.8	52.4	59	75	61	71
	May	15.1	14.5	15.8	16.8	38.0	50.1	44.1	91.8	63	71	70	74
	Jun	18.7	17.6	19.8	19.6	40.7	99.4	70.2	38.5	61	80	66	73
	Jul	22.6	20.1	20.3	22.1	16.5	47.9	54.4	21.1	54	70	70	52
	Aug	21.6	19.7	19.3	20.9	26.9	21.2	57.2	48.8	64	69	75	65
Bad Kreuznach	Apr	10.5	9.1	9.3	13.4	25.6	58.6	1.4	9.7	65	79	65	71
	May	14.3	14.2	15.3	16.8	11.6	66.2	71.8	106.3	67	76	77	70
	Jun	17.7	17.5	19.4	19.2	60.8	133.9	50.3	73.5	66	85	70	67
	Jul	21.7	19.9	19.7	22.0	15.2	18.8	92.8	13.6	63	77	76	54
	Aug	21.1	19.3	18.6	20.8	29.6	15.0	76.3	40.9	69	74	81	60
Hahnheim	Apr	- -	- -	9.1	13.2	- -	- -	6.9	22.5	- -	- -	61	68
	May	- -	- -	15.1	16.7	- -	- -	69.8	48.9	- -	- -	73	69
	Jun	- -	- -	19.1	19.5	- -	- -	58.0	45.2	- -	- -	66	68
	Jul	- -	- -	19.8	22.2	- -	- -	103.4	16.0	- -	- -	70	55
	Aug	- -	- -	18.9	20.9	- -	- -	76.9	23.3	- -	- -	74	59

Results

Incidence of Esca as a function of the age and cultivar

During the four years survey the most severe prevalence of Esca foliar symptoms was observed in 2016 (Fig. 2): the older vineyards containing Silvaner (planted 1982; max. 15.7% for SMPH in 2016), Müller-Thurgau (1984; max. 17.6% for SMPH in 2016), and Riesling (1990; max. 16.5% for SMPH in 2016) showed the highest foliar expression in our study. For Pinot Noir (1988; max. 2.0% for SMPH in 2016), Riesling (2008; max. 1.0% for SMPH in 2016), Chardonnay (2008; max. 0.5% in all three years) and Acolon (2003; max. 1.5% for VSP in 2018) foliar symptom frequency was very low; Felicia (2001) was free of symptoms throughout the monitoring period. Incidence level for Villaris (2001; max. 4.7% for SMPH in 2016), Gf-844-59-988 (2001; max. 6.4% for SMPH in 2016), Reberger (2001; max. 6.3% for VSP in 2016) and Dornfelder (2003; max. 8.4% for VSP in 2018) was considered medium, compared to the vineyards with a severe and minor rate of leaf-symptomatic vines.

Fig. 2: Incidence of Esca (chronic and apoplectic) in monitored vineyards (sorted by year of plantation) as a function of the cultivar and training system in the years 2015 to 2018. NA = data not available.

Occurrence of Esca over the course of the season

From 2015 through 2018, the number vines expressing Esca foliar symptoms was recorded at CW 28, 30, 32, 34 and 36 (Fig. 3). First symptoms developed at CW28 (i.e. the beginning of July; early summer); latest emergence of newly symptomatic plants was at CW36 (i.e., beginning of September).

With a proportion of approx. 30 to 40%, a maximum of newly symptomatic vines for every year was observed in CW 32 (i.e. beginning of August; midsummer), except for 2018. Here, the highest peak of newly leaf-symptomatic plants was observed in CW 28 (i.e. beginning

of July) with 68.5% for VSP and 60.4% for SMPH. No significant differences were noted between the training systems in all vineyards.

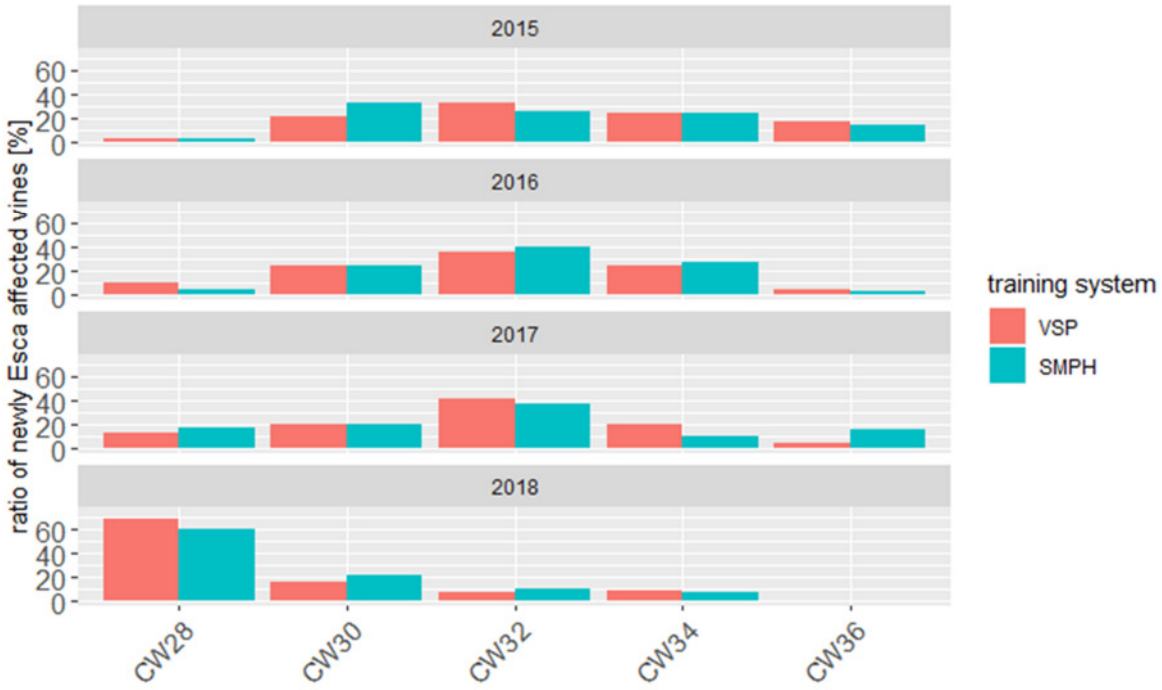

Fig. 3: Ratio of newly Esca affected vines, both chronic and apoplectic, in CW28 – CW36 monitored in the years 2015 to 2018 as a function of the training system.

Incidence of Esca as a function of training system

In 2015 the appearance of symptoms in general was very low and except for Riesling (planted 1990), for which the percentage of symptomatic vines was clearly higher in VSP (6.2%) than in SMPH vineyards (0.6%), no significant differences between the training systems were found (VSP = 1.9%, SMPH = 1.8%; Fig. 4). The highest value in 2015 was recorded in SMPH trained Müller-Thurgau (1984) with 8.7%.

In 2016, the mean value of affected vines increased drastically to approx. double the number of 2015; SMPH plants had significantly more symptoms (6.9%) than VSP plants (4.9%). This difference is mainly due to the higher incidence rate in older SMPH fields (year of planting 1982 – 1990). The highest rate of symptomatic plants in 2016 was again noted in SMPH trained Müller-Thurgau (1984, 17.6%).

In 2017, VSP trained vines, with a mean value of 4.5%, showed almost the same incidence level as in 2016. The value for SMPH trained vines however decreased strongly from 6.9% to 2.6% which was mainly related to the older vineyards: from 2016 to 2017 the mean value of SMPH trained Silvaner (1982) dropped from 16.5% to 10.9%, Riesling (1990) dropped from 16.5% to 5.6% and Müller-Thurgau (1984) from 17.6% to 3.8%. Also in the younger SMPH vineyards of Villaris (2001) and Gf-844-59-988 (2001) the number of symptomatic vines strongly declined, from 4.7% to 1.6% and from 6.4% to 1.4%, respectively. The maximum incidence rate of 13.3% was found in VSP trained Silvaner (1982).

In 2018, the monitoring results are nearly identical to the previous year; intensively pruned vines expressed significantly more foliar symptoms with a mean value of 3.6% compared to minimally pruned vines with 2.4%.

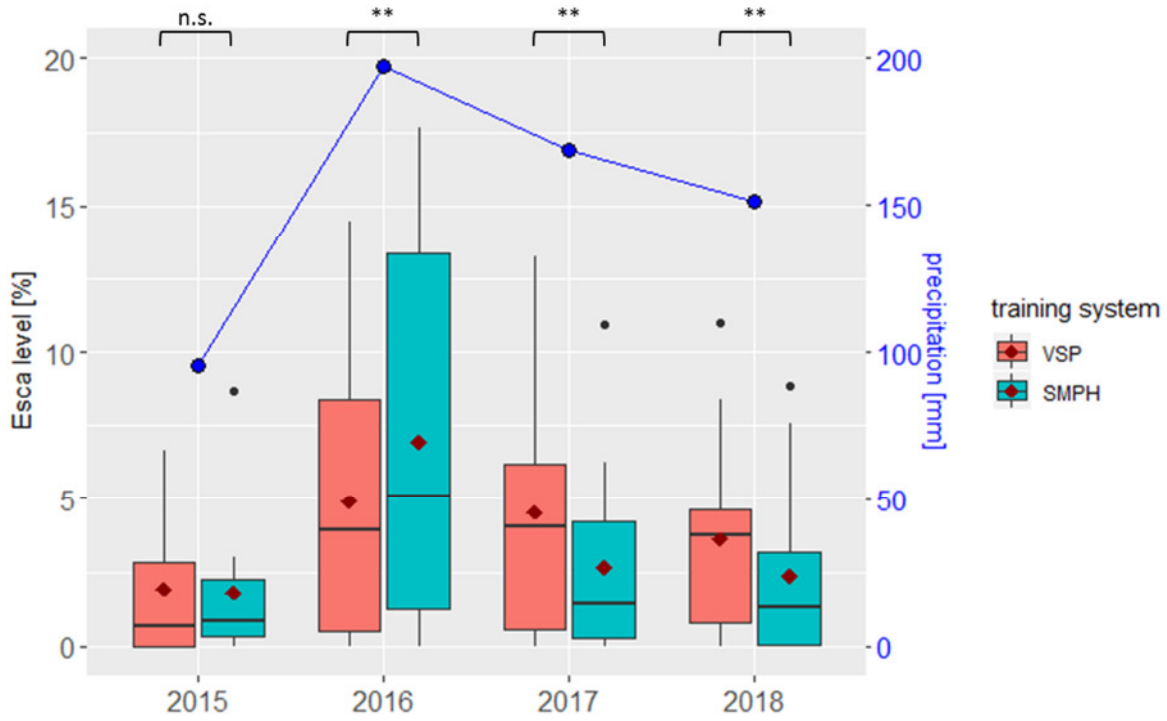

Fig. 4: Incidence of Esca (chronic and apoplectic) in VSP and SMPH trained vineyards in the years 2015 to 2018. The total precipitation [mm] from May to July is illustrated by the blue line. Asterisks illustrate significant differences (p<0.001) between the training systems according to Tukey´s post-hoc test for GLMM.**

Impact of precipitation on the Esca incidence

The amount of precipitation from early to mid-summer (May to July) and the number of Esca affected vines correlated well for all years (Fig. 4). In seasons with elevated

precipitation, such as in 2016 (197.4 mm), 2017 (168.7 mm) and 2018 (151.8 mm), the incidence of symptoms was more severe with a mean rate of 5.9% for 2016, 3.9% for 2017 and 3.0% for 2018, while in 2015, with decreased rainfall (95.2 mm), the incidence rate was low (1.9%). This relation was most evident in VSP trained vineyards, where in three years with higher rainfall the number of Esca affected plants was approx. the same (4.9% – 4.5% – 3.6%). In contrast, the number of symptomatic SMPH vines was much lower in the years 2017 (2.6%) and 2018 (2.4%) than in 2016 (6.9%) with the highest amount of precipitation during this study.

Multiple symptom occurrences

Monitoring results for single vines showed that especially in vineyards with a severe Esca situation (Silvaner, 1982; Müller-Thurgau, 1984; Riesling, 1990) the rate of repeated occurrence of Esca chronic was higher for VSP than for SMPH (Tab. 3). In Silvaner (1982) VSP, for instance, 79.7% of affected vines expressed symptoms once within the three years, 18.6% twice and 1.7% three times. For SMPH vines, the rates were 89.4%, 11.5%, and 0%, respectively. Similar observations were made for Müller-Thurgau (1984), Riesling (1990), Gf-844-59-988 (2001), Reberger (2001) and Dornfelder (2008).

Tab. 3: Rate of multiple observations of Esca chronic and apoplectic on the same vine plant 2015-2018.

Cultivar – year of planting	Training system	total number of plants expressing Esca chronic	Esca chronic [%]				total number of plants expressing Esca apoplexy	Esca apoplexy [%]			
			1x	2x	3x	4x		1x	2x	3x	4x
Silvaner – 1982	VSP	59	79.7	18.6	1.7	- -	7	100.0	0.0	0.0	- -
	SMPH	66	89.4	11.5	0.0	- -	24	100.0	0.0	0.0	- -
Müller-Thurgau – 1984	VSP	61	70.5	27.9	1.6	0.0	3	100.0	0.0	0.0	0.0
	SMPH	93	78.5	20.4	1.1	0.0	9	100.0	0.0	0.0	0.0
Pinot Noir – 1988	VSP	5	100.0	0.0	0.0	0.0	0	0	0.0	0.0	0.0
	SMPH	8	100.0	0.0	0.0	0.0	2	100.0	0.0	0.0	0.0
Riesling – 1990	VSP	89	71.9	23.6	4.5	0.0	22	100.0	0.0	0.0	0.0
	SMPH	75	86.7	13.3	0.0	0.0	12	100.0	0.0	0.0	0.0
Villaris – 2001	VSP	40	95.0	5.0	0.0	0.0	1	100.0	0.0	0.0	0.0
	SMPH	36	94.4	5.6	0.0	0.0	1	100.0	0.0	0.0	0.0
Felicia – 2001	VSP	0	0.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0
	SMPH	0	0.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0
Gf-844-58-988 – 2001	VSP	18	83.3	16.7	0.0	0.0	9	100.0	0.0	0.0	0.0
	SMPH	28	92.9	7.1	0.0	0.0	10	90.0	10.0	0.0	0.0
Reberger – 2001	VSP	63	88.9	11.1	0.0	0.0	11	100.0	0.0	0.0	0.0
	SMPH	48	95.8	4.2	0.0	0.0	8	100.0	0.0	0.0	0.0
Riesling – 2008	VSP	3	100.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0
	SMPH	3	100.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0
Chardonnay – 2008	VSP	4	100.0	0.0	0.0	0.0	0	0.0	0.0	0.0	0.0
	SMPH	0	0.0	0.0	0.0	0.0	1	100.0	0.0	0.0	0.0
Dornfelder – 2008	VSP	234	85.0	15.0	- -	- -	15	100.0	0.0	- -	- -
	SMPH	72	90.3	9.7	- -	- -	3	100.0	0.0	- -	- -
Acolon – 2008	VSP	15	100.0	0.0	- -	- -	1	100.0	0.0	- -	- -
	SMPH	2	100.0	0.0	- -	- -	0	0.0	0.0	- -	- -

Repeated occurrence of Esca apoplectic on the same vine was only observed once in the Gf-844-59-988 (2001) field on a SMPH trained vine. About half of the grapevines that had suffered apoplexy did not sprout in the subsequent season and ended up dead; the remaining affected vines however showed normal vigor. Here, no difference was noted between the two training systems. In 2016 it was noted that, a few weeks after wilting of apoplectic vines, some of the plants had developed new shoots (Fig. 5). This was observed both for VSP and SMPH trained plants.

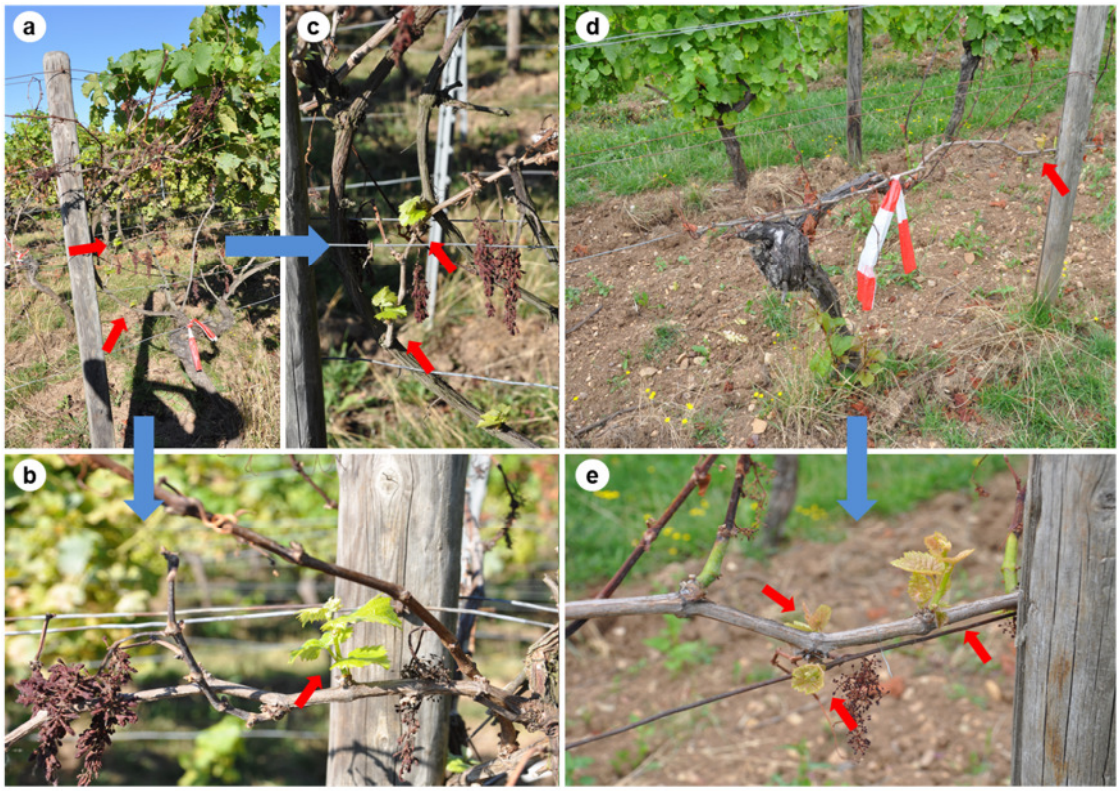

Fig. 5: Development of new shoots on vines suffering from Esca apoplectic (a – e).

Sanitary status of the vineyards

A look at the sanitary status of the vineyards revealed that the amount of missing or dead vines is higher for VSP trained vines compared to SMPH trained, except for Riesling (1990; Tab. 4). In the young Riesling (2008) and Chardonnay (2008) fields no missing or dead vines were found. The highest difference between the training systems was found in the Gf-844-59-988 (2001) field, where 38.2% of the intensively pruned vines were missing or dead and 10.9% of the minimally pruned vines.

Tab. 4: Sanitary status of the vineyards selected for this study. Shown is the total number of plant sites, the percentage of missing and dead vines at the end of the study and the percentage of plants expressing Esca chronic or apoplexy, in relation to the number of plant sites.

Cultivar – year of planting	Training system	Total number of plant sites	missing or dead vines at the end of the study [%]	plants expressing Esca chronic during the study [%]	plants expressing Esca apoplexy during the study [%]
Silvaner – 1982	VSP	430	30.5	13.7	1.6
	SMPH	428	28.0	15.4	5.6
Müller-Thurgau – 1984	VSP	456	37.3	13.4	0.7
	SMPH	448	29.2	20.8	2.0
Pinot Noir – 1988	VSP	373	12.1	1.3	0.0
	SMPH	376	9.3	2.1	0.5
Riesling – 1990	VSP	345	8.1	25.8	6.4
	SMPH	346	11.6	21.7	3.5
Villaris – 2001	VSP	437	3.2	9.2	0.2
	SMPH	454	1.5	7.9	0.2
Felicia – 2001	VSP	492	9.3	0.0	0.0
	SMPH	473	3.2	0.0	0.0
Gf-844-58-988 – 2001	VSP	304	38.2	5.9	3.0
	SMPH	311	10.9	9.0	3.2
Reberger – 2001	VSP	546	23.6	11.5	2.0
	SMPH	425	12.0	11.3	1.9
Riesling – 2008	VSP	200	0.0	1.5	0.0
	SMPH	200	0.0	1.5	0.0
Chardonnay – 2008	VSP	200	0.0	2.0	0.0
	SMPH	200	0.0	0.0	0.5
Dornfelder – 2008	VSP	2132	1.4	11.0	0.7
	SMPH	2277	0.5	3.2	0.1
Acolon – 2008	VSP	755	1.2	2.0	0.1
	SMPH	807	0.4	0.2	0.0

Concerning the training system, it was noted for older vineyards (Silvaner, 1982; Müller-Thurgau, 1984; Pinot Noir, 1988; Riesling, 1990) and Gf-844-59-988 (2001), that the percentage of plants expressing foliar symptoms, related to the number of plant sites, is contrary to the percentage of missing or dead vines. For example, in Müller-Thurgau (1984), 37.3% of VSP trained vines and 29.2% of SMPH trained vines are missing or dead, while 14.1% and 22.8%, respectively, of the vines expressed foliar symptoms during the study. However, for the vineyards Villaris (2001), Reberger (2001), Dornfelder (2008) and Acolon (2008) these numbers correlated positively with the particular training systems; high incidence of foliar symptoms means increased number of missing or dead vines.

Discussion

In our survey 2015 – 2018, the incidence of Esca in twelve selected plots changed from year to year. The highest occurrence of symptomatic vines was noted in older vineyards, i.e. Silvaner (planted 1982), Müller-Thurgau (1984) and Riesling (1988). This correlation between symptom frequency and age is in agreement with former studies undertaken in different countries (Surico *et al*., 2006; Kuntzmann *et al*., 2013; Fontaine *et al*., 2016). In contrast, incidences were low in the young vineyards Riesling (2008), Chardonnay (2008), and only slightly increased in middle-aged Villaris (2001), Gf-844-59-988 (2001), and Reberger (2001). Possibly due to some tolerance against the disease, Pinot Noir (1988) and Felicia (2001) showed marginal or no symptoms. In accordance, Pinot Noir in a previous long term study on a variety of cultivars had shown the lowest percentage of Esca related symptoms (Bruez *et al*., 2013). While for Felicia (2001) no other data are available, it was surprising that the particular vineyard of this new breeding, representing a progeny of the crossing Sirius and Vidal blanc, was fully free of symptoms. At the same time, in Villaris (2001) (progeny of the same crossing) symptomatic vines were found every year.

The temporal emergence of Esca leaf-symptomatic vines during the season expressed a clear pattern, which was evident for almost all years and apparently is not influenced by the training system. First symptomatic plants appear by the beginning of July. Subsequently, the number of newly symptomatic plants is steadily increasing until the beginning of August, when the maximum rate is reached. Newly affected vines can occur until September, however, in reduced numbers. This pattern is similar to two other observations made in the Bordeaux (Lecomte *et al*., 2012) and the Florence (Surico *et al*., 2006) region. Here, however, the maximum peak of newly symptomatic plants was reached about two to three weeks earlier (by the middle or end of July). Several reasons may account for this temporal shift. The ones most likely are the local Mediterranean climate (Fraga *et al*., 2016), and/or the distinct local

precipitation and temperature, both of which are assumed to play a major role in the epidemiology of Esca (Surico *et al*., 2000, 2006; Bertsch *et al*., 2012). Interestingly, in 2018 the maximum peak of newly symptomatic vines was reached within the first week of monitoring, in the beginning of July. After that the number of newly affect vines was marginal. This four weeks shift of the maximum peak is probably connected with the physiological development of the vines. Spring 2018 was characterized by warm temperatures and high sun exposure, which accelerated plant development. As a result, the physiological development stage of the vines were accomplished about two to three weeks earlier compared to the previous years 2015–2017. If the symptom appearance is indeed related to the physiological stage of the plant, this could be one possible explanation for the early outbreak of Esca foliar symptoms in 2018.

The data collected in or four years study confirm a possible correlation between precipitation in early summer and severity of the disease. In 2015, when precipitation from May to July was low, the appearance of symptoms in vineyard was reduced. In 2016, 2017 and 2018, with higher amounts of rain, an increased severity of Esca was observed. The same connection between rainfall in early summer and incidence of Esca was reported by two studies from Italy, which also emphasize the significance of this abiotic factor for symptom expression (Surico *et al*., 2000; Marchi *et al*., 2006). However, an exact explanation for this relation is still unknown and needs more attention in further investigations.

An extra focus in our study was on the possible impact of the training system on leaf-expression of Esca. In 2016, 2017 and 2018 significant differences between the two training systems VSP and SMPH were found. However, results were contradictory. While minimally pruned vines showed the most symptoms in 2016, the incidence of foliar symptoms in 2017 and 2018 was higher in intensively pruned vineyards. Our results suggest that the training system, especially in older vineyards, may have some effect on the occurrence of Esca. However, because of the complexity of the diseases and the influence of multiple factors, it is difficult to

conclude in which direction exactly the training system influences disease incidence (see also Becker 2010; Travadon *et al*., 2016). In general, it is proposed that minimal pruning produces fewer wounds and therefore reduces the risk of infection with Esca associated fungi.

It is hypothesized that also xylem embolism in the vascular system, caused by a disruption of water translocation after drought followed by rainfall, leads to better growth of pathogens and, consequently, the development of symptoms (Lecomte *et al.*, 2012; Pouzoulet *et al*., 2014). Grapevine cultivars with small xylem vessels, with a reduced tendency to form cavitations, would therefore be less susceptible to Esca (Pouzoulet *et al*., 2014, 2017). Intensive pruning of the vines probably leads to disturbance in the vascular system and thus promotes cavitations and xylem embolism.

Based on the percentage of missing or dead vines and the percentage of vines expressing Esca chronic or apoplexy, sanitary status of the examined vineyards was analyzed to compare the particular damage as a function of the training system. With this background it is suggested, that minimal pruning compared to intensive pruning may lead to an extended lifespan of vines. This assumption is indicated by the number of missing or dead vines, which was higher for VSP trained vines than for SMPH trained vines in almost all examined vineyards. Because VSP trained vines seem to be more susceptible to Esca foliar symptoms, the vines die earlier and are removed from the vineyard, causing a reduction of Esca affected vines. SMPH trained vines, on the other hand, are less susceptible and can persist longer to the disease. This could explain why in older vineyards the amount of missing or dead vines was higher for intensively pruned vines, but the percentage of plants expressing foliar symptoms, related to the number of plant sites, was higher for minimally pruned vines. However, in young vineyards, where plant death due to the disease is yet marginal, a high number of plants expressing both foliar symptoms correlates with a high number of missing or dead vines, and reverse. The reasons for missing or dead vine are diverse and cannot be reduced to Esca alone. Therefore, our conclusion about

the sanitary status of the vineyards in connection with the training system and the incidence of Esca foliar-symptoms is only one of several possibilities.

In summary, our Esca monitoring 2015 – 2018 revealed that in vineyards of southwest Germany the highest number of new leaf-symptomatic vines was observed in three out of four years by the beginning of August and is possibly linked to the physiological status of the vine. Already known influence factors of Esca like plant age, grapevine cultivar and precipitation could be supported with exact data and were found to apply both for VSP and SMPH training systems. In addition, the training system itself was identified as a possible factor, even though no consistent results were obtained throughout the years. Physiological differences due to pruning intensity might act as relevant parameters in this respect.

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References

Andolfi, A., Mugnai, L., Luque, J., Surico, G., Cimmino, A. & Evidente, A. (2011) Phytotoxins produced by fungi associated with grapevine trunk diseases. Toxins 3, 1569–1605.

Armijo, G., Schlechter, R., Agurto, M., Muñoz, D., Nuñez, C. & Arce-Johnson, P. (2016) Grapevine pathogenic microorganisms: Understanding infection strategies and host response scenarios. Front. Plant. Sci. 7, 1–18.

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015) Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1.

Becker, A. (2010) Esca: Der Schnitt macht die Musik. Der Deutsche Weinbau 1, 14–16.

Bertsch, C., Ramírez-Suero, M., Magnin-Robert, M., Larignon, P., Chong, J., Abou-Mansour, E., Spagnolo, A., Clément, C. & Fontaine, F. (2012) Grapevine trunk diseases: complex and still poorly understood. Plant Pathol. 62, 243–265.

Bruez, E., Lecomte, P., Grosman, J., Doublet, B., Bertsch, C., Fontaine, F., Ugaglia, A., Teissedre, P.L., Da Costa, J.P., Guerin-Dubrana, L. & Rey, P. (2013) Overview of grapevine trunk diseases in France in the 2000s. Phytopathol. Mediterr. 52, 262–275.

Bruez, E., Vallance, J., Gerbore, J., Lecomte, P., Da Costa, J.-P., Guérin-Dubrana, L. & Rey, P. (2014) Analyses of the temporal dynamics of fungal communities colonizing the healthy wood tissues of Esca leaf-symptomatic and asymptomatic vines. PLoS One 9, e95928.

Bruez, E., Baumgartner, K., Bastien, S., Travadon, R., Guérin-Dubrana, L. & Rey, P. (2016) Various fungal communities colonise the functional wood tissues of old grapevines externally free from grapevine trunk disease symptoms. Aust. J. Grape Wine Res. 22, 288–295.

Clingelefer, P.R. (1984) Production and growth of minimal pruned Sultana vines. Vitis 23, 42–54.

Cloete, M., Fischer, M., Mostert, L. & Halleen, F. (2014). A novel *Fomitiporia* species associated with esca on grapevine in South Africa. Mycol. Progr. 13, 303–311.

Crous, P.W. & Gams, W. (2000) *Phaeomoniella chlamydospora gen. et comb. nov*., a causal organism of Petri grapevine decline and esca. Phytopathol. Mediterr. 39, 112–118.

Díaz, G.A. & Latorre, B.A. (2013) Efficacy of paste and liquid fungicide formulations to protect pruning wounds against pathogens associated with grapevine trunk diseases in Chile. Crop Prot. 46, 106–112.

Deepayan, S. (2008) Lattice: Multivariate data visualization with R. Springer, New York.

FAO (Food and Agriculture Organization of the United Nations) (2018) FAOSTAT, http://www.fao.org/faostat/en/#data/QC (accessed 28th Feb 2018).

Fischer, M. (2002) A new wood-decaying basidiomycete species associated with esca of grapevine: *Fomitiporia mediterranea* (*Hymenochaetales*). Mycol. Progr. 1, 315–324.

Fischer, M. (2006) Esca der Weinrebe – eine Krankheit auf dem Vormarsch? Deutsches Weinbaujahrbuch 57, 87–94.

Fischer, M. & Kassemeyer, H.H. (2003) Fungi associated with esca disease of grapevine in Germany. Vitis 42, 109–116.

Fontaine, F., Gramaje, D., Armengol, J., Smart, R., Nagy, Z.A., Borgo, M., Rego, C. & Corio-Costet, M.-F. (2016) Grapevine trunk diseases. A review. OIV Publications, Paris. http://www.oiv.int/public/medias/4650/trunk-diseases-oiv-2016.pdf (accessed 28th Feb 2018).

Fraga, H., Garcia de Cortazar-Atauri, I., Malheiro, A.C. & Santos J.A. (2016) Modelling climate change impacts on viticultural yield, phenology and stress conditions in Europe. Glob. Change Biol. 22, 3774–3788.

Gramaje, D., Úrbez-Torres, J.R. & Sosnowski, M.R. (2018) Managing grapevine trunk diseases with respect to etiology and epidemiology: Current strategies and future prospects. Plant Dis. 102, 12–39.

Hofstetter, V., Buyck, B., Croll, D., Viret, O., Couloux, A. & Gindro, G. (2012) What if esca disease of grapevine were not a fungal disease? Fungal Divers. 54, 51–67.

Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous inference in general parametric models. Biom. J. 50, 346–363.

Intrieri, C., Poni, S., Lia, G. & Del Campo, M.G. (2001) Vine performance and leaf physiology of conventionally and minimally pruned Sangiovese grapevines. Vitis 40, 123–130.

Intrieri, C., Filippetti, I., Allegro, G., Valentini, G., Pastore, C. & Colucci, E. (2011) The semi-minimal-pruned hedge: A novel mechanized grapevine training system. Am. J. Enol. Vitic. 62, 312–318.

Kraus, C., Pennington, T., Herzog, K., Hecht, A., Fischer, M., Voegele, R.T., Hoffmann C., Töpfer, R. & Kicherer A. (2018) Effects of canopy architecture and microclimate on grapevine health in two training systems. Vitis 57, 53–60.

Kuntzmann, P., Villaume, S., Larignon, P. & Bertsch, C. (2010) Esca, BDA, Eutypiosis: foliar symptoms, trunk lesions and fungi observed in diseased vinestocks in two vineyards in Alsace. Vitis 49, 71–76.

Kuntzmann, P., Barbe, J., Maumy-Bertrand, M. & Bertrand, F. (2013) Late harvest as factor affecting esca and Botryosphaeria dieback prevalence of vineyards in the Alsace region of France. Vitis 52, 197–204.

Larignon, P. & Dubos, B. (2000) Preliminary studies on the biology of *Phaeoacremonium*. Phytopathol. Mediterr. 39, 184–189.

Lecomte, P., Darrieutort, G., Liminana, J.M., Comont, G., Muruamendiaraz, A., Legorburu, F.J., Choueiri, E., Jreijiri, F., El-Amil, R. & Fermaud, M. (2012) New insights into esca of grapevine: the development of foliar symptoms and their association with xylem discoloration. Plant Dis. 96, 924–934.

Marchi, G. (2001) Susceptibility to esca of various grapevine ("*Vitis vinifera*") cultivars grafted on different rootstocks in a vineyard in the province of Siena (Italy). Phytopathol. Mediterr. 40, 27–36.

Marchi, G., Peduto, F., Mugnai, L., Di Marco, S., Calzarano, F. & Surico, G. (2006) Some observations on the relationship of manifest and hidden esca to rainfall. Phytopathol. Mediterr. 45 (Supplement), S117–S126.

Mugnai, 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.

Murolo, S. & Romanazzi, G. (2014) Effects of grapevine cultivar, rootstock and clone on Esca disease. Australas. Plant Pathol. 43, 215–221.

Pertot, I., Caffi, T., Rossi, V., Mugnai, L., Hoffmann, C., Grando, M.S., Gary, C., Lafond, D., Duso, C., Thiery, D., Mazzoni, V. & Anfora, G. (2017) A critical review of plant protection tools for reducing pesticide use on grapevine and new perspectives for the implementation of IPM in viticulture. Crop Prot. 97, 70–84.

Pouzoulet, J., Pivovaroff, A.L., Santiago, L.S. & Rolshausen, P.E. (2014) Can vessel dimension explain tolerance toward fungal vascular wilt diseases in woody plants? Lessons from Dutch elm disease and esca disease in grapevine. Front. Plant Sci. 5, 253.

Pouzoulet, J., Scudiero, E., Schiavon, M. & Rolshausen, P.E. (2017) Xylem vessel diameter affects the compartmentalization of the vascular pathogen *Phaeomoniella chlamydospora* in grapevine. Front. Plant Sci. 8, 1442.

Quaglia, M., Covarelli, L. & Zazzerini, A. (2009) Epidemiological survey on esca disease in Umbria, central Italy. Phytopathol. Mediterr. 48, 84–91.

Romanazzi, G., Murolo, S., Pizzichini, L. & Nardi, S. (2009). Esca in young and mature vineyards, and molecular diagnosis of the associated fungi. Eur. J. Plant. Pathol. 125, 277–290.

RStudio Team (2016). RStudio: Integrated development for R. RStudio, Inc., Boston, MA URL http://www.rstudio.com/ (accessed 28th Feb 2018).

Serra, S., Mannoni, M.A. & Ligios, V. (2008) Studies on the susceptibility of pruning wounds to infection by fungi involved in grapevine wood diseases in Italy. Phytopathol. Mediterr. 47, 234–246.

Surico, G., Marchi, G., Braccini, P. & Mugnai, L. (2000) Epidemiology of esca in some vineyards in Tuscany (Italy). Phytopathol. Mediterr. 39, 190–205.

Surico, G., Mugnai, L. & Marchi, G. (2006) Older and more recent observations on esca: a critical review. Phytopathol. Mediterr. 45, S68–S86.

Surico, G. (2009) Towards a redefinition of the diseases within the esca complex of grapevine. Phytopathol. Mediterr. 48, 5–10.

Travadon, R., Lecomte, P., Diarra, B., Lawrence, D.P., Renault, D., Ojeda, H., Rey, P. & Baumgartner, K. (2016) Grapevine pruning system and cultivars influence the diversity of wood- colonizing fungi. Fungal Ecol. 24, 82–93.

Vasquez S.J., Gubler, W.D. & Leavitt, G.M. (2007). Economic loss in California's table grape vineyards due to measles. Phytopathol. Mediterr. 46, 118.

White, C.L., Halleen, F. & Mostert, L. (2011) Symptoms and fungi associated with esca in South African vineyards. Phytopathol. Mediterr. 50 (Supplement), S236–S246.

Wickham, H. (2009). ggplot2: Elegant graphics for data analysis. Springer, New York.

Manuscript IV

New species of *Phaeomoniellales* from a German vineyard and their potential threat to grapevine (*Vitis vinifera*) health.

Kraus, C., Damm, U., Voegele, R.T. & Fischer, M. (2019). IMA Fungus. Submitted.

Abstract

Recently, the order *Phaeomoniellales* was established that includes fungi closely related to *Phaeomoniella chlamydospora*, a phytopathogen assumed to be the main causal agent of the two most destructive grapevine trunk diseases, Petri disease and Esca. Other species of this order are reported as pathogens of other economically important crops, like olive, peach, apricot, rambutan, lichee or langsat. However, they are rarely isolated and hence, little is known about their ecological traits and pathogenicity. During a one-year period of spore trapping in a German vineyard divided in minimally and intensively pruned grapevines, 23 fungal strains of the *Phaeomoniellales* were isolated. Based on morphological and molecular analyses, performed with the gene regions ITS, LSU and *tub2*, the isolated strains were assigned to eight different species. Two species could be identified as *P. chlamydospora* and *Neophaeomoniella zymoides,* respectively. The remaining six species displayed morphological and molecular differences to known species of the *Phaeomoniellales* and are newly described, namely *Aequabiliella palatina*, *Minutiella simplex*, *Moristroma ampulliforme*, *Mo. germanicum*, *Neophaeomoniella constricta* and *N. ossiformis*. A pathogenicity test conducted in the greenhouse revealed that except for *P. chlamydospora* none of the species of the *Phaeomoniellales* isolated from spore traps is able to induce lesions in grapevine wood.

Keywords: Esca, grapevine, *Phaeomoniellales*, *Phaeomoniella*, phylogeny, spore trapping, training system

Introduction

To separate *Phaeomoniella chlamydospora* (synonym *Phaeoacremonium chlamydosporum*; Crous *et al*., 1996) from the genus *Phaeoacremonium* based on significant morphological and molecular differences, Crous and Gams (2000) established the hyphomycete genus *Phaeomoniella*. *Phaeomoniella chlamydospora* is considered to be one of the main causal agents of Petri disease and Esca, two grapevine trunk diseases (GTDs), which leads to high yield losses in grapevine industry all over the world (Bertsch *et al*., 2012; Fontaine *et al*.; 2016; Gramaje *et al*., 2018). After the introduction of the genus *Phaeomoniella*, further species in this genus were described (Lee *et al*., 2006; Crous *et al*., 2008; Damm *et al*., 2010; Crous *et al*., 2011). Additionally, further unidentified *Phaeomoniella* species and related fungi appeared in many surveys focusing on fungal endophytes (Arnold *et al*., 2007; Botella & Diez, 2010; Sánchez Márquez *et al.*, 2011; Gueidan *et al*., 2014). Chen *et al.* (2015) established the order *Phaeomoniellales* to assemble all fungi with close affinity to the genus *Phaeomoniella*. Shortly after, Crous *et al*. (2015) combined several *Phaeomoniella* species in new genera, and to date, the order *Phaeomoniellales* comprises eighteen species in the following eleven genera: *Aequabiliella*, *Celerioriella*, *Celothelium*, *Dolabra*, *Minutiella*, *Moristroma*, *Neophaeomoniella*, *Paraphaeomoniella*, *Phaeomoniella*, *Pseudophaeomoniella* and *Xenocylindrosporium* (Chen *et al*., 2015; Crous *et al.*, 2015, 2016). Kirk (2015) erected the Phaeomoniellaceae as the single family having an identical circumscription to the order *Phaeomoniellales*, apparently not being aware of the family *Celotheliaceae* (Aptroot *et al*., 2008) with the type genus *Celothelium* that is included in this order as well. As the older name of the family has priority, Phaeomoniellaceae is an illegitimate name (Art. 52.1).

All known species of the *Phaeomoniellales* were isolated from plants. Several species, besides *P. chlamydospra*, are associated with wood diseases of economically important fruit crops. For example, *Pseudophaeomoniella olea* and *Ps. oleicola* were isolated from discolored

xylem of wilting olive trees in Italy (Nigro *et al*., 2013; Crous *et al*., 2015). *Aequabiliella effusa*, *Minutiella tardicola*, *Celerioriella dura*, *C. prunicola,* and *Neophaeomoniella zymoides* were isolated from necrotic wood of *Prunus* trees in South Africa (Damm *et al*., 2010). *Dolabra nepheliae* is associated with stem canker disease of rambutan (*Nephelium lappaceum*; Booth & Ting, 1964) and lychee (*Litchi chinensis*; Rossman *et al*., 2010), as well as corky bark disease of langsat (*Lansium domesticum*; Keith *et al*., 2013). Other species, such as *Paraphaeomoniella capensis*, *Xenocylindrosporium kirstenboschense*, *N. niveniae* and *C. petrophiles* were isolated from symptomatic leaves of various host plants (Crous *et al*., 2008, 2009, 2011, 2016). Other species were reported from wood or other substrates without association to a symptom or disease. For example, ascostromata of *Moristroma quercinum* and *Mo. japonicum* were discovered by Nordén *et al.* (2005) on canes and old stumps of oak trees. Moreover, *N. zymoides* and *P. pinifoliorum* as well as *N. eucalypti* were isolated from pine needles (*Pinus densiflora*) and stems of *Eucalyptus globulus*, respectively (Lee *et al*., 2006; Crous *et al*., 2015). Furthermore, unknown species with affinity to the *Phaeomoniellales* were detected in a screening for lichen-associated fungi and multiple times in leaves of pine trees in Arizona (Peršoh & Rambold, 2012; Bowman & Arnold, 2018).

Despite efforts in revealing the ecological role and phylogenetic origin of the *Phaeomoniellales*, the number of isolates and thus the information currently available is insufficient for concrete conclusions (Chen *et al*., 2015). Consequently, the isolation and examination of more fungi belonging to the *Phaeomoniellales* is indispensable.

During a one-year period of spore trapping in a German vineyard planted with minimally and intensively pruned grapevines cv. Riesling, several fungi were isolated that were presumed to belong to the *Phaeomoniellales* based on preliminary blastn searches with ITS sequences. Therefore, one objective of this study was to clarify the relationship of these fungi based on molecular data, to characterise the species morphologically and by means of DNA sequence data and monitor their occurrence depending on season and pruning method. In

addition, since many species of the *Phaeomoniellales* can induce necrosis in woody tissue of their hosts and since the fungi were collected from spore traps located in vineyards, their potential threat to grapevine (*Vitis vinifera*) was investigated.

Materials and methodes

Isolation: Fungal spores were trapped using glass slides coated with petroleum jelly (Balea Vaseline, DM, Karlsruhe, Germany) attached close (2 cm; Fig. 1) to branches of grapevine (*Vitis vinifera*) cv. Riesling. The vineyard was located close to the Julius Kühn-Institute in Siebeldingen, Germany (49°13'11.5"N 8°02'32.3"E). Half of the grapevines were trained in semi minimal pruned hedge (SMPH; Fig. 1 a) and the other half in vertical shoot positioning (VSP; Fig. 1 b). For each training system, four spore traps were placed randomly in the field.

Fig. 1: Spore traps attached to grapevine plants cv. Riesling trained in SMPH (a) and VSP (b).

Spore trapping was carried out from February 2016 to February 2017. Each week, glass slides were replaced by new ones. A sterile washing solution (NaCl 136.9 mM; KCl 2.7 mM; Na_2HPO_4 7.9 mM; KH_2PO_4 1.5 mM; Tween® 80, 0.01%) was used to release the spores from the coated glass slides. Under sterile conditions, 25 mL washing solution was added to a 50-ml-reaction tube containing one glass slide each. The tube was shaken vigorously by hand for about 30 seconds. Subsequently, the washing solution was passed through a filter system consisting of a 5.0 µm and a 0.45 µm filter (mixed cellulose ester membrane filter, ADVANTEC MFS Inc., Japan). Since fungi of the order *Phaeomoniellales* produce small

conidia (< 5.0 µm), the 0.45 µm filter was used for further analysis. The filter was placed into a 2-ml-reaction tube and washed with 500 µL washing solution. Subsequently, the washing solution was spread equally on two plates of malt-yeast-agar (MYA; 20 g/L malt extract, 1 g/L yeast extract, 20 g/L agar, 2.5 µg/mL chloramphenicol; Carl Roth, Karlsruhe, Germany) and incubated for two weeks at 20 °C. Growing colonies were transferred to new MYA plates and identified by morphological and molecular analyses.

Cultures of newly described species are maintained in the culture collections of the Julius Kühn-Institute (JKI; www.julius-kuehn.de), the Westerdijk Fungal Biodiversity Institute, Ultrecht, the Netherlands (CBS; www.westerdijkinstitute.nl) and the Senckenberg Museum of Natural History, Görlitz, Germany (GLMC; www.senckenberg.de). Type specimens of the species studied are deposited in the fungarium of the Senckenberg Museum of Natural History Görlitz, Germany (GLMC). All descriptions are based on ex-holotype cultures, if not stated otherwise.

Local weather data (precipitation and temperature) were provided by the DLR Rhineland-Palatinate (www.dlr.rlp.de).

DNA extraction and sequencing: DNA extraction was performed according to the method of Tillett & Neilan (2000) from two weeks old cultures growing on MYA at 20 °C. Quality and quantity of the DNA were determined using a Spectrophotometer (Nanodrop 2000c, Thermo Fisher Scientific, Waltham, MA, USA). DNA was diluted to a final concentration of 100 µg/mL in distilled water.

For phylogenetic studies, three primer pairs were used to perform a polymerase chain reaction (PCR) amplifying the internal transcribed spacer regions 1 and 2 and intervening 5.8S rRNA (ITS: ITS5 and ITS4; White *et al.*, 1990), the 28S rRNA (LSU: NL1 and NL4; O´Donnell, 1993), and the beta tubulin gene (*tub2*: BT2A and BT2B; Glass & Donaldson, 1995). PCR reactions were set up as described in the KAPAHiFi™ hot start polymerase user manual (PEQLAB Biotechnologie GmbH, Erlangen, Germany). The reaction mixture consisted

of 15.25 µL H_2O, 5.0 µL 5x KAPAHiFi™ buffer, 0.75 µL KAPAHiFi™ dNTP mix (10 mM), 0.75 µL forward primer (10 µM), 0.75 µL reverse primer (10 µM), 0.5 µL KAPAHiFi™ HotStart DNA polymerase (1 U/µL) and 2 µl DNA template (5 ng/µL). For DNA amplification the following reaction steps were implemented on a SimpliAmp™ thermal cycler (Applied Biosystems, Darmstadt, Germany): 95 °C initial denaturation (5 min), 98 °C denaturation (20 sec), 58 °C annealing (15 sec), 72 °C extension (20 sec), 72 °C final extension (1 min). The main amplification steps (denaturation - annealing - extension) were repeated thirty-five times. Afterwards electrophoresis was carried out for 45 min at 110 V using a 1.5% agarose gel to check the quality and quantity of the amplicons. Amplicons were purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. Sequencing reactions were set up using the ABI Prism Big Dye Terminator v3.1 cycle sequencing ready reaction kit (PE Biosystems, Foster City, CA, USA). Sequencing was performed on an ABI Prism 3130XL DNA sequencer. Sequence analyses and alignments were done with the program CLC Main Workbench Version 8.0 (Qiagen). ITS, LSU and *tub2* sequences of the newly described fungi were deposited at NCBI GenBank (www.ncbi.nlm.nih.gov/genbank/).

Phylogenetic analyses: A concatenated ITS-LSU sequence alignment was constructed by separately aligning the sequences of the two loci and manually trimming the ends to achieve an uniform length of all sequences. Before the two loci were linked, a separator sequence of ten "N"s was added. *tub2* was excluded from the phylogenetic analyses, since sequences of only a few reference strains were available.

MEGA7 (Molecular Evolutionary Genetics Analysis version 7.0; Kumar *et al*., 2015) was used for Maximum Likelihood (ML) and Maximum Parsimony (MP) analyses. A test was performed with MEGA 7 to find the most robust model for ML. Then a ML tree using tree bisection and reconnection (TBR) as branch-swapping algorithm was generated. Tree length, consistency index, retention index and composite index were calculated for the resulting tree.

Finally, a ML tree containing bootstrap values (1,000 replications) calculated by both ML and one of four MP analyses was chosen to visualise the phylogenetic relationship of the fungi investigated. Alignment and phylogenetic tree were lodged in TreeBase (www.treebase.org).

Morphological characterisation: To improve sporulation and pycnidia formation, fungal strains were cultivated on autoclaved grapevine wood pieces and autoclaved pine needles placed on SNA medium (Nirenberg 1976) and incubated at 25 °C for up to 3 months. Thin sections were made with a Cryostat Microm HM 525 (Thermo Fisher Scientific). After embedding fungal structures in Tissue-Tek® O.C.T.™ Compound (Sakura Finetek, CA, USA) on an object plate and deep-freezing to -20 °C, 20 µm thin slices were trimmed and immediately put on a microscope slide. Microscopic preparations were done in lactic acid with a Zeiss Axio Imager Z1 microscope under 63x magnification (Carl Zeiss Microscopy GmbH, Jena, Germany) using differential interference contrast (DIC), or with a Leica DFC450 C dissecting microscope (DM) under 20x or 40x magnification (Leica Microsystems, Wetzlar, Germany). Interpretation of colony characters was done after two weeks of growth on potato dextrose agar (PDA; 40 g/L potato extract glucose agar, 5 µg/mL chloramphenicol; Carl Roth, Karlsruhe, Germany), oat meal agar (OA; 30 g/L oatmeal infusion, 20 g/L agar, 2.5 µg/mL chloramphenicol; Carl Roth, Karlsruhe, Germany) and MYA at 25 °C in the dark. The colour chart of Rayner (1970) was used to determine the colour of fungal colonies. To determine thermo tolerance and growth optima, the radial growth on MYA (three replicates) was measured at different temperatures (5 °C, 10 °C, 15 °C, 20 °C, 25 °C, 30 °C, and 35 °C) after two weeks in the dark.

Pathogenecity test: To investigate the potential threat of the trapped fungi to grapevine health, pathogenicity studies were performed on potted grapevines cv. Pinot noir and Müller-Thurgau in a greenhouse. Cuttings were hot water (50 °C) treated for 30 min and then stored for ten days at 10 °C for recovery. They were cut into smaller pieces with three buds each and

planted into plastic boxes containing sterile soil. Incubation in the greenhouse was 16 °C night time temperature and 24 °C day time temperature and 30% relative humidity for five months.

Young plants with shoot development were surface wounded with a sterilized scalpel by a diagonal cut of about 1 cm in length between the second and the tird bud. After inoculating the wound with a 20 µL spore suspension (~1,000 spores per 20 µL sterilised rainwater), they were sealed with plastic film to avoid evaporation and cross infection. One isolate of each species was tested, including *Phaeomoniella chlamydospora* as positive control. As negative control, plants were inoculated with sterile rainwater. Five plants were inoculated with each strain, and the experiment was repeated three times. Six months after inoculation, plants were cut longitudinally and lesions in the wood were measured up- and down-wards from the inoculation point. Plants, which had dried out at the inoculation point, were excluded from the experiment. Material from the lesion was removed with a sterile scalpel under the hood and placed on two MYA plates to verify the presence of the particular fungus. Fungi growing out from the lesion material were identified microscopically based on morphological characteristics.

For statistical evaluation of lesion length from the pathogenicity test, an analysis of variance (ANOVA) was conducted using the program RStudio Version 1.1.383 (RStudio Team, 2016). Additionally, the re-isolation rate of the tested fungi, i.e. the percentage of the plants of which the fungus could be re-isolated, was determined.

Results

Phylogenetic analysis

In total 23 fungal strains of the *Phaeomoniellales* were isolated in this study (Tab. 1). The phylogenetic analyses of the concatenated ITS-LSU sequence alignment comprised 41 taxa, including reference strains and the outgroup *Capronia epimyces* (Tab. 2) and 919 characters. The ML tree with both ML and MP bootstrap values (1,000 replicates) is shown in Figure 2 and consists of eleven main clades representing the ten genera of the *Phaeomoniellales* and *'Phaeomoniella' pinifoliorum. Celothelium cinchonarum* was excluded from the analysis due to missing sequence data. Strains JKI-Mz56, JKI-S09, JKI-Mz21, JKI-Mz20, JKI-Mz40, JKI-Mz34, JKI-Mz38, and JKI-Mz41 grouped with *N. zymoides* (ML/ MP bootstrap support values: 91/ 93) in the *Neophaeomoniella* clade (100/ 100). Strains JKI-Mai02, JKI-Mai03 and JKI-Mai30 formed a sub-clade (99/ 83) within this clade, sister to the single strain sub-clade JKI-Mz35, and the sub-clade formed by *N. eucalypti*, *N. niveniae* and *N. zymoides*. The isolates JKI-Feb08, JKI-Mai05 and JKI-Ap04 grouped with *P. chlamydospora* in the *Phaeomoniella* clade (100/ 100). Strains JKI-Mz48, JKI-Mai29 and JKI-Ap36 grouped with *Aequabiliella effusa* in the *Aequabiliella* clade (100/ 100). However, the latter represented a single strain sub-clade. The same situation was found for strains JKI-Jn27 and JKI-Jn48 that formed a clade with *Minutiella tardicola*, which formed a single strain sub-clade within the *Minutiella* clade (100/ 100). Together with *Moristroma japonicum* and *Mo. quercium*, strains JKI-Au02, JKI-Feb17 and JKI-Feb06 formed the *Moristroma* clade, in which isolate JKI-Feb06 grouped with *Mo. quercinum* and *Mo. japonicum* (95/ 93), while isolates JKI-Au02 and JKI-Feb17 formed a separate sub-clade within *Moristroma* (–/ 98). The *Moristroma* clade (100/ 100) was on a long branch, basal to all other genera in the *Phaeomoniellales*, except for *Dolabra nepheliae* strain CBS 122120.

Tab. 1: Fungi of the *Phaeomoniellales* studied with collection details and GenBank accession numbers.

Species	Accession no[a]			Collection date	Training system of the grapevine at which the trap was attached to[b]	Genbank no.[c]		
	JKI	CBS	GLMC			ITS	LSU	TUB2
Aequabiliella palatina	JKI-Mz48	CBS 145007	GLMC 1904	10. March 2016	SMPH	MH999505	MH999528	MK070468
	JKI-Ap36*	CBS 145018	GLMC 1905	21. April 2016	SMPH	MH999506	MH999529	MK070469
	JKI-May29	--	GLMC 1906	5. May 2016	SMPH	MH999507	MH999530	MK070470
Minutiella simplex	JKI-Jn27*	CBS 145008	GLMC 1907	9. June 2016	SMPH	MH999508	MH999531	MK070471
	JKI-Jn38	CBS 145009	GLMC 1908	9. June 2016	SMPH	MH999509	MH999532	MK070472
Moristroma ampulliforme	JKI-Feb17*	CBS 145010	GLMC 1909	23. February 2017	SMPH	MH999510	MH999533	MK070473
	JKI-Au2	CBS 145011	GLMC 1910	9. August 2016	VSP	MH999511	MH999534	MK070474
Mo. germanicum	JKI-Feb06*	CBS 145012	GLMC 1911	3. February 2017	SMPH	MH999512	MH999535	MK070475
Neophaeomoniella ossiformis	JKI-May02	CBS 145014	GLMC 1912	6. May 2016	SMPH	MH999513	MH999536	MK070476
	JKI-May03*	CBS 145013	GLMC 1913	6. May 2016	VSP	MH999514	MH999537	MK070477
	JKI-May30	--	GLMC 1914	6. May 2016	SMPH	MH999515	MH999538	MK070478
N. constricta	JKI-Mz35*	CBS 145015	GLMC 1915	3. March 2016	SMPH	MH999516	MH999539	MK070479
N. zymoides	JKI-Mz20	--	GLMC 1916	3. March 2016	SMPH	MH999517	MH999540	MK070480
	JKI-Mz21	CBS 145156	GLMC 1917	3. March 2016	SMPH	MH999518	MH999541	MK070481
	JKI-S09	--	GLMC 1918	11. February 2016	VSP	MH999519	MH999542	MK070482
	JKI-Mz56	--	GLMC 1919	17. March 2016	SMPH	MH999520	MH999543	MK070483
	JKI-Mz38	--	GLMC 1920	3. March 2016	VSP	MH999521	MH999544	MK070484
	JKI-Mz40	--	GLMC 1921	3. March 2016	VSP	MH999522	MH999545	MK070485
	JKI-Mz41	CBS 145155	GLMC 1922	3. March 2016	VSP	MH999523	MH999546	MK070486
	JKI-Mz34	--	GLMC 1923	3. March 2016	VSP	MH999524	MH999547	MK070487
Phaeomoniella chlamydospora	JKI-Ap04	CBS 145016	GLMC 1924	7. April 2016	SMPH	MH999525	MH999548	MK070488
	JKI-Feb08	CBS 145017	GLMC 1925	9. February 2017	SMPH	MH999526	MH999549	MK070489
	JKI-May05	--	GLMC 1926	6. May 2016	VSP	MH999527	MH999550	MK070490

[a] JKI: Culture collection of the Julius Kühn-Institute, Siebeldingen, Germany; CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands, GLMC: Culture collection of the Senckenberg Museum of Natural History Görlitz, Görlitz, Germany

[b] SMPH: semi minimal pruned hedge; VSP: vertical shoot positioning trained vineyards

[c] ITS: internal transcribed spacers and intervening 5.8S nrDNA; LSU: 28S rDNA; *tub2* : partial beta-tubulin gene

* Ex-type cultures.

Tab. 2: Reference strains of the *Phaeomoniellales* with collection details and GenBank accession numbers.

Species	Accession no[a]	Host	Country	Genbank no.[b]		
				ITS	LSU	TUB2
Aequabiliella effusa	CBS 120883*	*Prunus salicina*	South Africa	GQ154598	GQ154618	KR260451
Celerioriella dura	CBS 120882*	*Pr. salicina*	South Africa	GQ154597	GQ154617	--
C. petrophiles	CBS 142115*	*Petrophile teretifolia*	Australia	KY173394	KY173487	--
C. prunicola	CBS 120876*	*Pr. salicina*	South Africa	GQ154590	GQ154615	--
Dolabra nepheliae	CBS 122120*	*Nephelium lappaceum*	Malaysia	JQ004281	GU332516	--
Minutiella tardicola	CBS 121757*	*Pr. armeniaca*	South Africa	GQ154599	GQ154619	KR260454
Moristroma japonicum	BN1674*	*Quercus mongolica*	Japan	AY254052	AY254052	--
Mo.quercinum	BN1678*	*Quercus robur*	Sweden	AY254051	AY254051	--
Neophaeomoniella eucalypti	CBS 139919*	*Eucalyptus globulus*	USA	NR_138001	KR476782	--
N. niveniae	CBS 131316*	*Nivenia stokoei*	South Africa	JQ044435	JQ044454	--
N. zymoides	CBS 114904*	*Pinus densiflora*	Korea	GQ154600	GQ154620	KR260455
Paraphaeomoniella capensis	CBS 123535*	*Encephalartos altensteinii*	South Africa	NR_137711	FJ372408	--
Phaeomoniella chlamydospora	CBS 229.95*	*Vitis vinifera*	Italy	FJ530942	AF353609	--
P. pinifoliorum	CBS 114903*	*Pi. densiflora*	Korea	DQ270240	DQ270250	--
Pseudophaeomoniella oleae	CBS 139191*	*Olea europaea*	Italy	NR_137966	KP635971	--
Ps. oleicola	CBS 139192*	*Olea europaea*	Italy	KP411807	KP635970	--
Xenocylindrosporium kirstenboschense	CBS 125545*	*En. friderici-guilielmi*	South Africa	NR_132841	GU229891	--
Capronia epimyces[c]	CBS 601.96*	--	--	AY156968	AF050245	--

[a] BN: Botanical Institute, Göteborg University, Göteborg, Sweden; CBS: Culture collection of the Westerdijk Fungal Biodiversity Institute, Utrecht, The Netherlands

[b] ITS: internal transcribed spacers and intervening 5.8S nrDNA; LSU: 28S rDNA; *tub2*: partial beta-tubulin gene

[c] Outgroup

* Types and ex-type cultures.

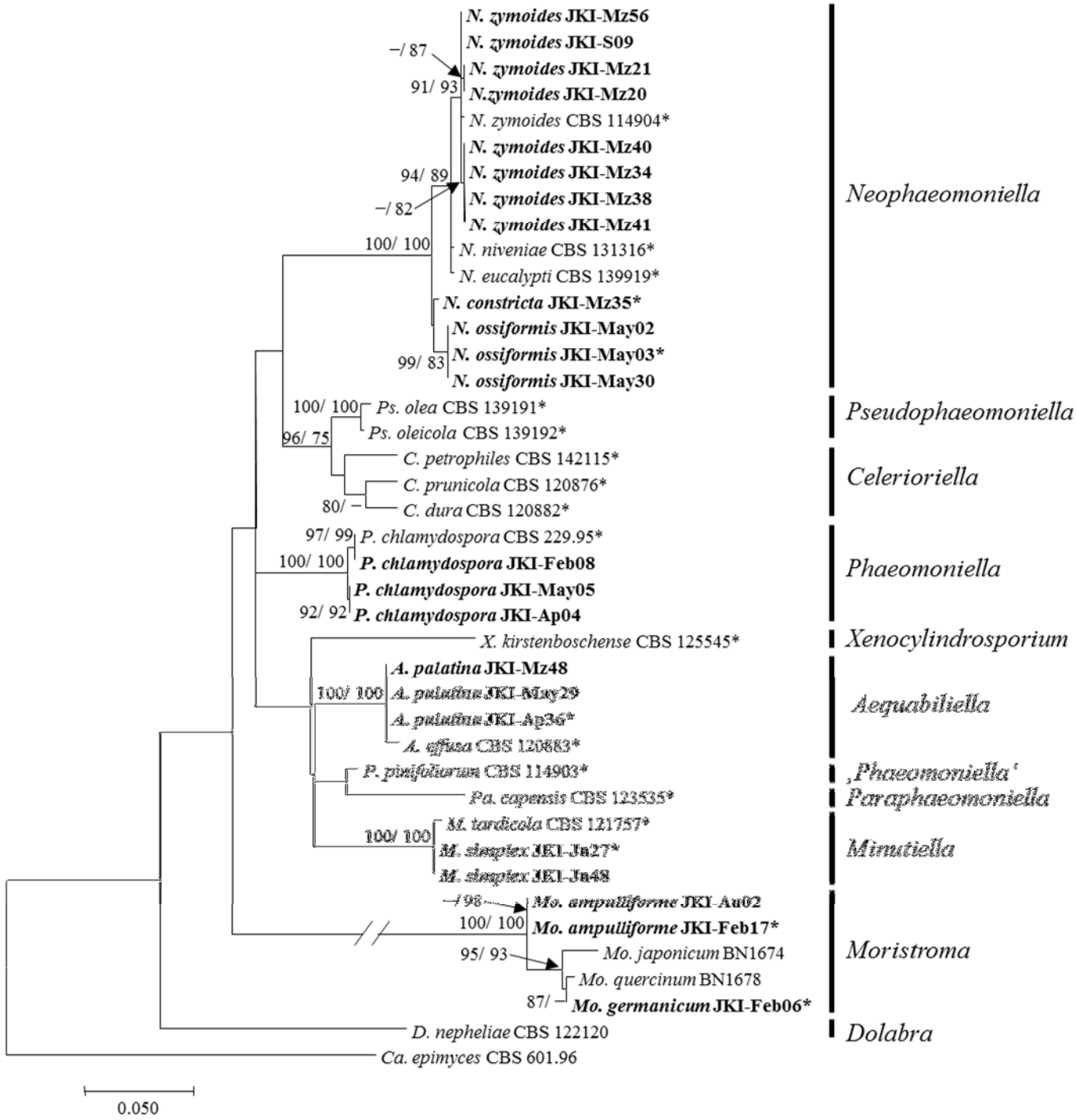

Fig. 2: Maximum likelihood tree based on the concatenated ITS-LSU sequence alignment of the *Phaeomoniellales*. Bootstrap support values above 70% of ML/MP analyses are shown at the nodes. *Capronia epimyces* strain CBS 601.96 was used as outgroup. Isolates analysed in this study are emphasised in bold. Numbers of ex-type cultures are marked with an asterisk. Branches that are crossed by diagonal lines are shortened by 50%.

Diversity and occurrence

The strains were assigned to eight species in five genera: *Aequabiliella*, *Minutiella*, *Moristroma, Neophaeomoniella* and *Phaeomoniella*. Fifteen strains belonging to eight species originated from spore traps attached to SMPH trained grapevines and eight strains belonging to four species were isolated from traps attached to VSP trained grapevines. Spores were trapped between February and June, with one exception in August (JKI-Au2; Fig. 3).

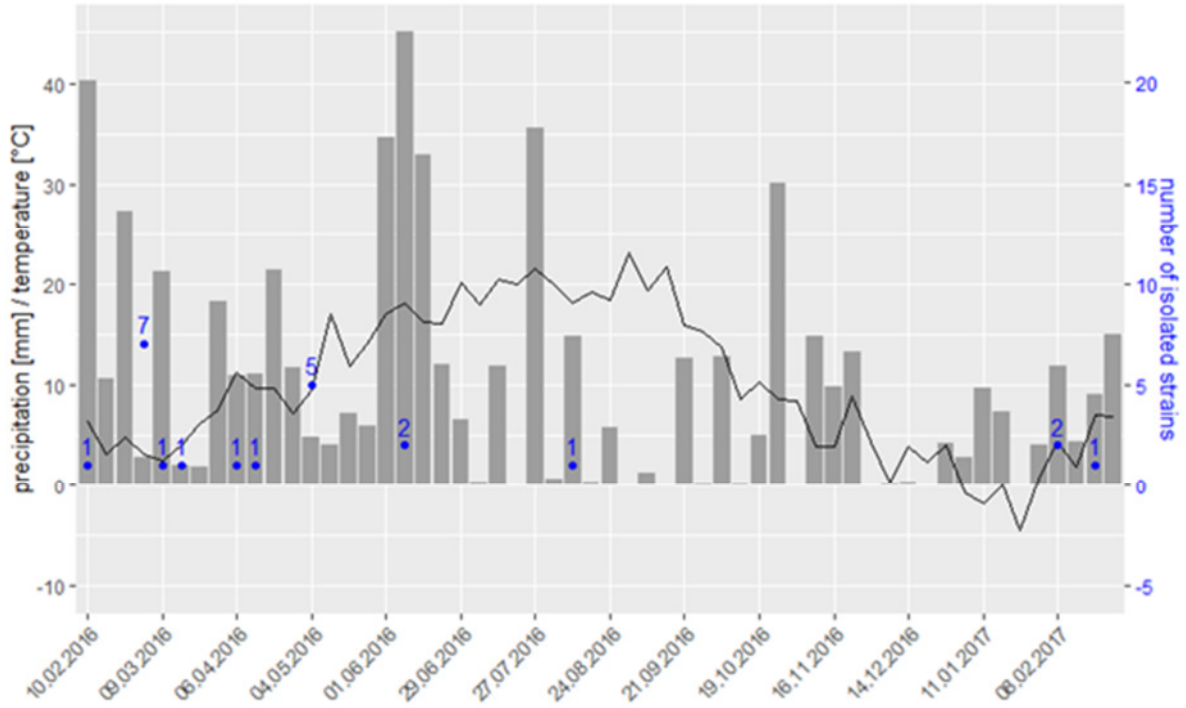

Fig. 3: Weekly precipitation [mm] (grey columns), average temperature [°C] (black line) and number of fungal strains collected (emphasized in blue) during the time of spore trapping.

Taxonomy

Six of the eight *Phaeomoniellales* species found in this study exhibited significant morphological and molecular differences to known species and are therefore described as new species (Figs. 4–10).

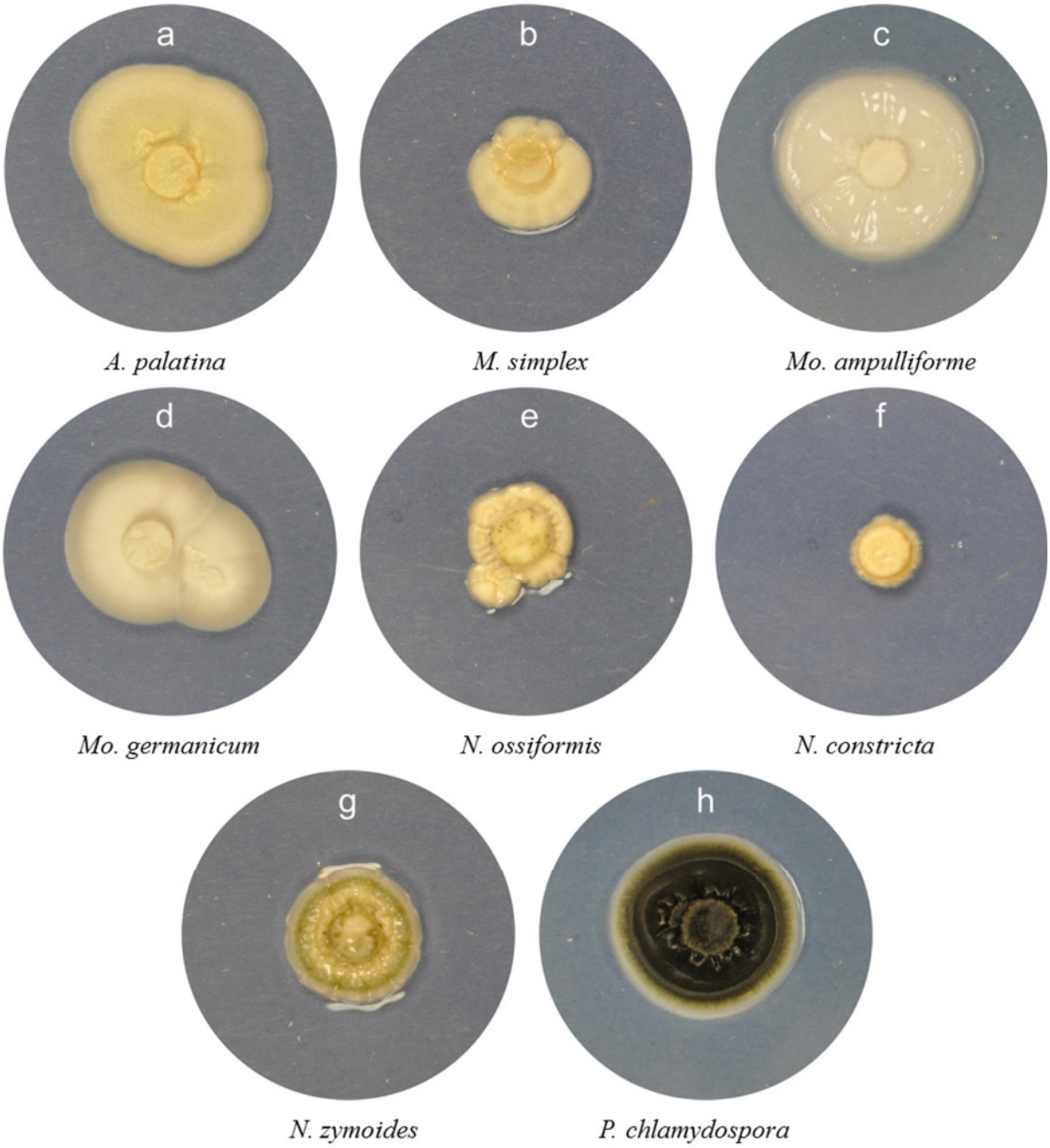

Fig. 4: Cultures of the *Phaeomoniellales* studied grown on PDA for 14 d at 20 °C in the dark. *A palatina* JKI-Ap36 (a), *M. simplex* JKI-Jn27, (b), *Mo. ampulliforme* JKI-Feb17 (c), *Mo. germanicum* JKI-Feb06 (d), *N. ossiformis* JKI-May02 (e), *N. constricta* JKI-Mz35 (f), *N. zymoides* JKI-Mz41 (g), *P. chlamydospora* JKI-Ap04 (h). The diameter of the pictures corresponds to 45 mm.

Aequabiliella palatina (Kraus, Damm, Voegele & Fischer), ***sp. nov.*** MycoBank MB 828284.

Figure 5.

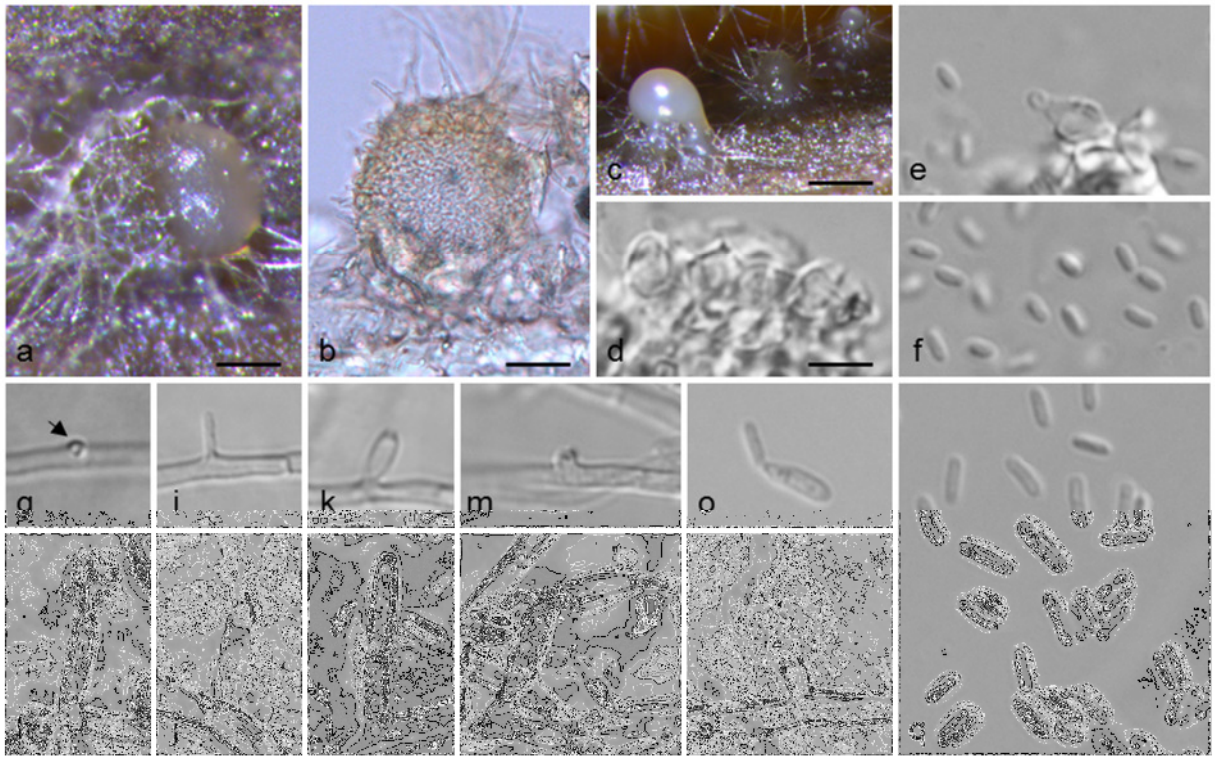

Fig. 5: ***Aequabiliella palatina*** **(ex-type culture JKI-Ap36). a, c. conidia oozing from pycnidia on pine needle; b. longitudinal section through a pycnidium; d, e. conidiogenous cells lining the inner cell wall of pycnidia; f. conidia formed in pycnidia; g–n, p. conidiogenous cells on hyphal cells (arrows indicate a conidiogenous opening on a hyphal cell); o. microcyclic conidiation; q. conidia generated on hyphal cells. a, c: DM; b, d–q: DIC. Scale bars: a = 100 µm; b = 25 µm; c = 200 µm, d = 5 µm; d applies to d–q.**

Etymology. Named after the federal state of Germany, Rhineland-Palatinate, in which the species was isolated (palatinus, adjective of Palatinatus = Pfalz).

Vegetative hyphae hyaline, smooth-walled, 1.5–2.5 µm wide, septate, chlamydospores not observed. **Sporulation** abundant; conidia formed on hyphae, in pycnidia and by microcyclic conidiation. **Conidiophores on hyphae** hyaline, smooth-walled, mainly reduced to conidiogenous cells, rarely 2- or 3-celled, cylindrical to lanceolate, 13–41.5 × 2–2.5 µm. **Conidiogenous cells** enteroblastic, hyaline, smooth-walled, most frequently reduced to openings formed directly on hyphal cells, sometimes discrete phialides, rarely adelophialides; collarettes inconspicuous; openings on hyphal cells 0.5–1.5 µm wide, periclinal thickening visible; discrete phialides cylindrical to cigar-shaped, 4.5–14 × 1.5–3 µm, adelophialides cylindrical, 1–8 × 1–3 µm. **Conidia** accumulated in heads around conidiogenous openings, hyaline, smooth-walled, aseptate, elliptical to oblong-elliptical, sometimes slightly curved, smooth-walled, (3–)4(–6) × (1–)1.5(–2) µm, L/W ratio = 2.7.

Microcyclic conidiation observed on one side of swollen mother cells developed from primary conidia; conidiogenous cells hyaline, smooth-walled, aseptate, elongated obovate to oblong-elliptical, (5–)6(–6.5) × (1–)2(–2.5) µm, L/W ratio = 2.7.

Conidiomata pycnidial produced superficially on pine needles, grapevine wood and immersed in SNA medium after four weeks, solitary or in groups, globose to subglobose, 52–330 µm diameter, unilocular, opening by irregular rupture, pycnidial wall composed of textura angularis, 6–13 µm thick, 2–4 cell layers. **Conidiophores** reduced to conidiogenous cells lining the inner wall of pycnidia. **Conidiogenous cells** enteroblastic, hyaline, smooth-walled, discrete phialides, obpyriform 3.5–6.5 × 2.5–4 µm, collarettes inconspicuous; opening 0.5–2 µm. **Conidia** hyaline, smooth-walled, aseptate, elliptical to oblong-elliptical, (2.5–)3(–3.5) × (1–)1.5(–2) µm, L/W ratio = 1.9.

Culture characteristics – Colonies on **PDA** flat to raised, with crenated margin, moist, buff to primrose, sparse, whitish, funiculose aerial mycelium in centre; reverse same colours. Colonies on **OA** flat, with crenated margin, moist, olivaceous buff to olivaceous grey, olivaceous black in centre, buff at the margin, aerial mycelium sparse; reverse same colours. Colonies on **MYA** flat, with crenated margin, moist, honey in centre, with a grey olivaceous to olivaceous black ring and a buff margin, aerial mycelium sparse, reverse same colours; 32–32.5 mm diameter after 14 d on MYA (25 °C, in the dark), min 10 °C, max 30 °C, opt 25 °C.

Specimens examined: Germany, Rhineland-Palatinate, Siebeldingen, °13'11.5"N 8°02'34.6"E, isolated from a spore trap attached to a grapevine shoot, 21. April 2016, C. Kraus, GLMC GLM-F117490 holotype, culture ex-type JKI-Ap36 = CBS 145018 = GLMC 1905; Germany, Rhineland-Palatinate, Siebeldingen, °13'11.5"N 8°02'34.6"E, isolated from a spore trap attached to a grapevine shoot, 10. March 2016, C. Kraus, JKI-Mz48 = CBS 145007 = GLMC 1904; Germany, Rhineland-Palatinate, Siebeldingen, °13'11.5"N 8°02'34.6"E, isolated from a spore trap attached to a grapevine shoot, 6. May 2016, C. Kraus, JKI-May29 = GLMC 1906.

Notes - *Aequabiliella palatina* is closely related to *A. effusa*, however *tub2*, ITS and LSU sequences of the ex-type strain are only 94% (21 nucleotides different), 98% (9 nucleotides different) and 99% (1 nucleotid different), respectively, identical to that of *A. effusa* (Damm *et al*. 2010, Úrbez-Torres *et al*. 2015). A blastn search of the ITS sequence resulted in 99% (2–3 nucleotides different) accordance with unidentified fungal isolates from wood samples of Norway spruce and Scots pine taken in Finland (MG190556, Müller *et al*. 2018) and Western white pine taken in Montana (USA; JF705946, Larkin *et al*. 2012). Colonies of *A. effusa* are herbage-green, dark herbage-green to olivaceous on PDA, while those of *A. palatina* are primrose. Microcyclic conidiation was only observed in *A. palatina*. Additionally, the temperature depending growth range differs between the two species: *A. effusa* can grow at temperatures between 5 °C and 35 °C, with an optimum at 30 °C, while the growth range of *A. palatina* is narrower, ranging from 10 °C to 30 °C, with an optimum at 25 °C.

Minutiella simplex (Kraus, Damm, Voegele & Fischer), ***sp. nov.*** MycoBank MB 828285. **Figure 6.**

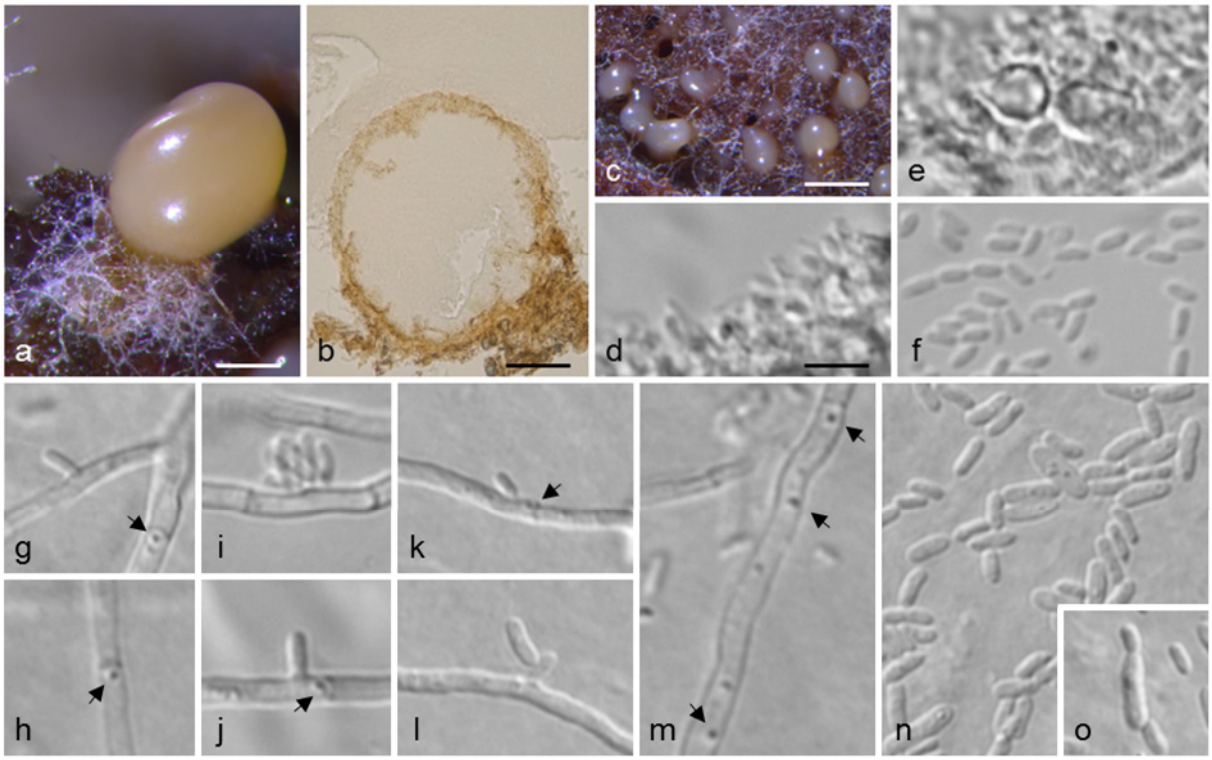

Fig. 6: ***Minutiella simplex*** **(ex-type culture JKI-Jn27). a, c. conidia oozing from pycnidia on grapevine wood; b. longitudinal section through a pycnidium; d, e. conidiogenous cells lining the inner cell wall of pycnidia; f. conidia formed in pycnidia; g–m. conidiogenous cells on hyphal cells (arrows indicate conidiogenous openings on hyphal cells); n. conidia generated on hyphal cells; o. microcyclic conidiation. a, c: DM; b, d–o: DIC. Scale bars: a, b = 100 µm; c = 300 µm, d = 5 µm; d applies to d–o.**

Etymology. Named after the simple conidiogenous cells on hyphae.

Vegetative hyphae hyaline, smooth-walled, 2–3.5 µm wide, septate, chlamydospores not observed. **Sporulation** abundant, conidia formed on hyphae, in pycnidia and by microcylic conidiation. **Conidiophores on hyphae** hyaline, smooth-walled, reduced to conidiogenous cells. **Conidiogenous cells** enteroblastic, almost exclusively simple openings on hyphal cells, rarely discrete phialides; collarettes inconspicuous; openings on hyphal cells 0.5–1.0 µm wide. **Conidia** accumulated in heads around conidiogenous openings, hyaline, smooth-walled, aseptate, oblong-elliptical, sometimes slightly curved, smooth-walled, (3–)4(–5) × (1–)1.5(–2) µm, L/W ratio = 2.4.

Microcyclic conidiation observed on one side of swollen mother cells developed from primary conidia; conidiogenous cells, hyaline, smooth-walled, aseptate, obovate to oblong-elliptical, (5–)6(–7) × (1.5–)2(–2.5) µm, L/W ratio = 3.1.

Conidiomata pycnidial produced superficially on pine needles, grapevine wood and immersed in SNA medium after four to eight weeks, mainly solitary, globose, subglobose to ellipsoidal, 128–286 µm diameter, unilocular, opening by irregular rupture, pycnidial wall composed of textura angularis, 8–18 µm thick, 3–6 cell layers. **Conidiophores** reduced to conidiogenous cells. **Conidiogenous cells** enteroblastic, hyaline, discrete phialides; collarettes visible; discrete phialides obpyriform to ampulliform, 4–7 × 2–5 µm, openings 0.5–1.5 µm. **Conidia** hyaline,

smooth-walled, aseptate, oblong-elliptical, sometimes slightly curved, (2.5–)3(–4) × (1–)1.5(–1.5) µm, L/W ratio = 2.2.

Culture characteristics – Colonies on **PDA** raised, with lobate margin, moist, buff to straw, lacking aerial mycelium, reverse same colours; on **OA** flat, with crenated margin, moist, buff, whitish, funiculose to felty aerial mycelium in centre; reverse same colours; on **MYA** flat to raised, with crenated margin, moist, buff, hyaline, sparse aerial mycelium; reverse buff to luteous; 8–11.5 mm diameter after 14 d on MYA (25 °C, in the dark), min 10 °C, max 25 °C, opt 20 °C.

Specimens examined: Germany, Rhineland-Palatinate, Siebeldingen, °13'11.5"N 8°02'34.6"E, isolated from a spore trap attached to a grapevine shoot, 9. June 2016, C. Kraus, GLMC GLM-F117492 holotype, culture ex-type JKI-Jn27 = CBS 145008 = GLMC 1907; Germany, Rhineland-Palatinate, Siebeldingen, °13'11.5"N 8°02'34.6"E, isolated from a spore trap attached to a grapevine shoot, 9. June 2016, C. Kraus, culture ex-type JKI-Jn38 = CBS 145009 = GLMC 1908.

Notes - *Minutiella simplex* displays strong similarities with *M. tardicola*, however, differences in *tub2*, ITS and LSU sequences were found. The alignment of *tub2* displays 99% (1 nucleotide different), ITS 99% (6 nucleotides different) and LSU 99% (4 nucleotides different) identity of the two species (Damm *et al*. 2010; Úrbez-Torres *et al*. 2015). Furthermore, colonies of the former have a faster growth rate than *M. tardicola* and form larger pycnidia. Additionally, *M. simplex* can grow between 10 °C and 25 °C with an optimum at 20 °C, while *M. tardicola* grows between 15 °C and 30 °C, preferring 25 °C.

Moristroma ampulliforme (Kraus, Damm, Voegele & Fischer), ***sp. nov.*** MycoBank MB 828286. **Figure 7.**

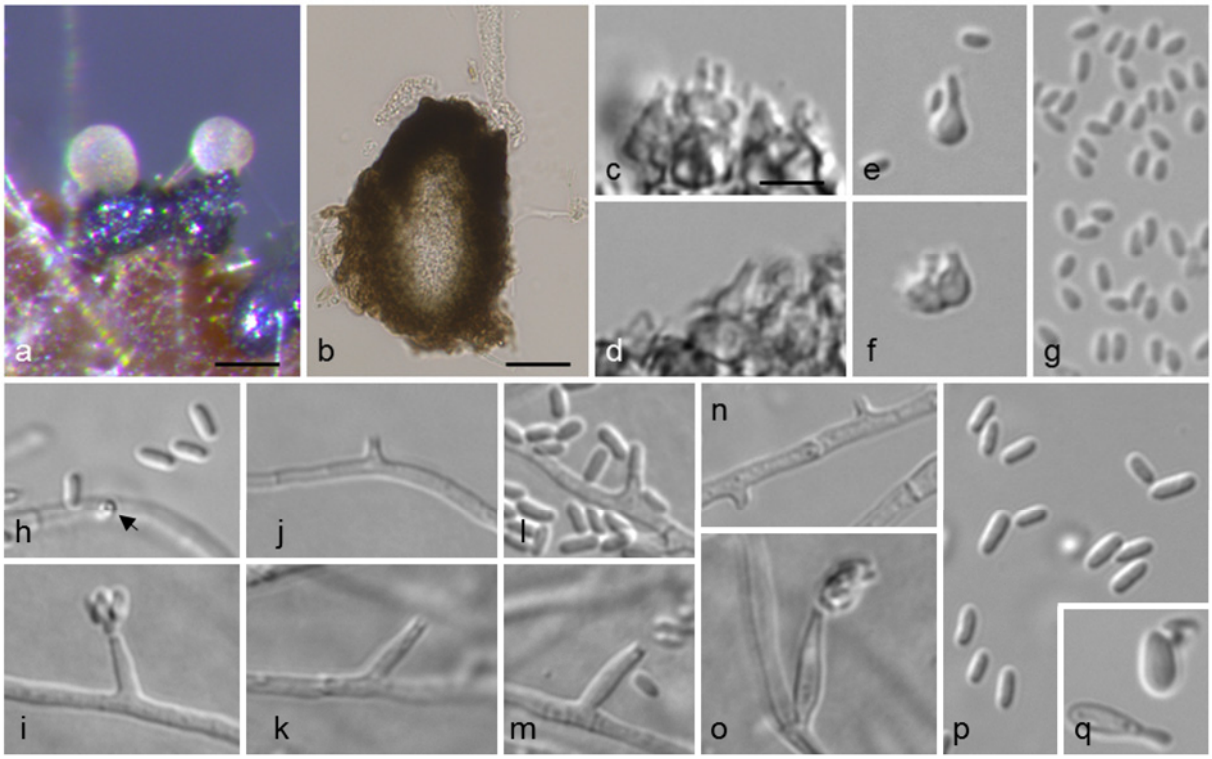

Fig. 7: ***Moristroma ampulliforme*** **(ex-type culture JKI-Feb17). a. conidia oozing from pycnidia on pine needle; b. longitudinal section through pycnidium; c, d. conidiogenous cells lining the inner cell wall of pycnidia; e, f. conidiogenous cells from pycnidia; g. conidia formed in pycnidia; h–o. conidiogenous cells on hyphal cells (arrows indicate openings on hyphal cells); p. conidia generated on hyphal cells; q. microcyclic conidiation. a: DM; b–q: DIC. Scale bars: a = 60 µm; b = 30 µm; c = 5 µm; c applies to c–q.**

Etymology. Named after the shape of the conidiogenous cells from pycnidia that are ampulliform.

Vegetative hyphae hyaline, smooth-walled, 1–2.5 µm wide, septate, no chlamydospores observed. **Sporulation** abundant, conidia formed on hyphae, in pycnidia and by microcyclic conidiation. **Conidiophores on hyphae**, hyaline, smooth-walled, reduced to conidiogenous cells. **Conidiogenous cells** enteroblastic, hyaline, smooth-walled, mostly reduced to openings directly formed on hyphael cells and adelophialides, discrete phialides rare; collarettes conspicuous, cylindrical, 0.5–1.5 µm long, opening 0.5–1.5 µm diameter; adelophialides cylindrical to lanceolate, sometimes conical, 1.5–13 × 1.0–2.5 µm; discrete phialides cylindrical to lanceolate. **Conidia** accumulated in heads around conidiogenous opening, hyaline, smooth-walled, aseptate, elliptical to oblong-elliptical to obovate, smooth-walled, (2–)3(–3.5) × (1–)1.5(–2) µm, L/W ratio = 1.9.

Microcyclic conidiation observed on one side of swollen mother cells developed from primary conidia; conidiogenous cells, hyaline, smooth-walled, aseptate, ellipsoidal to ovoidal, sometimes cylindrical, (5–)5.5(–6.5) × (2.5–)3(–4) µm, L/W ratio = 1.9.

Conidiomata pycnidial, rarely observed only on pine needles after four weeks, mainly solitary, superficial, ovoid, 39–86 µm diameter, unilocular, with a central ostiole, pycnidial wall composed of textura angularis, 7–15 µm thick, 2–4 cell layers. **Conidiophores** reduced to

conidiogenous cells lining the inner wall of the pycnidia. **Conidiogenous cells** enteroblastic, hyaline, smooth-walled, discrete phialides; collarettes conspicuous, cylindric, 0.5–1 µm long; discrete phialides ampulliform, 3.5–6.5 × 1.5–3.5 µm, opening 0.5–1.5 µm. **Conidia** hyaline, smooth-walled, aseptate, oblong-elliptical to obovate, (2–)2.5(–3) × (1–)1.5(–2.0) µm, L/W ratio = 1.7.

Culture characteristics – Colonies on **PDA** flat, with undulate margin, moist to slimy, buff, aerial mycelium sparse; reverse same colours; on **OA** flat, with entire margin, moist, buff, dense, funiculose aerial mycelium in centre; reverse same colours; on **MYA** flat to raised, with undulate margin, moist, buff to primrose, sparse aerial mycelium; reverse same colours; 20–23 mm diameter after 14 d on MYA (25 °C, in the dark), min 10 °C, max 30 °C, opt 25 °C.

Specimens examined: Germany, Rhineland-Palatinate, Siebeldingen, °13'11.5"N 8°02'34.6"E, isolated from a spore trap attached to a grapevine shoot, 23. February 2017, C. Kraus, GLMC GLM-F117495 holotype, culture ex-type JKI-Feb17 = CBS 145010 = GLMC 1909; Germany, Rhineland-Palatinate, Siebeldingen, °13'11.5"N 8°02'34.6"E, isolated from a spore trap attached to a grapevine shoot, 9. August 2016, C. Kraus, JKI-Au02 = CBS 145011 = GLMC 1910.

Notes - The ITS and LSU sequences of *Moristroma ampulliforme* are 93% and 98% identical with *Mo. germanicum* (31 and 11 nucleotides different), 91% and 98% with *Mo. quercinum* (42 and 13 nucleotides different) and 92% and 97% with *Mo. japonicum* (35 and 16 nucleotides different; Nordén et al., 2005), respectively. Additionally, the *tub2* sequence of *Mo. ampulliforme* differs from *Mo. germanicum* by 15 nucelotides (95% identical). Moreover, the abscence of inflated hyphal cells and the lesser occurrence of discrete phialides distinguish *Mo. ampulliforme* from *Mo. germanicum*. The pycnidia of *Mo. ampulliforme* are smaller and conidiogenous cells in pycnidia are longer than those of *Mo. quercinum* and *Mo. japonicum*. Due to missing sequence data and morphological data of the asexual morph, *Mo. multisporum* and *Mo. polysporum* that were described from dead wood of *Terminalia arjuna* in India and from decorticated wood of *Eucalyptus viminalis* in Argentina, cannot be compared with *Mo. ampulliforme* (Sivanesan *et al*. 1988, Romero & Samuels 1991, Boonmee *et al*. 2011, Zhang *et al*. 2012).

Moristroma germanicum (Kraus, Damm, Voegele & Fischer), ***sp. nov.*** MycoBank MB 828287. **Figure 8.**

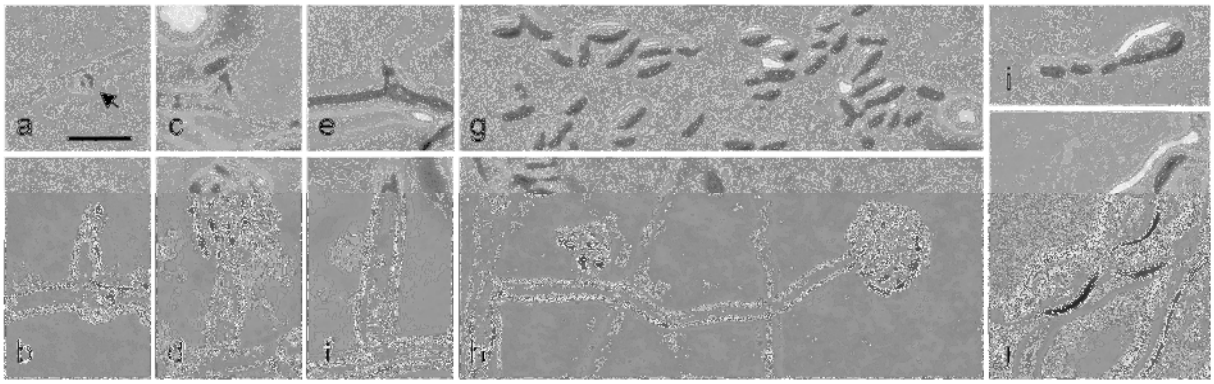

Fig. 8: ***Moristroma germanicum*** **(ex-type culture JKI-Feb06). a–f, h. Conidiogenous cells on hyphal cells (arrow indicate conidiogenous opening on a hyphal cell); g. conidia formed on hyphal cells; i. microcyclic conidiation; j. inflated hyphal cells. a–j: DIC. Scale bars: a = 5 µm; a applies to a–j.**

Etymology. Named after the country the species was found in, Germany.

Vegetative hyphae hyaline, smooth-walled, septate, 1–2.5 µm wide, partly inflated up to 5.5 µm. **Sporulation** abundant, conidia formed on hyphae and by microcyclic conidiation. **Conidiophores on hyphae** hyaline, smooth-walled, mainly reduced to conidiogenous cells, rarely 2–7-celled, cylindrical, 17–71 × 1–2 µm. **Conidiogenous cells** enteroblastic, hyaline, smooth-walled, mostly adelophialides, often discrete phialides; adelophialides cylindrical to lanceolate, sometimes conical, 2–21 × 1–2 µm; discrete phialides cylindrical to naviculate, 2.5–15.5 × 1.5–4 µm, collarettes conspicuous, cylindrical, 0.5–1.5 µm long, opening 0.5–1 µm, periclinal thickening observed. **Conidia** accumulated in heads around conidiogenous openings, hyaline, smooth-walled, aseptate, oblong-elliptical to obovate, (2.5–)3(–5) × (1–)1.5(–2) µm, L/W ratio = 2.1.

Microcyclic conidiation observed on one side of swollen mother cells developed from primary conidia; conidiogenous cells, hyaline, smooth-walled, aseptate, sometimes 2-celled, obovate (3–)4(–5) × (2–)2.5(–3) µm, L/W ratio = 1.6.

Conidiomata not observed.

Culture characteristics – Colonies on **PDA** flat to raised, with entire margin, moist, buff, aerial mycelium sparse; reverse same colour; on **OA** flat, with undulate margin, moist, buff, aerial mycelium sparse; reverse same colour; on **MYA** flat, with entire margin, moist, buff to primrose, aerial mycelium in the centre dense, whitish, funiculose; reverse same colours; 17.5–19 mm diameter after 14 d on MYA (25 °C, in the dark), min 15 °C, max 30 °C, opt 25 °C.

Specimens examined: Germany, Rhineland-Palatinate, Siebeldingen, °13'11.5"N 8°02'34.6"E, isolated from a spore trap attached to a grapevine shoot, 3. February 2017, C. Kraus, GLMC GLM-F117494 holotype, culture ex-type JKI-Feb06 = CBS 145012 = GLMC 1911.

Notes – Frequently occurring discrete phialides distinguishes *Mo. germanicum* from *Mo. ampulliforme*. The *tub2* sequence of *Mo. germanicum* shows 96% identity with *Mo. ampulliforme* (15 nucleotides different). The alignment displays 97% identity (14 nucleotides

different) of ITS sequences of *Mo. germanicum* with that of *Mo. quercinum*, 93% (31 nucleotides different) with that of *Mo. ampulliforme* and 93% (33 nucleotides different) with that of *Mo. japonicum*. The LSU sequence of *Mo. germanicum* is two nucleotides different from *Mo. quercinum* (99% identical), 9 nucleotides different (98% identical) from *Mo. japonicum* and 11 nucleotides different (98% identical) from *Mo. ampulliforme* (Nordén *et al*. 2005).

Neophaeomoniella constricta (Kraus, Damm, Voegele & Fischer), ***sp. nov.*** MycoBank MB 828288. **Figure 9.**

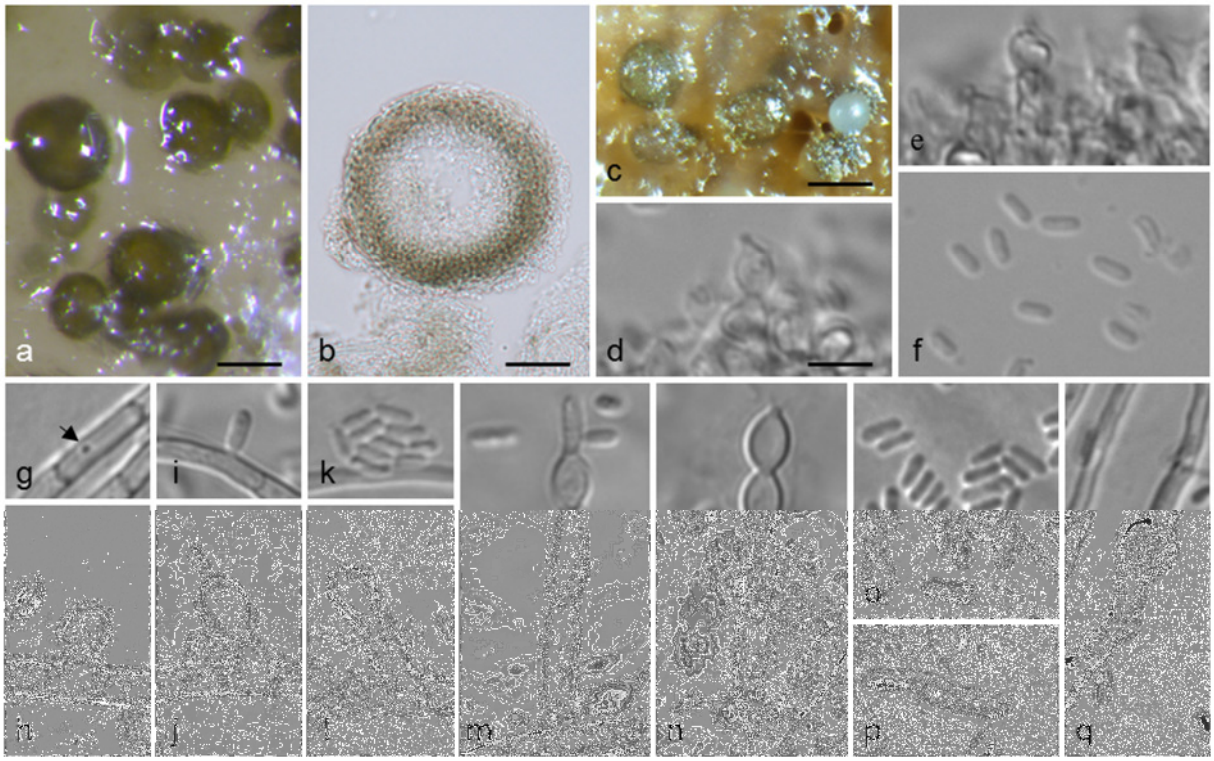

Fig. 9: ***Neophaeomoniella constricta*** **(ex-type culture JKI-Mz35). a. Pycnidia formed on OA; b. longitudinal section through a pycnidium from OA; c. Pycnidia formed on grapevine wood; d, e. conidiogenous cells from the inner cell wall of pycnidia formed on OA; f. conidia formed in pycnidia; g–n. conidiogenous cells on hyphal cells; o. conidia formed on hyphal cells; p. microcyclic conidiation; q. inflated hyphal cell. a, c: DM; b, d–q: DIC. Scale bars: a = 25 µm; b = 30 µm; c = 150 µm, d = 5 µm; d applies to d–q.**

Etymology. Named after the conidiogenous cells that are often constricted at the septa.

Vegetative hyphae hyaline, smooth-walled, 1–3 µm wide, septate, partly inflated up to 5.5 µm. **Sporulation** abundant; conidia formed on hyphae, in pycnidia and by microcyclic conidiation. **Conidiophores on hyphae**, hyaline, smooth-walled, mainly reduced to conidiogenous cells, but also 2–6-celled conidiophores observed, often constricted at the septa, 4.5–32.5 × 2.5–3.5 µm. **Conidiogenous cells** enteroblastic, hyaline, smooth-walled, mostly openings formed directly on hyphael cells, sometimes discrete phialides; ellipsoidal, ovoidal to navicular, rarely cylindrical, 3.5–7.5 × 2.5–4 µm, collarettes sometimes visible, periclinal thickening inconspicuous. **Conidia** accumulated in groups around conidiogenous openings, hyaline, smooth-walled, aseptate, oblong-elliptical, sometimes slightly curved, (3–)4(–5) × (1–)1.5(–2) µm, L/W ratio = 2.7.

Microcyclic conidiation observed on one side of swollen mother cells developed from primary conidia; conidiogenous cells, hyaline, smooth-walled, aseptate, rarely 2-celled, oblong-elliptical, (5–)6.5(–8) × (1.5–)2(–3) µm, L/W ratio = 3.0.

Conidiomata pycnidial, observed on pine needles, grapevine wood and immersed in SNA and OA after four weeks, on OA solitary or in groups, superficial or immersed, globose, subglobose to ellipsoidal, 21–41 µm diameter, unilocular, opening by irregular rupture, pycnidial wall composed of textura angularis, 8–19 µm thick, 2–6 cell layers. **Conidiophores** reduced to

conidiogenous cells lining the inner cavity of the conidiomata. **Conidiogenous cells** enteroblastic, hyaline, smooth-walled, phialides discrete, ampulliform, 3.5–8 × 2.5–4 µm, collarettes inconspicuous, opening 0.5–1.5 µm wide. **Conidia** hyaline, smooth-walled, aseptate, oblong-elliptical, sometimes slightly curved, (3–)4(–5) × (1–)1.5(–2) µm, L/W ratio = 2.3.

Culture characteristics – Colonies on **PDA** raised, radially striate with lobate margin, moist, saffron to luteous, aerial mycelium sparse; reverse saffron; on **OA** flat, with entire margin, moist, in the centre greenish olivaceous to dark herbage green due to pycnidia formation, with a buff margin, aerial mycelium abundant, sometimes funiculose; reverse same colours; on **MYA** flat, with lobate margin, moist, saffron, aerial mycelium sparse; reverse same colours; 7–9.5 mm diameter after 14 d on MYA (25 °C, in the dark), min 10 °C, max 25 °C, opt 20 °C.

Specimens examined: Germany, Rhineland-Palatinate, Siebeldingen, °13'11.5"N 8°02'34.6"E, isolated from a spore trap attached to a grapevine shoot, 3. March 2016, C. Kraus, GLMC GLM-F117500 holotype, culture ex-type JKI-Mz35 = CBS 145015 = GLMC 1915.

Notes – The formation of up to 6-celled conidiophores, typically constricted at the septa, distinguishes *N. constricta* from its close relatives *N. ossiformis*, *N. eucalypti*, *N. niveniae* and *N. zymoides*. Within this group of slow-growing fungi studied here, *N. constricta* was the one with the slowest growth, which is an additional feature of this fungus (Fig. 4). Comparison of the *tub2*, ITS and LSU sequences showed that *N. constricta* is 93% (24 nucleotides different), 98% (10 nucleotides different) and 99% (6 nucleotides different), respectively, identical with its closest relative *N. ossiformis*, while *N. zymoides* is 82%, 95% and 97% identical. A blastn search with the ITS sequence of *N. constricta* resulted in 97–99% identity (8–16 nucleotides different) with sequences of eight fungal strains (identified as *Eurotiomycetes*, *Phaeomoniella* sp. or referred to as uncultured fungus), most of them described as endophytes of trees in Arizona (USA), New Zealand, New Mexico (USA) and Canada (KP202999, GQ999270, JN225892, KT264520, KT264593, KF742578, GQ153143, GQ153196; Hoffman & Arnold 2010, Johnston *et al*. 2012, Bérubé & Nicolas 2015, Chen *et al*. 2015). Additionally, 38 endophytic fungi isolated from leaves of pine trees in Arizona (USA) display a high accordance (98–99%; 7–11 nucleotides different) with *N. constricta* (Bowman & Arnold 2018).

Neophaeomoniella ossiformis (Kraus, Damm, Voegele & Fischer), ***sp. nov.*** MycoBank MB 828289. **Figure 10.**

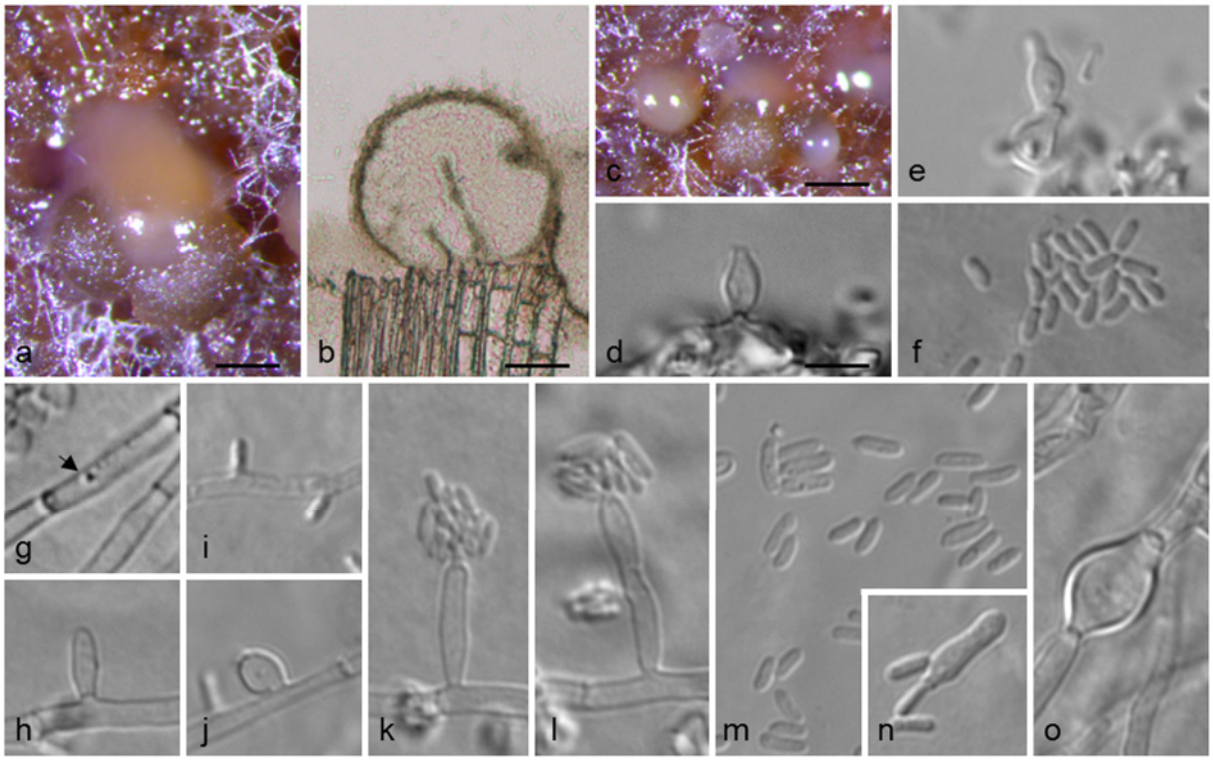

Fig. 10: ***Neophaeomoniella ossiformis*** **(ex-type culture JKI-May02). a, c. conidia oozing from pycnidia on grapevine wood; b. longitudinal section through a pycnidium; d, e. conidiogenous cells lining the inner cell wall of pycnidia; f. conidia generated in pycnidia; g–l. conidiogenous cells on hyphal cells; m. conidia generated on hyphal cells; n. microcyclic conidiation; o. chlamydospore. a, c: DM; b, d–o: DIC. Scale bars: a = 100 µm; b = 80 µm; c = 200 µm, d = 5 µm; d applies to d–o.**

Etymology. Named after the shape of the conidia mother cells of the microcyclic conidiation that are sometimes ossiform.

Vegetative hyphae hyaline, smooth-walled, 1.5–3 µm wide, septate, chlamydospores hyaline, limoniform, 6–12 × 5–8 µm. **Sporulation** abundant; conidia formed on hyphae, in pycnidia and by mycrocyclic conidiation. **Conidiophores on hyphae** hyaline, smooth-walled, mainly reduced to conidiogenous cells, 2-3-celled conidiophores observed, 6.5–22.5 × 2–3.5 µm. **Conidiogenous cells** enteroblastic, hyaline, smooth-walled, mostly reduced to openings directly formed on hyphal cells, discrete phialides sometimes observed, subglobose to elongate navicular, 4.5–11.5 × 2.0–3.5 µm, collarettes rarely observed, periclinal thickening inconspicuous. **Conidia** accumulated in heads around conidiogenous openings, hyaline, smooth-walled, aseptate, oblong-elliptical, sometimes slightly curved, (2.5–)3.5(–4.5) × (1–)1.5(–2) µm, L/W ratio = 2.7.

Microcyclic conidiation observed on one side of swollen mother cells developed from primary conidia; conidiogenous cells, hyaline, smooth-walled, aseptate, obovate to oblong-elliptical, sometimes ossiform, (6–)8(–10) × (1.5–)2.5(–3) µm, L/W ratio = 3.3.

Conidiomata pycnidial, produced on pine needles, grapevine wood and immersed in SNA medium after four weeks, mainly solitary, superficial, globose, subglobose to ellipsoidal, 46–

353 µm diameter, unilocular, opening by irregular rupture, pycnidial wall composed of textura angularis, 10–16 µm thick, 3–6 cell layers. **Conidiophores** reduced to conidiogenous cells lining the inner cavity of the pycnidium. **Conidiogenous cells** enteroblastic, hyaline, smooth-walled, phialides discrete, ampulliform to flask-shaped, 3.5–7 × 2.5–5 µm, opening 0.5–1.5 µm wide, collarettes inconspicuous, periclinal thickening not observed. **Conidia** hyaline, smooth-walled, aseptate, oblong-elliptical, sometimes slightly curved, (3–)3.5(–4) × (1–)1.5(–2) µm, L/W ratio = 2.3.

Culture characteristics – Colonies on **PDA** raised, radially striate with lobate margin, moist, buff, grey olivaceous in centre due to pycnidia formation, aerial mycelium in centre sparse, funiculose; reverse buff; on **OA** flat, radially striate with lobate margin, moist, buff to rosy buff, grey olivaceous in centre due to pycnidia formation, aerial mycelium sometimes formed in centre sparse, funiculose; reverse same colours; on **MYA** raised, radially striate with lobate margin, moist, buff, grey olivaceous in centre due to pycnidia formation, aerial mycelium abundant, whitish, funiculose; reverse buff; 12–14 mm diameter after 14 d on MYA (25 °C, in the dark), min 10 °C, max 25 °C, opt 20 °C.

Specimens examined: Germany, Rhineland-Palatinate, Siebeldingen, °13'11.5"N 8°02'34.6"E, isolated from a spore trap attached to a grapevine shoot, 6. May 2016, C. Kraus, GLMC GLM-F117498 holotype, culture ex-type JKI-May03 = CBS 145013 = GLMC 1913; Germany, Rhineland-Palatinate, Siebeldingen, °13'11.5"N 8°02'34.6"E, isolated from a spore trap attached to a grapevine shoot, 6. May 2016, C. Kraus, JKI-May02 = CBS 145014 = GLMC 1912; Germany, Rhineland-Palatinate, Siebeldingen, °13'11.5"N 8°02'34.6"E, isolated from a spore trap attached to a grapevine shoot, 6. May 2016, C. Kraus, JKI-May30 = GLMC 1914.

Notes – *Neophaeomoniella ossiformis* can be distinguished from the closely related *N. constricta* by its faster growth and shorter conidiophores that are usually less strongly constricted at the septa. The *tub2*, ITS and LSU sequences of *N. ossiformis* are 93% (24 nucleotides different), 98% (10 nucleotides different) and 99% (6 nucleotides different) identical with the sequences of *N. constricta*. A blastn search of the ITS sequence of *N. ossiformis* produced similar results as for *N. constricta* (see above).

Pathogenicity test

Until the day of evaluation, infected plants showed no external symptoms of Esca or other GTD. However, longitudinal sections of the trunks unveiled discolorations at the inoculation site in all plants. The measured mean lesion length revealed significant differences between *P. chlamydospora* (22.7 ± 13.8 mm and 20.4 ± 11.2 mm, respectively) and the water control (6.1 ± 0.9 mm and 6.5 ± 1.7 mm, respectively) for both cultivars, Pinot noir and Müller-Thurgau (Table 3). However, the lesion length of the seven other examined species of the *Phaeomoniellales* showed no differences to the water control. For Pinot noir the produced mean lesions of the tested fungi ranged from 6.0 ± 1.2 mm (*Mo. ampulliforme*) to 7.5 ± 2.4 mm (*N. zymoides*). Similarly, for Müller-Thurgau the mean lesion varied between 6.2 ± 1.5 mm (*Mo. ampulliforme*) and 10.5 ± 4.1 mm (*N. zymoides*).

Tab. 3: Results of the pathogenicity test performed with eight species of the *Phaeomoniellales*. The test was conducted with grapevine varieties Pinot noir and Müller-Thurgau in a greenhouse. Shown is the mean total lesion length measured up- and downwards from the inoculation point in mm and the re-isolation rate. Significant differences ($p<0.001$) between mean lesion lengths according to an ANOVA are indicated by different letters.

		Pinot noir		Müller-Thurgau	
Accession no[a]	Fungal species	Mean lesion length [mm]	Re-isolation rate [%]	Mean lesion length [mm]	Re-isolation rate [%]
JKI-Ap04	*P. chlamydospora*	22.7 ± 13.8[a]	46.7	20.4 ± 11.2[a]	66.7
JKI-Ap36	*A. palatina*	6.1 ± 2.4[b]	14.3	7.2 ± 3.8[b]	7.7
JKI-Jn27	*M. simplex*	6.6 ± 2.6[b]	21.4	8.2 ± 2.9[b]	61.5
JKI-Feb17	*Mo. ampulliforme*	6.0 ± 1.2[b]	0	6.2 ± 1.5[b]	6.7
JKI-Feb06	*Mo. germanicum*	6.0 ± 1.4[b]	30	6.8 ± 1.1[b]	38.5
JKI-Mz35	*N. constricta*	7.2 ± 4.0[b]	15.4	8.1 ± 2.2[b]	14.3
JKI-May03	*N. ossiformis*	7.1 ± 1.9[b]	27.3	8.6 ± 3.1[b]	50
JKI-Mz42	*N. zymoides*	7.5 ± 2.4[b]	15.4	10.5 ± 4.1[b]	50
--	Control (water)	6.1 ± 0.9[b]	0	6.5 ± 1.7[b]	0

[a] JKI: Culture collection of the Julius Kühn-Institute, Siebeldingen, Germany

Wood pieces from the induced lesions were placed on MYA plates to verify the presence of the inoculated fungi in order to fulfil Koch's postulates. *Phaeomoniella chlamydospora* was re-isolated most frequently (46.7%) from infected Pinot noir plants. The seven other studied fungi reached a re-isolation rate between 14.3% (*A. palatina*) and 30.0% (*Mo. germanicum*). *Moristroma ampulliforme* could not be re-isolated from Pinot noir plants. Concerning the Müller-Thurgau vines, *P. chlamydospora* again showed the highest re-isolation rate with

66.7%. The remaining fungi were recovered from 6.7% (*Mo. ampulliforme*) to 61.5% (*M. simplex*).

Discussion

During this study, spore traps made of sticky glass slides were positive for fungi of the *Phaeomoniellales* in eleven out of 56 monitored weeks (20%). A similar trapping method was applied in Californian vineyards that were monitored from February to July; spores of *Phaeomoniella chlamydospora* were detected in nine out of 24 weeks (38%) (Eskalen & Gubler, 2001), while Larignon and Dubos (2000) detected *P. chlamydospora* spores in fifteen weeks (15%) during a two-year study with a sampling size of thirty collected glass slides each week. Both studies focused on *P. chlamydospora* spores and do not report the detection of other *Phaeomoniellales*. Since the detection rate was low in all three studies, it is assumed that in general the spore concentration of *Phaeomoniellales* in the air is relatively low or that the applied spore trapping method does not enable a higher trapping rate. Especially the trapping technique seems to be a critical point for successful monitoring of *Phaeomoniellales*. Although *P. chlamydospora* is common in South African vineyards, it was not detected by spore trapping, which could be due to the use of air sampling traps specifically made for the collection of wind distributed spores, e.g. Hirst or Burkard spore traps (Hirst, 1952; Mostert *et al*., 2006; van Niekerk *et al*., 2010). However, slimy spores like those of *P. chlamydospora* and its relatives are more likely to be distributed by rain droplets, pruning scissors or even insects than by air (Aroca *et al*., 2010; Moyo *et al*., 2014). That may be the reason why glass slides with a sticky surface, closely attached to grapevines, are more effective in trapping spores of *Phaeomoniellales* than strict air sampling devices.

The amount of isolated fungal strains and species diversity of *Phaeomoniellales* tended to be higher in SMPH than in VSP trained vines indicating that this group of fungi might preferably appear around perennial branches of minimal pruned vines and lesser around annual branches of intensive pruned vines. However, the small number of *Phaeomoniellales* spores

from traps attached to branches of either SMPH or VSP trained vines does not allow a clear explanation for the incidence of these fungi in the two different training systems.

All spores except for one were trapped between February and June, when precipitation was abundant. Eskalen and Gubler (2001) also trapped spores of *P. chlamydospora* mostly after rain events from February to July indicating that rainfall and/or high relative humidity is important for sporulation. Unfortunately, the spore trapping was conducted only in six months and so no comparison of the seasonal occurrence of the spores can be made. Larignon and Dubos (2000) collected spores of *P. chlamydospora* throughout the year. However, the authors did not compare the incidence of the spores with the local climate.

Only two of the eight identified species of the family *Phaeomoniellales*, namely *P. chlamydospora* and *N. zymoides*, were known species. The former is considered to be the main causal agent of Esca (Crous & Gams, 2000; Bertsch *et al*., 2012). This pathogen is known to be almost exclusively associated with grapevine; only once it was isolated from symptomatic wood of olive trees (Úrbez-Torres *et al*., 2013; Farr & Rossman, 2018). *N. zymoides* was first described from healthy pine needles and later isolated from necrotic wood of *Prunus salicina* (Lee *et al*., 2006; Damm *et al*., 2010). Two of the newly described species, *N. constricta* and *N. ossiformis*, are closely related to to *N. eucalypti*, *N. niveniae* and *N. zymoides*, while *Aequabiliella palatina* and *Minutiella simplex* are closely related to *A. effusa* and *M. tardicola*, respectively. The latter two were also associated with symptomatic wood of *Prunus* trees (Damm *et al*., 2010). *Moristroma ampulliforme* and *Mo. germanicum* were assigned to the genus *Moristroma*; the other two species in this genus, *Mo. quercinum* and *Mo. japonicum*, had been identified based on their ascostroma found on oak wood (Nordén *et al*., 2005).

This is the first report of *Phaeomoniellales* other than *P. chlamydospora* in vineyards. Due to the harm, which this Esca associated pathogen can cause, the question emerged, if the species detected are also pathogenic to grapevine. However, the pathogenicity test showed that only *P. chlamydospora* is able to induce significant necrosis in the wood of potted grapevine

plants. This led to the conclusion that these species are not pathogenic to grapevine. Furthermore, it is likely that grapevine wood is not the natural habitat of these species, since they have to date never been found in or on any parts of grapevine plants (Casieri *et al*., 2009; González & Tello, 2011; Hofstetter *et al*., 2012; Pancher *et al*., 2012; Bruez *et al*., 2014; Pinto *et al*., 2014; Bruez *et al*., 2016; Travadon *et al*., 2016; Farr & Rossman, 2018; Kraus *et al*., 2018). Based on the habitats of previously described *Phaeomoniellales* species, the new species might originate from woody plants. Five species belonging to four genera of the *Phaeomoniellales* have previously been collected from wood of *Prunus* trees in South Africa (Damm *et al*., 2010). One of these species, *N. zymoides,* was originally described from pine needles in Korea (Lee *et al*., 2006) and was the most abundant species in our study. Moreover, Bien and co-workers (unpublished) recently isolated a fungus with full accordance to the ITS sequence of *M. simplex* from necrotic wood of *P. domestica* in the region of Baden-Württemberg, Germany. Therefore, the *Prunus* trees planted close (in about 100 m distance) to our vineyard could be one possible origin of the collected spores in this study. Two independent investigations on endophytic fungi in wood of pine trees in Montana, USA and Finnland revealed unidentified fungal strains with high similarity (99%, 2–3 nucleotides different) to the ITS sequence of *A. palatina* (Larkin *et al*., 2012; Müller *et al*., 2018). These findings may indicate that *A. palatina* also appears endophytically and that pine trees represent a possible host genus.

With the data generated in this study, the comparatively new order *Phaeomoniellales* will be extended by six new species. However, with the information at hand, the origin of the trapped fungal spores and their ecological traits remain basically unknown. The pathogenicity test conducted in this study does not support them to be pathogens of grapevine. However, this is no prove that these fungi do not live in or on grapevine wood. Further studies are necessary to reveal the origin and life style of the newly described species and their possible impact for example on economically important fruit crops.

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References

Aptroot, A., Lücking, R., Sipman, H.J.M., Umana, L. & Chaves, J.L. (2008) Pyrenocarpous lichens with bitunicate asci. Bibl. Lichenol. 97, 1–162.

Aroca, A., Gramaje, D., Armengol, J., García-Jiménez, J. & Raposo, R. (2010) Evaluation of the grapevine nursery propagation process as a source of *Phaeoacremonium* spp. and *Phaeomoniella chlamydospora* and occurrence of trunk disease pathogens in rootstock mother vines in Spain. Eur. J. of Plant Pathol. 126, 165–174.

Arnold, A.E., Henk, D.A., Eells, R.L., Lutzoni, F. & Vilgalys, R. (2007) Diversity and phylogenetic affinities of foliar fungal endophytes in loblolly pine inferred by culturing and environmental PCR. Mycologia 99, 185–206.

Bertsch, C., Ramírez-Suero, M., Magnin-Robert, M., Larignon, P., Chong, J., Abou-Mansour, E., Spagnolo, A., Clément, C. & Fontaine, F. (2012) Grapevine trunk diseases: complex and still poorly understood. Plant Pathol. 62, 243–265.

Bérubé, J.A. & Nicolas, G.G. (2015) Alien fungal species on asymptomatic live woody plant material imported into Canada. Can. J. Plant Pathol. 37, 67–81.

Boonmee, S., Zhang, Y., Chomnunti, P., Chukeatirote, E., Tsui, C.K.M., Bahkali, A.H., & Hyde, K.D. (2011) Revision of lignicolous Tubeufiaceae based on morphological reexamination and phylogenetic analysis. Fungal Divers. 51, 63–102.

Booth, C. & Ting, T.P. (1964) *Dolabra nepheliae gen. nov. sp. nov.* associated with canker of *Nephelium lappaceum*. Trans. Brit. Mycol. Soc. 47, 235–237.

Botella, L. & Diez, J.J. (2010) Phylogenic diversity of fungal endophytes in Spanish stands of Pinus halepensis. Fungal Divers. 47, 9–18.

Bowman, E.A. & Arnold, A.E. (2018) Distributions of ectomycorrhizal and foliar endophytic fungal communities associated with *Pinus ponderosa* along a spatially constrained elevation gradient. Am. J. Bot. 105, 687–699.

Bruez, E., Vallance, J., Gerbore, J., Lecomte, P., Da Costa, J.-P., Guérin-Dubrana, L. & Rey, P. (2014) Analyses of the temporal dynamics of fungal communities colonizing the healthy wood tissues of Esca leaf-symptomatic and asymptomatic vines. PLoS One 9: e95928 doi:101371/journalpone0095928.

Bruez, E., Baumgartner, K., Bastien, S., Travadon, R., Guérin-Dubrana, L. & Rey, P. (2016) Various fungal communities colonise the functional wood tissues of old grapevines externally free from grapevine trunk disease symptoms. Aust. J. Grape Wine Res. 22, 288–295.

Casieri, L., Hofstetter, V., Viret, O. & Gindro, K. (2009) Fungal communities living in the wood of different cultivars of young *Vitis vinifera* plants. Phytopathol. Mediterr. 48, 73–83.

Chen, K.-H., Miadlikowska, J., Molnár, K., Arnold, A.E., U'Ren, J.M., Gaya, E., Gueidan, C. & Lutzoni, F. (2015) Phylogenetic analyses of eurotiomycetous endophytes reveal their close affinities to *Chaetothyriales*, *Eurotiales*, and a new order – *Phaeomoniellales*. Mol. Phylogenet. Evol. 85, 117–130.

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) *Phaeomoniella chlamydospora gen. et comb. nov.* a causal organism of Petri grapevine decline and esca. Phytopathol. Mediterr. 39, 112–118.

Crous, P.W., Wood, A.R., Okada, G. & Groenewald, J.Z. (2008) Foliicolous microfungi occurring on *Encephalartos*. Persoonia 21, 135–146.

Crous, P.W., Verkley, G.J.M. & Groenewald, J.Z. (2009) *Xenocylindrosporium kirstenboschense*. Persoonia 23, 200–201.

Crous, P.W., Summerell, B.A., Shivas, R.G., Romberg, M., Mel´nik, V.A., Verkley, G.J.M. & Groenewald, J.Z. (2011) Fungal planet description sheets: 92-106. Persoonia 27, 130–162.

Crous, P.W. *et al*. (2015) Fungal planet description sheets: 320-370. Persoonia 34, 167–266.

Crous, P.W. *et al*. (2016) Fungal planet description sheets: 469-557. Persoonia 37, 218–403.

Damm, U., Fourie, P.H. & Crous, P.W. (2010) *Coniochaeta* (*Lecythophora*) *Collophora gen. nov.* and *Phaeomoniella* species associated with wood necroses of *Prunus* trees. Persoonia 24, 60–80.

Eskalen, A. & Gubler, W.D. (2001) Association of spores of *Phaeomoniella chlamydospora*, *Phaeoacremonium inflatipes* and *Pm. aleophilum* with grapevine cordons in California. Phytopathol. Mediterr. 40, S429–S432.

Farlow, W.G. (1876) The American grape mildew. Bussy Institut Bulletin 1, 423.

Farr, D.F. & Rossman, A.Y. (2018) Fungal Databases US National Fungus Collections ARS USDA https://ntars-gringov/fungaldatabases/ (accessed 16th July 2018).

Fontaine, F., Gramaje, D., Armengol, J., Smart, R., Nagy, Z.A., Borgo, M., Rego, C. & Corio-Costet, M.-F. (2016) Grapevine trunk diseases A review 1st Edition OIV publications Paris ISBN: 979-10-91799-60-7.

Glass, L.N. & Donaldson, G.C. (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous Ascomycetes. Appl. Environ. Microb. 61, 1323–1330.

González ,V. & Tello, M.L. (2011) The endophytic mycota associated with *Vitis vinifera* in central Spain. Fungal Divers. 47, 29–42.

Gramaje, D., Úrbez-Torres, J.R. & Sosnowski, M.R. (2018) Managing grapevine trunk diseases with respect to etiology and epidemiology: current strategies and future prospects. Plant Dis.102, 12–39.

Gueidan, C., Aptroot, A., Cáceres, M.E.S., Badali, H. & Stenroos, S. (2014) A reappraisal of orders and families within the subclass *Chaetothyriomycetidae* (*Eurotiomycetes Ascomycota*). Mycol. Prog. 13, 1027–1039.

Hirst, J.M. (1952) An automatic volumetric spore trap. Ann. App. Biol. 39, 257–265.

Hoffman, M.T. & Arnold, A.E. (2010) Diverse bacteria inhabit living hyphae of phylogenetically diverse fungal endophytes. Appl. Environ. Microb. 76, 4063–4075

Hofstetter, V., Buyck, B., Croll, D., Viret, O., Couloux, A. & Gindro, G. (2012) What if esca disease of grapevine were not a fungal disease? Fungal Divers. 54, 51–67.

Johnston, P.R., Johansen, R.B., Williams, A.F., Paula Wikie, J. & Park, D. (2012) Patterns of fungal diversity in New Zealand Nothofagus forests. Fungal Biol. 116, 401–412.

Keith, L.M., Matsumoto, T.K. & McQuate, G.T. (2013) First report of *Dolabra nepheliae* associated with corky bark disease of langsat in Hawaii. Plant Dis. 97, 990.

Kirk, P.M. (2015) Phaeomoniellaceae Nomenclatural novelties Index Fungorum 265, 1–1.

Kraus, C., Voegele, R.T. & Fischer, M. (2018) Temporal development of the culturable endophytic fungal community in healthy grapevine branches and occurrence of GTD associated fungi. Microb. Ecol. DOI: https://doi.org/10.1007/s00248-018-1280-3 (accessed 4th December 2018).

Kumar, S., Stecher, G. & Tamura, K. (2015) MEGA7: molecular evolutionary genetics analysis version 70 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.

Larignon, P. & Dubos, B. (2000) Preliminary studies on the biology of *Phaeoacremonium*. Phytopathol. Mediterr. 39, 184–189.

Larkin, B.G., Hunt, L.S. & Ramsey, P.W. (2012) Foliar nutrients shape fungal endophyte communities in Western white pine (*Pinus monticola*) with implications for white-tailed deer herbivory. Fungal Ecol. 5, 252–260.

Lee, H.B., Park, J.Y., Jung, H.S. & Summerbell, R.C. (2006) *Phaeomoniella zymoides* and *Phaeomoniella pinifoliorum* spp. *nov.* new acid-tolerant epiphytic fungi isolated from pine needles in Korea. Mycologia 98, 598–611.

Mostert, L., Abeln, E.C.A., Halleen, F. & Crous, P.W. (2006) Genetic diversity among isolates of *Phaeomoniella chlamydospora* on grapevines. Australas. Plant Pathol. 32, 47–52.

Moyo, P., Allsopp, E., Roets, F., Mostert, L. & Halleen, F. (2014) Arthropods vector grapevine trunk disease pathogens. Phytopathology 104, 1063–1069.

Müller, M.M., Henttonen, H.M., Penttilä, R., Kulju, M., Helo, T. & Kaitera, J. (2018) Distribution of Heterobasidion butt rot in northern Finland. For. Ecol. Manage. 425, 85–91.

Nigro, F., Boscia, D., Antelmi, I. & Ippolito, A. (2013) Fungal species associated with a severe decline of olive in Southern Italy. J. Plant Pathol. 95, 659–668.

Nirenberg, H. (1976) Untersuchungen über die morphologische und biologische Differenzierung in der Fusarium-Sektion. Liseola Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem 169: 1–117.

Nordén, B., Sunhede, S. & Larsson, E. (2005) New species of *Moristroma* (Ascomycetes) and phylogenetic position of the genus. Mycol. Prog. 4, 325–332.

O´Donnell, K. (1993) *Fusarium* and its near relatives, pp. 225–233. In: Reynolds, D.R. and Taylor, J.W. (eds) The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics, CAB International Wallingford.

Pancher, M., Ceol, M., Corneo, P.E., Longa, C.M.O., Yousaf, S., Pertot, I. & Campisano, A. (2012) Fungal endophytic communities in grapevines (*Vitis vinifera L.*) respond to crop management Appl. Environ. Microbiol. 78, 4308–4317.

Peršoh, D. & Rambold, G. (2012) Lichen-associated fungi of the *Letharietum vulpinae*. Mycol. Prog. 11, 753–760.

Pinto, C., Pinho, D., Sousa, S., Pinheiro, M., Egas, C. & Gomes, A.C. (2014) Unravelling the diversity of grapevine microbiome. PLoS One 9 e85622 doi:101371/journalpone0085622.

Rayner, R.W. (1970) A mycological colour chart. Commonwealth Mycological Institute Kew UK.

Romero, A.I. & Samuels, G.J. (1991) Studies on xylophilous fungi from Argentina: VI Ascomycotina on *Eucalyptus viminalis* (Myrtaceae). Sydowia 43, 228–248.

RStudio Team (2016) RStudio: Integrated development for R RStudio Inc Boston MA URL http://wwwrstudiocom/ (accessed 16th July 2018).

Rossman, A.Y., Schoch, C.L., Farr, D.F., Nishijima, N., Keith, L. & Coenaga, R. (2010) *Dolabra nepheliae* on rambutan and lychee represents a novel lineage of phytopathogenic *Eurotiomycetes*. Mycoscience 51, 300–309.

Sánchez Márquez, S., Bills, G.F. & Zabalgogeazcoa, I. (2011) Fungal species diversity in juvenile and adult leaves of *Eucalyptus globulus* from plantations affected by Mycosphaerella leaf disease. Ann. Appl. Biol. 158, 177–187.

Sivanesan, A., Rajak, R.C. & Gupta, R.C. (1988) Thaxterina a new tubeufiaceous genus with multispored asci from India. Trans. Brit. Mycol. Soc. 90, 662–665.

Tillett, D. & Neilan, B.A. (2000) Xanthogenate nucleic acid isolation from cultured and environmental cyanobacteria. J. Phycol. 36, 251–258.

Travadon, R., Lecomte, P., Diarra, B., Lawrence, D.P., Renault, D., Ojeda, H., Rey, P. & Baumgartner, K. (2016) Grapevine pruning system and cultivars influence the diversity of wood-colonizing fungi. Fungal Ecol. 24, 82–93.

Úrbez-Torres, J.R., Peduto, F., Vossen, P.M. & Gubler, W. (2013) Olive twig and branch dieback: Etiology incidence and distribution in California. Plant Dis. 97, 231–244.

Úrbez-Torres, J.R., Haag, P., Bowen, P., Lowery, T. & O'Gorman, D. (2015) Development of a DNA-macroarray for the detection and identification of fungal pathogens causing decline of young grapevines. Phytopathology 105, 1373–1388.

Van Niekerk, J.M., Calitz, F.J., Halleen, F. & Fourie, P.H. (2010) Temporal spore dispersal patterns of grapevine trunk pathogens in South Africa. Eur. J. Plant Pathol. 127, 375–390.

White, T.J., Bruns, T., Lee, S. & Taylor, J.W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ,

White TJ (eds) PCR protocols: a guide to methods and applications, pp. 315–322. Academic Press, Inc., New York.

Zhang, Y., Crous, P.W., Schoch, C.L. & Hyde, K.D. (2012) Pleosporales. Fungal Divers. 53, 1–221.

Chapter THREE

Conclusion

SMPH is a novel grapevine training system that has the ability to reduce production costs without compromising grape quality. Increasing interest of German grapevine growers in applying this system in their vineyards and so far marginal information about the impact of this system on the incidence of fungal grapevine diseases make it necessary to investigate the phytopathogenic aspects of SMPH. Therefore, one main objective of this thesis was to analyse the fungal community of SMPH trained grapevines and to compare it with the traditional VSP system. In this chapter, results and conclusions of the previously presented manuscripts are summarized and discussed. Furthermore, some additional data are shown to support the results from manuscript I ("Effects of canopy architecture and microclimate on grapevine health in two training systems").

Canopy architecture and microclimate: Impact on incidence of the major fungal grapevine diseases

Numerous abiotic and biotic factors, e.g. host physiology, temperature, and relative humidity influence life and incidence of fungi. For that reason, microclimate and several morphological characteristics of SMPH and VSP trained grapevines were studied during this thesis. The results of manuscript I show that canopies of minimal pruned vines have smaller, but more numerous leaves than VSP vines. Also based on an increased number of shoots this causes a bigger and denser canopy for SMPH trained plants compared to VSP trained plants. Furthermore, the number of bunches was also higher for SMPH vines than for VSP vines. SMPH bunches had smaller and fewer berries, creating a loose bunch architecture. In contrast, VSP bunches were loaded with a high number of large berries, creating a more dense structure.

Plant vigour of VSP trained vines was located in the lower zones (Fig. 1 a, zones 1-2) of the trellis, while vigour of SMPH trained vines was concentrated in the upper trellis zones (Fig. 1 b, zones 3-5). The results obtained during this thesis are in accordance with other studies, which compared the plant morphology of minimal and intensively pruned grapevines (Clingeleffer & Possingham, 1987; Sommer *et al.*, 1993; Schmid & Schultz, 2000; Wolf *et al.*, 2003; Intrieri *et al.*, 2011).

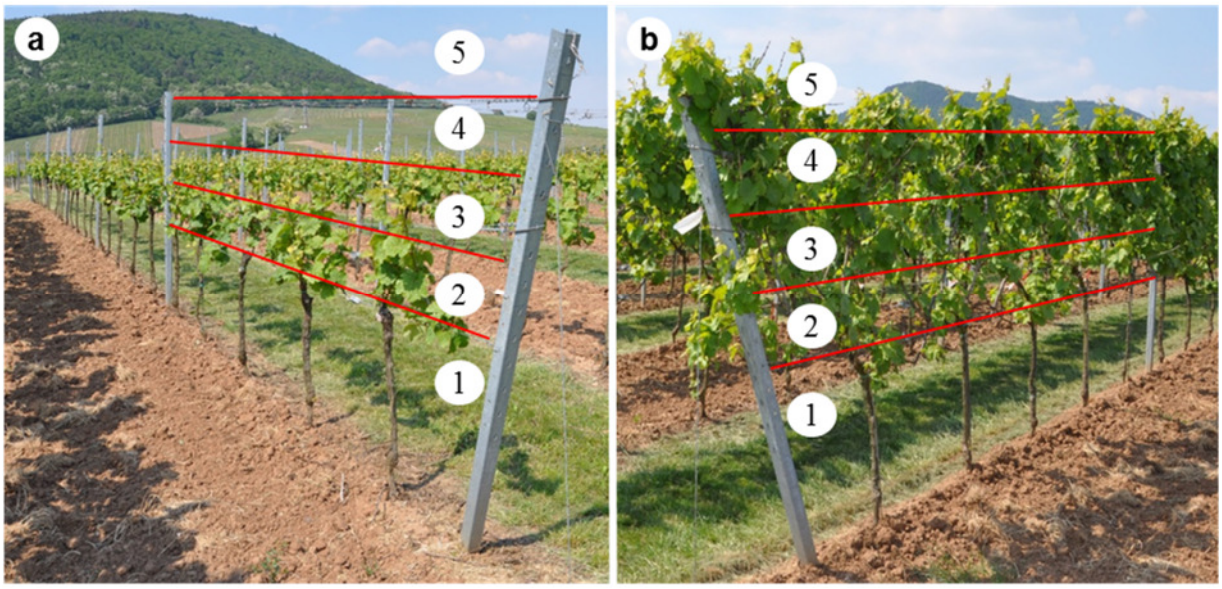

Fig. 1: Distribution of the trellis zones (1-5) in the canopy of VSP trained (a) and SMPH trained (b) grapevines at three leaves stadium.

In correlation with the differing plant architecture, the canopy microclimate was also changed by the pruning system. The seasonal, mean temperature was lower in SMPH canopies compared to VSP canopies, while the average relative humidity was higher for SMPH than for VSP. This divergence in microclimate was probably caused by the differing canopy architecture (Fig. 1). The big and densely packed canopy of SMPH vines prevents sunlight from reaching the inside and reduces air movement, which in sum impedes fast drying of the canopy. This effect could be observed after rain events or morning dew when the relative humidity in the canopy reached 100%. After the rain stopped, or after sunrise, respectively, the canopy of SMPH trained vines dried slower than VSP trained vines.

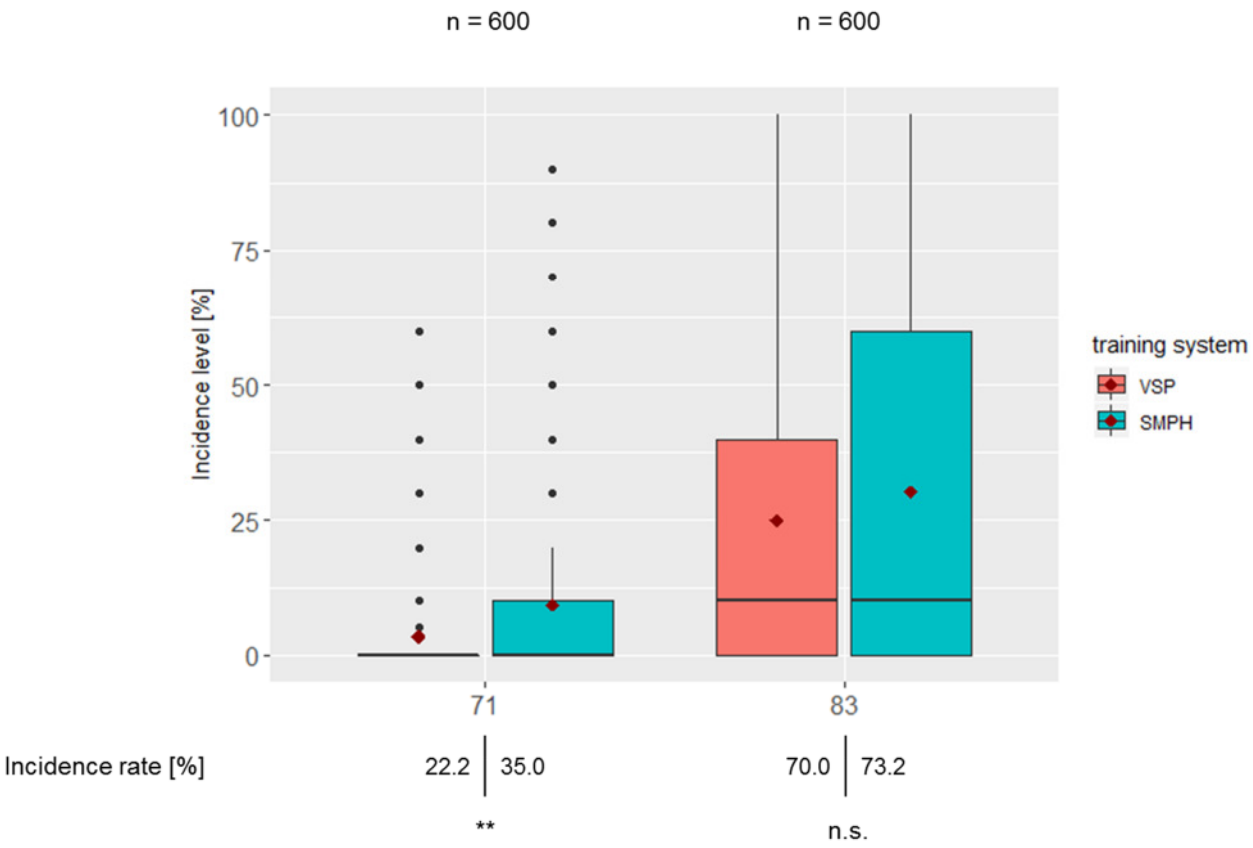

Fig. 2: Results of the Downy Mildew assessments for VSP and SMPH trained vines made in the year 2016 at BBCH 71 (left) and BBCH 83 (right). Six vineyards planted with different cultivars were screened. Incidence level is shown in the upper graph, Nemenyi post-hoc for Kruskal-Wallis test, * p<0.05; ** p<0.001. Incidence rate is shown in the lower table, Fisher´s exact test, * p<0.05; ** p<0.001. n = number of grapes per training system monitored.

In 2015, 2017 and 2018 no DM symptoms were noticed in the monitored vineyards, probably due to the hot and dry weather in these seasons. However, heavy and frequent rainfalls in 2016 led to a severe DM infection in the trial fields. Two assessments of inflorescences/bunches conducted after flowering (BBCH 71) and at the beginning of ripening (BBCH 83) demonstrated a significantly higher DM susceptibility of SMPH trained vines compared to VSP trained vines, at least in the first assessment (Fig. 2). In the second assessment, the significance test for the incidence level was less compelling and for the incidence rate no significant differences between the training systems were found. However, caused by the high infection pressure in the SMPH fields, many inflorescences had died and fallen to the ground, making the results of the second assessment delusive.

It is assumed that the higher DM susceptibility of SMPH vines originates from the slow drying of the canopy. For spreading and infection, *P. viticola* strongly depends on rain and a moist environment (Blaeser, 1978; Blaeser & Weltzien, 1978; 1979). The slower drying of the SMPH canopy after rain or morning dew extends the time frame for the pathogen to penetrate and thus increases the chances for a successful host infection.

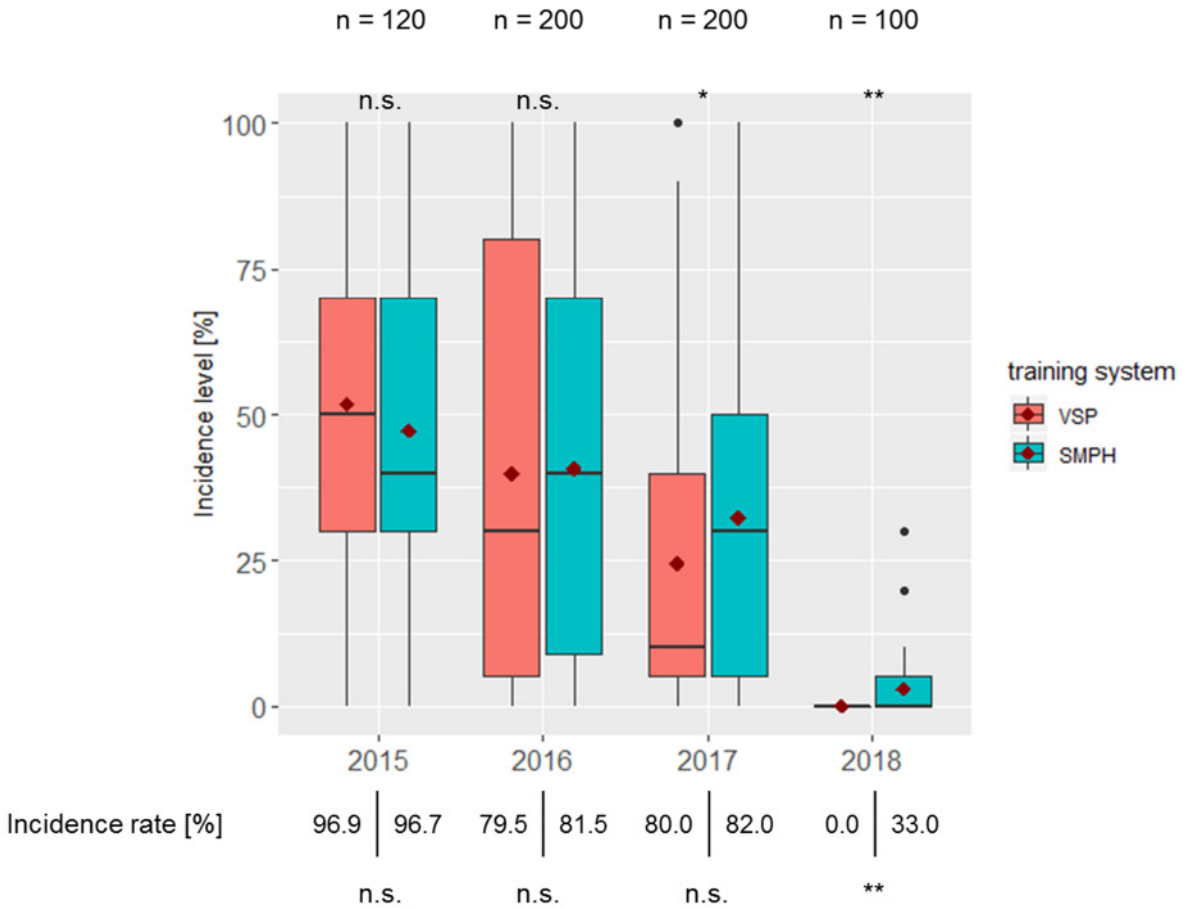

Fig. 3: Results of the Powdery Mildew assessments for VSP and SMPH trained vines made in the years 2015-2018. Two vineyards, Chardonnay and Riesling, were assessed. In 2018 only the Riesling field was assessed due to the absence of PM in the Chardonnay field. Incidence level is shown in the upper graph, Nemenyi post-hoc for Kruskal-Wallis test, * p<0.05; ** p<0.001. Incidence rate is shown in the lower table, Fisher´s exact test, * p<0.05; ** p<0.001. n = number of grapes per training system monitored.

No differences in PM incidence between SMPH and VSP were found in the years 2015 and 2016 (Fig. 3). It is assumed that the extraordinary high infection level of PM in these years and the use of contact pesticides (based on sulphur and potassium bicarbonate) which are less effective compared to systemic pesticides, blurred the differences between the training systems. After a profound cleaning of the trial fields with synthetic fungicides in the beginning of the season 2017 differences between SMPH and VSP were noted. According to the bunch

assessments made in this year, SMPH trained vines displayed a higher incidence level of PM than VSP trained vines. During the season 2018, the grapevines were treated with synthetic pesticides only and the assessment again revealed a higher incidence rate and level for SMPH trained vines compared to VSP trained vines, however based on overall low infection rates. This is in conflict with the results of Emmett *et al.* (1995) who noticed a higher incidence of PM in cane pruned vines compared to minimally pruned vines. The authors assumed that the microclimate in SMPH canopies is less favourable for disease development. However, the microclimate data collected in this work demonstrates that the canopies of SMPH trained vines express a higher relative humidity and lower temperatures compared to VSP. The SMPH microclimate seems therefore more favourable for PM than the VSP microclimate (Delp, 1954; Fessler & Kassemeyer, 1995; Carroll & Wilcox, 2003). In addition, sunlight exposure of *E. necator* can impede the growth of the pathogen, and training systems which promote light penetration of the grapes are less susceptible to an infection of PM (Austin, 2010; Austin & Wilcox, 2011; Austin *et al*., 2011). In contrast to VSP bunches, bunches of SMPH trained vines are mostly shaded by a mass of leaves in the canopy and so are subjected to a higher risk of PM infection. Another explanation for the different susceptibilities of the two training systems against PM could be pesticide deposition. Like sunlight exposure, the application of fungicides may be better in VSP trained vines because of an enhanced bunch accessibility.

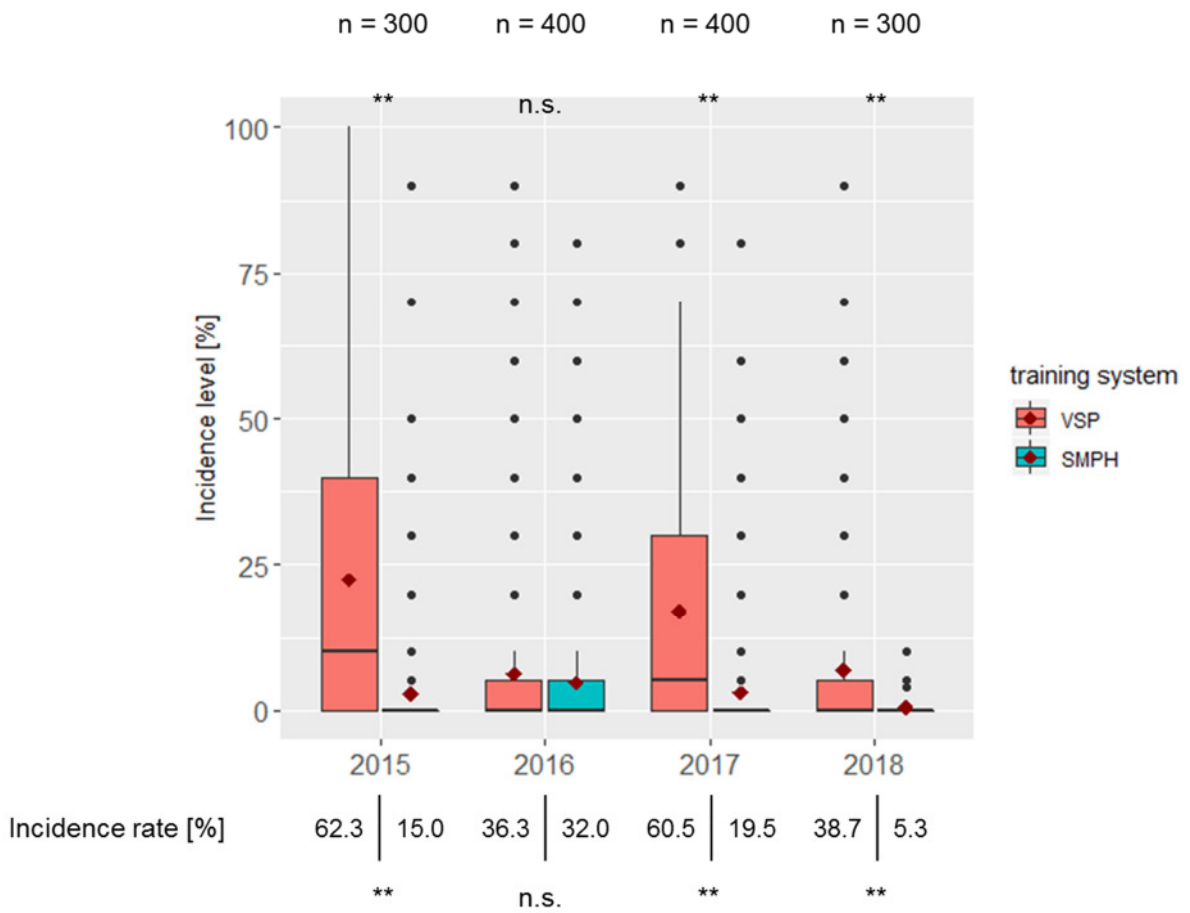

Fig. 4: Results of the Botrytis Bunch Rot assessments for VSP and SMPH trained vines made in the years 2015–2018. In 2015 and 2018 three vineyards were assessed. In 2016 and 2017 four vineyards. Incidence level is shown in the upper graph, Nemenyi post-hoc for Kruskal-Wallis test, * p<0.05; ** p<0.001. Incidence rate is shown in the lower table, Fisher´s exact test, * p<0.05; ** p<0.001. n = number of grapes per training system monitored.

In contrast to DM and PM, SMPH trained vines were less affected by BR infection than VSP trained vines, in 2015, 2017 and 2018 (Fig. 4). This is in line with the results of Emmett *et al.* (1995) and Ashley *et al.* (2005) who investigated the incidence of BR in bunches of minimally and intensively pruned grapevines. Both authors assumed that the reason for the different BR susceptibilities are the contrasting bunch architectures of the two training systems. VSP bunches carry many and large berries, forming a densely packed structure. This often leads to berry cracking and thus BR infection. SMPH bunches on the other hand display a loose structure due to fewer and smaller berries. As a result, the berries do not tend to crack and BR infection is reduced. Results of the BR assessment made in 2016 support the theory that bunch architecture influences the incidence of BR. In this year, severe DM infection caused a

reduction in the number of berries per bunch in both SMPH and VSP. Consequently, VSP as well as SMPH bunches displayed a loose structure and no significant differences in BR susceptibility between the training systems were noted.

Temporal development of the culturable, endophytic fungal community in healthy grapevine branches and occurrence of GTD-associated fungi.

The most outstanding feature of SMPH trained grapevines are the woody, perennial branches in the trellis. After conversion of VSP trained vines into SMPH, annual shoots from the pre-season remain in the trellis; they increase in size and lignify over the years. The occurrence of annual shoots in the trellis of VSP trained vines and perennial branches in the trellis of SMPH trained vines led to the question: How does the endophytic fungal community develop over time, i.e. from young shoots to old branches? Furthermore, it was questioned whether there are differences in community composition between the different ages of branches of the two training systems with special regard to grapevine trunk diseases (GTDs) associated fungi. Therefore, the endophytic fungal community in healthy grapevine branches of different age classes, between two months and eight years, was investigated to answer these questions.

The results of this study (manuscript II) showed that there is a group of fungi, which seems not be correlated with the branch age. This so called core community is especially characteristic for young branches (two months to one year) and consists of *Alternaria* spp., *Aureobasidium pullulans*, *Botrytis cinerea*, *Cladosporium* spp., *Diaporthe* spp., *Epicoccum nigrum*, *Penicillium* spp., and *Truncatella angustata*. With increasing age, this group of primary settlers is joined by another group of fungi, which we call late settlers. Besides harmless endophytes, such as the non-grapevine pathogenic *Neofabraea kienholzii* and *Paraphaeosphaeria neglecta*, the late settlers also comprise GTD-associated fungi, most strikingly *Cadophora luteo-olivacea*, *Diplodia seriata* and *Eutypa lata*. The fungi of the two

mentioned groups were highly abundant in the sampled branches. However, about half of the identified taxa in this study appeared rarely and were isolated only once or twice. This large group of uncommon endophytes is also known from other studies which analysed the fungal endophytic community in grapevine wood (Hofstetter *et al*., 2012; Bruez *et al*., 2016).

The majority of the isolated GTD-associated fungi are linked to the diseases Eutypa and Botryosphaeria dieback. For Esca, only *Phaeomoniella chlamydospora* could be isolated from five years old wood at a small rate. Other prominent pathogens associated with Esca, such as *Fomitiporia mediterranea* or *Phaeoacremonium* spp. could not be found in the branches. The rare occurrence or absence of these fungi is not surprising given that only apparently healthy grapevine wood was sampled. Esca associated fungi, however, dominate the endophytic community of necrotic or decaying wood tissue (Bruez *et al*., 2014, 2016).

In conclusion, grapevine wood becomes colonized very early in the shoot development by a high diversity of fungi, primarily by fast growing fungi, such as *Alternaria* spp., *A. pullulans*, *B. cinerea*, *Cladosporium spp*., *Diaporthe* spp., *E. nigrum*, *Penicillium* spp. and *T. angustata*, which are part of the core community and characteristic for branch ages up to one year. Later, in two years and older branches, late settlers join the endophytic community; among them are numerous GTD associated fungi, such as *C. luteo-olivacea*, *D. seriata* and *E. lata*. For SMPH trained vines, with their high amount of perennial branches, this means a higher threat for GTDs compared to VSP trained vines where branches are renewed every season. However, for minimal pruned vines it has been demonstrated that the incidence of Esca is lower, the expression of necrotic wood is reduced and the endophytic fungal community is associated with fewer virulent GTDs compared to intensive pruned vines (spur-pruned; Travadon *et al*., 2016). One explanation is that minimal pruning produces less and smaller wounds thus reducing the risk of infection by GTDs-associated fungi (Lecomte *et al*., 2012).

Impact of the grapevine training system on the incidence of Esca foliar symptoms

Vineyards subdivided into minimally and intensively pruned sections and located in three areas in Rhineland-Palatinate were chosen to investigate the impact of the training system on the external incidence of Esca (manuscript III). In addition to the training system also the year of planting, the cultivars as well as the year of pruning conversion differed between the vineyards. In each season from 2015 to 2018 a monitoring for vines expressing Esca related external symptoms, both chronic and apoplectic, took place every two weeks from calendar week 28 to 36.

Concerning the impact of the training system on the incidence of Esca the monitoring results were inconsistent over the four years. In the first year, 2015, the number of vines expressing foliar symptoms was nearly the same for both training systems and no significant difference was found. In the following year, 2016, SMPH trained vines were affected significantly more frequently than VSP trained vines. In contrast to 2016, in 2017 and 2018 VSP trained vines showed foliar symptoms more frequently than SMPH trained vines. As a consequence of these results it is not possible to clearly answer the question, which training system is more susceptible to Esca foliar symptoms. However, some influence of the training system on the incidence of external Esca symptoms may in fact exist. The conflicting monitoring results could be explained by the complexity of the diseases and the numerous influence factors, e.g. plant age, cultivar, plant physiological aspects, local climate, that probably interact with each other (Surico *et al.*, 2000; Marchi, 2001; Quaglia *et al.*, 2009; Bertsch *et al.*, 2012; Bruez *et al.*, 2013; Kuntzmann *et al.*, 2013; Fontaine *et al*., 2016; Gramaje *et al.*, 2018)

Esca monitoring in 13 German vineyards in 2009 revealed a higher incidence of external symptoms for VSP trained vines compared to MP trained vines (Becker, 2010). Furthermore, Travadon *et al*. (2016) found that spur-pruned vines showed external Esca symptoms and

internal trunk necrosis significantly more often than MP vines. Combining all data, VSP or intensively pruned vines are most likely more susceptible to external Esca symptoms than SMPH or minimally pruned vines, probably because of the increased number of pruning wounds, which goes along with a higher infection risk of Esca associated fungi (Lecomte *et al*., 2012, Travadon *et al*., 2016).

New species of *Phaeomoniellales* from a German vineyard and their potential threat to grapevine (*Vitis vinifera*) health

Phaeomoniella chlamydospora is a phytopathogenic fungus, which among others (e.g. *Fomitiporia* spp. and *Phaeoacremonium* spp.) is suspected to cause one of the most destructive grapevine trunk diseases, Esca (Crous & Gams, 2000; Bertsch *et al*., 2012; Fontaine *et al*., 2016; Gramaje *et al*., 2018). This species belongs to the recently established order *Phaeomoniellales,* comprising fungi that are associated with plants or lichens and have different trophic states, e.g. endophytic, saprotrophic or endolichenic (Nordén *et al*., 2005; Lee *et al*., 2006; Crous *et al*., 2008, 2009, 2011, 2015, 2016; Damm *et al*., 2010; Rossman *et al*., 2010; Peršoh & Rambold, 2012; Chen *et al*., 2015). A one-year survey of spore trapping in a German vineyard (variety ‘Riesling’, planted 2008) divided into SMPH and VSP trained vines was conducted to investigate the incidence of *P. chlamydospora* spores in the two different training systems (Manuscript IV). However, besides spores of *P. chlamydospora,* also spores from other species of the *Phaeomoniellales* were isolated from the traps. These comprised one known species, *Neophaeomoniella zymoides*, but also six unknown species, which were newly described here.

Neophaeomoniella zymoides was first described as an endophyte from pine needles by Lee and co-workers (2006). A few years later, this species was isolated from necrotic wood of *Prunus salicina* (Damm *et al*., 2010). Two of our newly described species, *N. constricta* and *N.*

ossiforme, display high genetic and morphologic similarities with *N. zymoides*, but also with *N. eucalypti* and *N. niveniae*. The two latter were isolated from stems of *Eucalyptus globulus* and leaves of *Niveniae stokoei*, respectively (Crous *et al*., 2011, 2015). Two other newly described species are *Aequabiliella palatina* and *Minutiella simplex*, both showing high affiliation to *A. effusa* and *M. tardicola*, respectively, which were also associated with symptomatic wood of *Prunus* trees, in this way similar to *N. zymoides* (Damm *et al*., 2010). The genus *Moristroma* previously consisted of the two species *Mo. quercinum* and *Mo. japonicum*, both identified based on their ascostroma found on oak wood (Nordén *et al*., 2005) and is now extended by the species *Mo. ampulliforme* and *Mo. germanicum.*

No clear conclusion about the incidence of *P. chlamydospora* spores in SMPH trained vines compared to VSP trained vines can be made due to the small number of trapped spores. However, the number of fungal strains and species of the *Phaeomoniellales* tend to be higher for spore traps attached to SMPH trained vines, which leads to the conclusion that this group of fungi appears around perennial branches of SMPH vines rather than on annual branches of VSP vines.

A pathogenicity test with greenhouse plants was done to investigate whether other species of the *Phaeomoniellales* besides *P. chlamydospora* can induce wood necrosis in grapevine. The results show that only *P. chlamydospora* is able to produce significant lesions in the wood and that other species of the *Phaeomoniellales* display no pathogenicity against grapevine. Additionally, since only *P. chlamydospra* could be isolated from grapevine wood it is assumed that grapevine is not the natural habitat for other species of the *Phaeomoniellales* (Casieri *et al*., 2009; González & Tello, 2011; Hofstetter *et al*., 2012; Pancher *et al*., 2012; Pinto *et al*., 2014; Bruez *et al*., 2014, 2016; Farr & Rossman, 2016; Travadon *et al*., 2016; Kraus *et al*., 2018). Recently, a fungus with full accordance to the *ITS* sequence of *M. simplex* was isolated from necrotic wood of *P. domestica* in the region of Baden-Württemberg, Germany,

indicating that *Prunus* trees or other woody plants could be natural hosts of this newly described species (Damm *et. al*, 2010; Bien *et al*., unpublished).

References

Andolfi, A., Mugnai, L., Luque, J., Surico, G., Cimmino, A. & Evidente, A. (2011) Phytotoxins produced by fungi associated with grapevine trunk diseases. Toxins 3, 1569–1605.

Aptroot, A., Lücking, R., Sipman, H.J.M., Umana, L. & Chaves, J.L. (2008) Pyrenocarpous lichens with bitunicate asci. Bibl. Lichenol. 97, 1–162.

Armijo, G., Schlechter, R., Agurto, M., Muñoz, D., Nuñez, C. & Arce-Johnson, P. (2016) Grapevine pathogenic microorganisms: Understanding infection strategies and host response scenarios. Front. Plant. Sci. 7, 1–18.

Arnold, A.E., Henk, D.A., Eells, R.L., Lutzoni, F. & Vilgalys, R. (2007) Diversity and phylogenetic affinities of foliar fungal endophytes in loblolly pine inferred by culturing and environmental PCR. Mycologia 99, 185–206.

Aroca, A., Gramaje, D., Armengol, J., García-Jiménez, J. & Raposo, R. (2010) Evaluation of the grapevine nursery propagation process as a source of *Phaeoacremonium* spp. and *Phaeomoniella chlamydospora* and occurrence of trunk disease pathogens in rootstock mother vines in Spain. Eur. J. Plant Pathol. 126, 165–174.

Arzanlou, M., Narmani, A., Moshari, S., Khodaei, S. & Babai-Ahari, A. (2013) *Truncatella angustata* associated with grapevine trunk disease in northern Iran. Arch. Phytopathol.Plant Protec. 46, 1168–1181.

Ashley, R., Clingeleffer, P., Emmett, R. & Dry, P. (2005) Effects of canopy and irrigation management on Shiraz production, quality and disease development in Sunraysia region. In: Finishing the job: Optimal ripening of Cabernet Sauvignon and Shiraz: Proceedings, ASVO Seminar, Mildura Arts Centre, Mildura, Victoria, Friday, 21 July 2006. Eds. D. Oag, K. DeGaris, S. Partridge, C. Dundon, M. Francis, R.S. Johnstone and R. Hamilton (Australian Society of Viticulture and Oenology: Adelaide) pp. 36–40.

Austin, C.N. (2010) Sunlight's influence on grapevine powdery mildew: direct effects on pathogen development and attendant consequences of canopy management and vineyard variability. PhD Thesis, Cornell University, Ithaca, NY.

Austin, C.N. & Wilcox, W.F. (2011) Effects of fruit-zone leaf removal, training system, and irrigation on the development of grapevine Powdery Mildew. Am. J. Enol. Vitic. 62, 193–198.

Austin, C.N., Meyers, J., Grove, G.G. & Wilcox, W.F. (2011) Powdery Mildew severity as a function of canopy density: Associated impacts on sunlight penetration and spray coverage. Am. J. Enol. Vitic. 62, 23–31.

Bates, D., Maechler, M., Bolker, B. & Walker, S. (2015) Fitting linear mixed-effects models using lme4. J. Stat. Softw. 67, 1.

Baumgartner, K., Fujiyoshi, P.T., Travadon, R., Castlebury, L.A., Wilcox, W.F. & Rolshausen, P.E. (2013) Characterization of species of *Diaporthe* from wood cankers of grape in eastern North American vineyards. Plant Dis. 97, 912–920.

Becker, A. (2010) Esca: Der Schnitt macht die Musik. Der Deutsche Weinbau 1, 14–16.

Becker, T. & Knoche, M. (2012a) Deposition, strain, and microcracking of the cuticle in developing 'Riesling' grape berries. Vitis. 51, 1–6.

Becker, T. & Knoche, M. (2012b) Water induces microcracks in the grape berry cuticle. Vitis. 51, 141–142.

Bellamy, D.E., Sisterson, M.S. & Walse, S.S. (2013) Quantifying host potentials: indexing postharvest fresh fruits for spotted wing drosophila, *Drosophila suzukii*. PLoS One 8, e61227.

Berg, G., Rybakova, D., Grube, M. & Köberl, M. (2016) The plant microbiome explored: implications for experimental botany. J. Exp. Bot. 67, 995–1002.

Bertsch, C., Ramírez-Suero, M., Magnin-Robert, M., Larignon, P., Chong, J., Abou-Mansour, E., Spagnolo, A., Clément, C. & Fontaine, F. (2012) Grapevine trunk diseases: complex and still poorly understood. Plant Pathol. 62, 243–265.

Bérubé, J.A. & Nicolas, G.G. (2015) Alien fungal species on asymptomatic live woody plant material imported into Canada. Can. J. Plant Pathol. 37, 67–81.

Blaeser, M. (1978) Untersuchungen zur Epidemiologie des Falschen Mehltaus an Weinrebe, *Plasmopara viticola* (Berk. et de Toni). PhD Thesis Universität Bonn Germany, 127 pp.

Blaeser, M. & Weltzien, H.C. (1978) Die Bedeutung von Sporangienbildung, -ausbreitung und -keimung für die Epidemiebildung von *Plasmopara viticola*. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 85, 155–161.

Blaeser M. & Weltzien, H.C. (1979) Epidemiological studies of *Plasmopara viticola* for improving determination of spraying dates. Zeitschrift für Pflanzenkrankheiten und Pflanzenschutz 86, 489–498.

Boonmee, S., Zhang, Y., Chomnunti, P., Chukeatirote, E., Tsui, C.K.M., Bahkali, A.H. & Hyde, K.D. (2011) Revision of lignicolous Tubeufiaceae based on morphological reexamination and phylogenetic analysis. Fungal Divers. 51, 63–102.

Booth, C. & Ting, T.P. (1964) *Dolabra nepheliae gen. nov. sp. nov.* associated with canker of *Nephelium lappaceum*. Trans. Br. Mycol. Soc. 47, 235–237.

Botella, L. & Diez, J.J. (2010) Phylogenic diversity of fungal endophytes in Spanish stands of *Pinus halepensis*. Fungal Divers. 47, 9–18.

Bowman, E.A. & Arnold, A.E. (2018) Distributions of ectomycorrhizal and foliar endophytic fungal communities associated with *Pinus ponderosa* along a spatially constrained elevation gradient. Am. J. Bot. 105, 687–699.

Bruez, E., Lecomte, P., Grosman, J., Doublet, B., Bertsch, C., Fontaine, F., Ugaglia, A., Teissedre, P.L., Da Costa, J.P., Guerin-Dubrana, L. & Rey, P. (2013) Overview of grapevine trunk diseases in France in the 2000s. Phytopathol. Mediterr. 52, 262–275.

Bruez, E., Vallance, J., Gerbore, J., Lecomte, P., Da Costa, J.-P., Guérin-Dubrana, L. & Rey, P. (2014) Analyses of the temporal dynamics of fungal communities colonizing the healthy wood tissues of Esca leaf-symptomatic and asymptomatic vines. PLoS One 9, e95928. doi:10.1371/journal.pone.0095928.

Bruez, E., Baumgartner, K., Bastien, S., Travadon, R., Guérin-Dubrana, L. & Rey, P. (2016) Various fungal communities colonise the functional wood tissues of old grapevines externally free from grapevine trunk disease symptoms. Aust. J. Grape Wine Res. 22, 288–295.

Burrack, H. J., Fernandez G.E., Spivey T. & Kraus D.A. (2013) Variation in selection and utilization of host crops in the field and laboratory by *Drosophila suzukii* Matsumara (Diptera: *Drosophilidae*), an invasive frugivore. Pest. Manag. Sci. 69, 1173–1180.

Campbell, C. (2006) The botanist and the vintner: How wine was saved for the world. Chapel Hill, NC: Algonquin Books. ISBN 978-1565125285.

Carbone, I. & Kohn, L.M. (1999) A method for designing pimer sets for speciation studies in filamentous ascomycetes. Mycologia 91, 553–558.

Carbonneau, A. (1991) Bilan de résultats d'analyse sensorielle de la qualité des vins de divers systèmes de conduite. Riv. Vitic. Enol. 4, 335–344.

Carroll, J.E. & Wilcox, W.F. (2003) Effects of humidity on the development of grapevine Powdery Mildew. Phytopathol. 93, 1137–1144.

Casieri, L., Hofstetter, V., Viret, O. & Gindro, K. (2009) Fungal communities living in the wood of different cultivars of young *Vitis vinifera* plants. Phytopathol. Mediterr. 48, 73–83.

Chen, K.-H., Miadlikowska, J., Molnár, K., Arnold, A.E., U'Ren, J.M., Gaya, E., Gueidan, C. & Lutzoni, F. (2015) Phylogenetic analyses of eurotiomycetous endophytes reveal their close affinities to *Chaetothyriales*, *Eurotiales*, and a new order – *Phaeomoniellales*. Mol. Phylogenet. Evol. 85, 117–130.

Choquer, M., Fournier, E., Kunz, C., Levis, C., Pradier, J.M., Simon, A. & Viaud, M. (2007) *Botrytis cinerea* virulence factors: new insights into a necrotrophic and polyphageous pathogen. FEMS Microbiol. Lett. 277, 1–10.

Cini, A., Ioriatti, C. & Anfora, G. (2012) A review of the invasion of *Drosophila suzukii* in Europe and a draft research agenda for integrated pest management. Bull. Insectology 65, 149–160.

Clingeleffer, P.R. (1984) Production and growth of minimal pruned Sultana vines. Vitis 23, 42–54.

Clingeleffer, P.R. & Possingham J.V. (1986) Minimal pruning - its role in canopy management and its adoption in Australian viticulture. In: Proceedings for the XIX Congreso Internacional de la Vina y el Vino, Santiago, Chile, pp. 615–631.

Clingeleffer, P.R. & Possingham, J.V. (1987) The role of minimal pruning of cordon trained vines (MPCT) in canopy management and its adoption in Australian viticulture. Aust. Grapegrow. Winemak. 280, 7–11.

Clingeleffer, P.R. (1988) Minimal pruning of Cordon trained vines, MPCT (in: Proceedings of the 2nd International Seminar on Mechanical Pruning of Vineyards, Villanova di Motta di Livenza, Treviso, pp. 112–120.

Clingeleffer, P.R. (1993) Development of management systems for low cost, high quality wine production and vigour control in cool climate Australian vineyards. Aust. Grapegrow. Winemak. 358, 43–48.

Clingeleffer, P.R. (2000) Mechanization of wine and raisin production in Australian vineyards. In: Proceedings for the ASEV 50th Anniversary Annual Meeting, Seattle Washington, U.S.A. American Society for Enology and Viticulture. pp.165–169

Clingeleffer, P.R., Petrie, P.R. & Ashley, R.M. (2005) Suitability of minimal pruning and other low-input systems for warm and cool climate grape production. In: Proceedings for the 14th GESCO Meeting, Geisenheim, Germany, pp. Vol. 1: pp. 2–9.

Clingeleffer, P.R. (2013) Mechanization in Australian vineyards. Acta Hort. Leuven. 978, 169–177.

Cloete, M., Fischer, M., Mostert, L. & Halleen, F. (2014). A novel *Fomitiporia* species associated with esca on grapevine in South Africa. Mycol. Progr. 13, 303–311.

Collado, I.G., Aleu, J., Hernandez-Galan, R. & Duran-Patron, R. (2000) *Botrytis* species: an intriguing source of metabolites with wide ranges of biological activities. Structure, chemistry and bioactivity of metabolites isolated from *Botrytis* species. Curr. Organic Chem. 4, 1261–1286.

Commenil, P., Brunet, L. & Audran, J.-C. (1997) The development of the grape berry cuticle in relation to susceptibility to bunch rot disease. J. Exp. Bot. 48, 1599–1607.

Coombe, B.G. & Dry, P.R. (1992) Viticulture, vol. 2, Practices, Aust. Indust. Publ. PTY LTD, Adelaide, 232–246.

Crespy, A. (2008) Practical handbook of grapevine pruning. Oenoplurimedia, Chateau du Chaintré, pp. 43–46.

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) *Phaeomoniella chlamydospora gen. et comb. nov*., a causal organism of Petri grapevine decline and esca. Phytopathol. Mediterr. 39, 112–118.

Crous, P.W., Wood, A.R., Okada, G. & Groenewald, J.Z. (2008) Foliicolous microfungi occurring on *Encephalartos*. Persoonia 21, 135–146.

Crous, P.W., Verkley, G.J.M. & Groenewald, J.Z. (2009) *Xenocylindrosporium kirstenboschense*. Persoonia 23, 200–201.

Crous, P.W., Summerell, B.A., Shivas, R.G., Romberg, M., Mel´nik, V.A., Verkley, G.J.M. & Groenewald, J.Z. (2011) Fungal planet description sheets: 92–106. Persoonia 27, 130–162.

Crous *et al*. (2015) Fungal planet description sheets: 320–370. Persoonia 34, 167–266.

Crous *et al*. (2016) Fungal planet description sheets: 469–557 *Persoonia* 37, 218–403.

Damm, U., Fourie, P.H. & Crous, P.W. (2010) *Coniochaeta* (*Lecythophora*), *Collophora gen. nov.* and *Phaeomoniella* species associated with wood necroses of *Prunus* trees. Persoonia 24, 60–80.

Deepayan, S. (2008) Lattice: Multivariate data visualization with R. Springer, New York.

Delp, C.J. (1954) Effects of temperature and humidity on the grape Powdery Mildew fungus. Phytopathology 44, 615–626.

Diaz, G.A. & Latorre, B.A. (2013) Efficacy of paste and liquid fungicide formulations to protect pruning wounds against pathogens associated with grapevine trunk diseases in Chile. Crop Prot. 46, 106–112.

Diepenbrock, L.M. & Burrack, H.J. (2017) Variation of within-crop microhabitat use by *Drosophila suzukii* (Diptera: *Drosophilidae*) in blackberry. J. Appl. Entomol. 141, 1–7.

Droby, A. & Lichter, A. (2004) Post-harvest *Botrytis* infection: etiology, development and management. In: Botrytis: Biology, pathology and control. (Elad, Y., Williamson, B., Tudzynski, P. and Delen, N., eds), pp. 349–367. Dordrecht, Netherlands: Kluwer Academic Press.

Emmett, R.W., Clingeleffer, P.R., Wicks, T.J., Nair, N.G., Hall, B., Hart, K.M., Clarke, K. & Somers, T. (1995) Influence of canopy architecture on disease development. In 'Canopy Management: Proceedings of a seminar organized by the Australian society of viticulture and oenology, Inc. and held on 18. August, 1994 at Mildura, Victoria', pp. 22–23. Eds. P. Hayes, Australian society of viticulture and oenology, Adelaide.

English, J.T., Kaps, M.L., Moore, J.F., Hill, J. & Nakova, M. (1993) Leaf removal for control of *Botrytis* bunch rot of wine grapes in the Midwest United States. Plant Dis. 77, 1224–1227.

Eskalen, A. & Gubler, W.D. (2001) Association of spores of *Phaeomoniella chlamydospora, Phaeoacremonium inflatipes* and *Pm. aleophilum* with grapevine cordons in California. Phytopathol. Mediterr. 40: S429–S432.

EUROSTAT EC. (2007) The use of plant protection products in the European Union. Data 1992-2003. Luxembourg: Office for Official Publications of the European Communities. ISBN 92-79-03890-7.

FAO (Food and Agriculture Organization of the United Nations). (2018) FAOSTAT, http://www.fao.org/faostat/en/#data/QC (accessed 4th December 2018).

Farlow, W.G. (1876) The American grape mildew. Busy Institute Bulletin 1, 423.

Farr, D.F. & Rossman, A.Y. (2016) Fungal Databases, U.S. National Fungus Collections, ARS, USDA. https://nt.ars-grin.gov/fungaldatabases/ (accessed 4th December 2018).

Fessler, C. & Kassemeyer, H.H. (1995) The influence of temperature during the development of conidia on the germination of *Uncinula necator*. Vitis 34, 63–64.

Fischer, M. (2002) A new wood-decaying basidiomycete species associated with esca of grapevine: *Fomitiporia mediterranea* (*Hymenochaetales*). Mycol. Progr. 1, 315–324.

Fischer, M. & Kassemeyer, H.H. (2003) Fungi associated with esca disease of grapevine in Germany. Vitis 42, 109–116.

Fischer, M. (2006) Esca der Weinrebe – eine Krankheit auf dem Vormarsch? Deutsches Weinbaujahrbuch 57, 87–94.

Fontaine, F., Gramaje, D., Armengol, J., Smart, R., Nagy, Z.A., Borgo, M., Rego, C. & Corio-Costet, M.-F. (2016) Grapevine trunk diseases. A review. OIV Publications, Paris. http://www.oiv.int/public/medias/4650/trunk-diseases-oiv-2016.pdf (accessed 4th December 2018).

Fraga, H., Garcia de Cortazar-Atauri, I., Malheiro, A.C. & Santos J.A. (2016) Modelling climate change impacts on viticultural yield, phenology and stress conditions in Europe. Glob. Change Biol. 22, 3774–3788.

Gabler, F.M., Smilanick, J.L., Mansour, M., Ramming, D.W. & Mackey, B.E. (2003) Correlations of morphological, anatomical, and chemical features of grape berries with resistance to *Botrytis cinerea*. Phytopathology 93, 1263–1273.

Gadoury, D.M., Cadle-Davidson, L., Wilcox, W. F., Dry, I. B., Seem, R.C. & Milgroom, M.G. (2012) Grapevine Powdery Mildew (*Erysiphe necator*): a fascinating system for the study of the biology, ecology and epidemiology of an obligate biotroph. Mol. Plant. Pathol. 13, 1–16.

Gessler, C., Pertot, I. & Perazzolli, M. (2011) *Plasmopara viticola*: a review of knowledge on Downy Mildew of grapevine and effective disease management. Phytopathol. Mediterr. 50, 3–44.

Glass, L.N. & Donaldson, G.C. (1995) Development of primer sets designed for use with the PCR to amplify conserved genes from filamentous Ascomycetes. Appl. Environ. Microb. 61, 1323–1330.

González, V. & Tello, M.L. (2011) The endophytic mycota associated with *Vitis vinifera* in central Spain. Fungal Divers. 47, 29–42.

Gramaje, D., Mostert, L. & Armengol, J. (2011) Characterization of *Cadophora luteo-olivacea* and *C. melinii* isolates obtained from grapevines and environmental samples from grapevine nurseries in Spain. Phytopathol. Mediterr. 50, S112–S126.

Gramaje, D., Úrbez-Torres, J.R. & Sosnowski, M.R. (2018) Managing grapevine trunk diseases with respect to etiology and epidemiology: Current strategies and future prospects. Plant Dis. 102, 12–39.

Gu, S., Cochran, R.C., Du, G., Hakim, A., Fugelsang, K.C., Ledbetter, J., Ingles, C.A. & Verdegaal, P.S. (2005) Effect of training-pruning regimes on *Eutypa dieback* and performance of 'Cabernet Sauvignon' grapevines. J. Hort. Sci. Biotechnol. 80, 313–318.

Gubler, W.D., Marois, J.J., Bledsoe, A.M. & Bettiga, L.J. (1987) Control of Botrytis Bunch Rot of grape with canopy management. Plant Dis. 71, 599–601.

Gueidan, C., Aptroot, A., Cáceres, M.E.S., Badali, H. & Stenroos, S. (2014) A reappraisal of orders and families within the subclass *Chaetothyriomycetidae* (*Eurotiomycetes, Ascomycota*). Mycol. Prog. 13, 1027–1039.

Halleen, F. & Holz, G. (2001) An overview of the biology, epidemiology and control of *Uncinula necator* (Powdery Mildew) on grapevine, with reference to South Africa. S. Afr. J. Enol. Vitic. 22, 111–121.

Halleen, F. & Fourie, P.H. (2016) An integrated strategy for the proactive management of grapevine trunk disease pathogen infections in grapevine nurseries. S. Afr. J. Enol. Vitic. 37, 104–114.

Hauser, M., Gaimari, S. & Damus, M. (2009) *Drosophila suzukii* new to North America. Fly Times 43, 12–15.

Herzog, K., Wind, R. & Töpfer, R. (2015) Impedance of the grape berry cuticle as a novel phenotypic trait to estimate resistance to *Botrytis cinerea*. Sensors 15, 12498–12512.

Hill, G. (1989) Neues zur Biologie der Rebenperonospora. Der Deutsche Weinbau. 44, 471–474.

Hirst, J.M. (1952) An automatic volumetric spore trap. Ann. Appl. Biol. 39, 257–265.

Hoffman, M.T. & Arnold, A.E. (2010) Diverse bacteria inhabit living hyphae of phylogenetically diverse fungal endophytes. App. Environ. Microbiol. 76, 4063–4075.

Hofstetter, V., Buyck, B., Croll, D., Viret, O., Couloux, A. & Gindro, G. (2012) What if esca disease of grapevine were not a fungal disease? Fungal Divers. 54, 51–67.

Hothorn, T., Bretz, F. & Westfall, P. (2008) Simultaneous inference in general parametric models. Biom. J. 50, 346–363.

Hauser, M., Gaimari, S. & Damus, M. (2009). *Drosophila suzukii* new to North America. Fly Times 43, 12–15.

Intrieri, C., Poni, S., Lia, G. & Del Campo, M.G. (2001) Vine performance and leaf physiology of conventionally and minimally pruned Sangiovese grapevines. Vitis, 40, 123–130.

Intrieri, C., Filippetti, I., Allegro, G., Valentini, G., Pastore, C. & Colucci, E. (2011) The semi-minimal-pruned hedge: A novel mechanized grapevine training system. Am. J. Enol. Vitic. 62, 312–318.

Intrieri, C. & Filippetti (2012) A new grape training system to suit machine pruning. Aust. N.Z. Grapegrow. Winemak. 580, 41–48.

Intrieri, C. (2013) Research and innovation for full mechanization of Italian vineyards at Bologna University Acta Hort. Leuven. 978, 151–168.

Ioriatti, C., Walton, V., Dalton, D., Anfora, G., Grassi, A., Maistri, S. & Mazzoni, V. (2015). *Drosophila suzukii* (Diptera: *Drosophilidae*) and its potential impact to wine grapes during harvest in two cool climate wine grape production regions. J. Econ. Entomol. 108, 1148–1155.

Jackson, D. (1996) Pruning and Training. Monographs in cool climate viticulture, pp. 37–41. Wellington, N.Z., Daphne Brasell Associates Ltd in association with Lincoln University Press.

Jarausch, B., Müller, T., Gramm, T. & Hoffmann C. (2017) Comparative evaluation of insecticide efficacy tests against *Drosophila suzukii* on grape berries in laboratory, semi-field and field trials. Vitis, 56, 133–140.

Jarvis, W.R. (1962a) The dispersal of spores of *Botrytis cinerea* Fr. in a raspberry plantation. Trans. Br. Mycol. Soc. 45, 549–559.

Jarvis, W.R. (1962b) Splash dispersal of spores of *Botrytis cinerea* Pers. Nature (London), 193, 599.

Jarvis, W.R. (1980a) The Biology of *Botrytis* (Coley-Smith, J.R., Verhoeff, K. and Jarvis, W.R., eds) – Epidemiology – 2 Dispersal, pp. 222–228. London: Academic Press.

Jarvis, W.R. (1980b) The Biology of *Botrytis* (Coley-Smith, J.R., Verhoeff, K. and Jarvis, W.R., eds) – Epidemiology – 4 The environment and infection, pp. 229–238. London: Academic Press.

Jarvis, W.R. (1980c) The Biology of *Botrytis* (Coley-Smith, J.R., Verhoeff, K. and Jarvis, W.R., eds) – *Botrytis cinerea* in enology – 2 Parasitism of *Botrytis cinerea* on grapes, pp. 252–259. London: Academic Press.

John, S., Wicks, T.J., Hunt, J.S., Lorimer, M.F., Oakey, H. & Scott, E.S. (2005) Protection of grapevine pruning wounds from infection by *Eutypa lata* using *Trichoderma harzianum* and *Fusarium lateritium*. Australas. Plant Pathol. 34, 569–575.

John, S., Wicks, T.J., Hunt, J.S. & Scott, E.S. (2008) Colonisation of grapevine wood by *Trichoderma harzianum* and *Eutypa lata*. Aust. J. Grape Wine Res. 14, 18–24.

Johnston, P.R., Johansen, R.B., Williams, A.F., Paula Wikie, J. & Park, D. (2012) Patterns of fungal diversity in New Zealand *Nothofagus* forests. Fungal Biol. 116, 401–412.

Keith, L.M., Matsumoto, T.K. & McQuate, G.T. (2013) First report of *Dolabra nepheliae* associated with corky bark disease of langsat in Hawaii. Plant Dis. 97, 990.

Kennelly, M.M., Gadoury, D.M., Wilcox, W.F., Magarey, P.A. & Seem, R.C. (2005) Seasonal development of ontogenic resistance to Downy Mildew in grape berries and rachises. Phytopathol. 95, 1445–1452.

Kernaghan, G., Mayerhofer, M. & Griffin, A. (2017) Fungal endophytes of wild and hybrid *Vitis* leaves and their potential for vineyard biocontrol. Can. J. Microbiol. 63, 583–595.

Kiefer, B., Riemann, M., Büche, C., Kassemeyer, H.H. & Nick, P. (2002) The host guides morphogenesis and stomatal targeting in the grapevine pathogen *Plasmopara viticola*. Planta. 215, 387–393.

Kirk, P.M. (2015) Phaeomoniellaceae. Nomenclatural novelties Index Fungorum 265, 1–1.

Kraus, C., Pennington, T., Herzog, K., Hecht, A., Fischer, M., Voegele, R.T., Hoffmann C., Töpfer, R. & Kicherer A. (2018) Effects of canopy architecture and microclimate on grapevine health in two training systems. Vitis 57, 53–60.

Kraus, C., Voegele, R.T. & Fischer, M. (2018) Temporal Development of the culturable, endophytic fungal community in healthy grapevine branches and occurrence of GTD-associated fungi. Microb. Ecol. DOI: https://doi.org/10.1007/s00248-018-1280–3. (accessed 4th December 2018).

Kuntzmann, P., Villaume, S., Larignon, P. & Bertsch, C. (2010) Esca, BDA, Eutypiosis: foliar symptoms, trunk lesions and fungi observed in diseased vinestocks in two vineyards in Alsace. Vitis 49, 71–76.

Kuntzmann, P., Barbe, J., Maumy-Bertrand, M. & Bertrand, F. (2013) Late harvest as factor affecting esca and Botryosphaeria dieback prevalence of vineyards in the Alsace region of France. Vitis 52, 197–204.

Kumar, S., Stecher, G. & Tamura, K. (2015) MEGA7: molecular evolutionary genetics analysis version 70 for bigger datasets. Mol. Biol. Evol. 33, 1870–1874.

Langcake, P. & Lovell, P.A. (1980) Light and electron microscopical studies of the infection of *Vitis* spp. by *Plasmopara viticola*, the Downy Mildew pathogen. Vitis 19, 321–337.

Larignon, P. & Dubos, B. (2000) Preliminary studies on the biology of *Phaeoacremonium*. Phytopathol. Mediterr. 39, 184–189.

Larkin, B.G., Hunt, L.S. & Ramsey, P.W. (2012) Foliar nutrients shape fungal endophyte communities in Western white pine (*Pinus monticola*) with implications for white-tailed deer herbivory. Fungal Ecol. 5, 252–260.

Lawrence, D.P., Travadon, R. & Baumgartner, K. (2015) Diversity of *Diaporthe* species associated with wood cankers of fruit and nut crops in northern California. Mycologia 107, 926–940.

Lawrence, D.P., Travadon, R. & Travadon, R. (2018) Novel *Seimatosporium* species from grapevine in northern California and their interactions with fungal pathogens involved in the trunk-disease complex. Plant Dis. 102, 1081–1092.

Lecomte, P., Darrieutort, G., Liminana, J.M., Comont, G., Muruamendiaraz, A., Legorburu, F.J., Choueiri, E., Jreijiri, F., El-Amil, R. & Fermaud, M. (2012) New insights into esca of grapevine: the development of foliar symptoms and their association with xylem discoloration. Plant Dis. 96, 924–934.

Lee, H.B., Park, J.Y., Jung, H.S. & Summerbell, R.C. (2006) *Phaeomoniella zymoides* and *Phaeomoniella pinifoliorum* spp. *nov*., new acid-tolerant epiphytic fungi isolated from pine needles in Korea. Mycologia 98, 598–611.

Lee, J.C., Bruck, D.J., Curry, H., Edwards, D.L., Haviland, D.R., Van Steenwyk, R. & Yorgey, B. (2011) The susceptibility of small fruits and cherries to the spotted wing drosophila, *Drosophila suzukii*. Pest. Manag. Sci. 67, 1358–1367.

Letaief, H., Rolle, L., Zeppa, G. & Gerbi, V. (2008) Assessment of grape skin hardness by a puncture test. J. Sci. Food Agric. 88, 1567–1575.

Lorenz, D.H., Eichhorn, K.W., Bleiholder, H., Klose, R., Meier, U. & Weber, E. (1995) Growth stages of the grapevine: Phenological growth stages of the grapevine (*Vitis vinifera L.* ssp. *vinifera*) - Codes and descriptions according to the extended BBCH scale. Austral. J. Grape Wine Res. 1, 100–103.

Luque, J., Garcia-Figueres, F., Legorburu, F.J., Muruamendiaraz, A., Armengol, J. & Trouillas, F.P. (2012) Species of *Diatrypaceae* associated with grapevine trunk diseases in Eastern Spain. Phytopathol. Mediterr. 51, 528–540.

Maharachchikumbura, S.S.N., Larignon, P., Hyde, K.D., Al-Sadi, A.M. & Liu, Z.Y. (2016) Characterization of *Neopestalotiopsis*, *Pestalotiopsis* and *Truncatella* species associated with grapevine trunk diseases in France. Phytopathol. Mediterr. 55, 380–390.

Maiguashca, F., Ferguson, H., Bahder, B., Brooks, T., O'Neal, S. & Walsh, D. (2010) SWD ovipositing on grapes in laboratory; partial maggot survival inconclusive. Washington State University Extension, Spotted Wing Drosophila Grape Update, 28 August. http://ipm.wsu.edu/small/pdf/NoChoiceSWDonGrapesAug28.pdf (accessed 4th December 2018).

Marchi, G. (2001) Susceptibility to esca of various grapevine ("*Vitis vinifera*") cultivars grafted on different rootstocks in a vineyard in the province of Siena (Italy). Phytopathol. Mediterr. 40, 27–36.

Marchi, G., Peduto, F., Mugnai, L., Di Marco, S., Calzarano, F. & Surico, G. (2006) Some observations on the relationship of manifest and hidden esca to rainfall. Phytopathol. Mediterr. 45 (Supplement), S117–S126.

Marois, J.J., Nelson, J.K., Morrison, J.C., Lile, L.S. & Bledsoe, A.M. (1986) The influence of berry contact within grape clusters on the development of *Botrytis cinerea* and epicuticular wax. Am. J. Enol. Viric. 37, 293–296.

Martínez de Toda, F. & Sancha J.C. (1998) Long-term effects of zero pruning on Grenache vines under drought conditions. Vitis 37, 155–157.

May, P. & Clingeleffer, P.R. (1977) Mechanical pruning of grape vines. Australian wine, brewing and spirit review 96, 36–38.

Mend, M. (2013) Erziehungssysteme – ein Kostenvergleich. Der Deutsche Weinbau. 24, 12–15.

de Mendiburu, F. (2017) agricolae: Statistical Procedures for Agricultural Research. R package version 1.2-8. https://CRAN.R-project.org/package=agricolae (accessed 4th December 2018).

Mostert, L., Abeln, E.C.A., Halleen, F. & Crous, P.W. (2006) Genetic diversity among isolates of *Phaeomoniella chlamydospora* on grapevines. Aust. Plant Pathol. 32, 47–52.

Moyo, P., Allsopp, E., Roets, F., Mostert, L. & Halleen, F. (2014) Arthropods vector grapevine trunk disease pathogens. Phytopathology 104, 1063–1069.

Moyo, P., Mostert, L., Spies, C., Damm, U. & Halleen, F. (2018) Diversity of *Diatrypaceae* species associated with dieback of grapevines in South Africa, with the description of *Eutypa cremea sp. nov*. Plant Dis. 102, 220–230.

Mondello, V., Lo Piccolo, S., Conigliaro, G., Alfonzo, A., Torta, L. & Burruano, S. (2013) First report of *Neofusiccoccum vitifusiforme* and presence of other *Botryosphaeriaceae* species associated with Botryosphaeria dieback of grapevine in Sicily (Italy). Phytopathol. Mediterr. 52, 388–396.

Mugnai, 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.

Müller, M.M., Henttonen, H.M., Penttilä, R., Kulju, M., Helo, T. & Kaitera, J. (2018) Distribution of *Heterobasidion* butt rot in northern Finland. For. Ecol. Manage. 425, 85–91.

Munkvold, G.P. & Marois, J.J. (1993) Efficacy of natural epiphytes and colonizers of grapevine pruning wounds for biological control of Eutypa dieback. Phytopathology 83, 624–629.

Murolo, S. & Romanazzi, G. (2014) Effects of grapevine cultivar, rootstock and clone on esca disease. Australas. Plant Pathol. 43, 215–221.

Mutawila, C., Halleen, F. & Mostert, L. (2015) Development of benzimidazole resistant *Trichoderma* strains for the integration of chemical and biocontrol methods of grapevine pruning wound protection. Biocontrol 60, 387–399.

Nelson, R.E. (1951) Factors influencing the infection of table Grapes by *Botrytis cinerea*. Phytopathology 41, 319–326.

Nigro, F., Boscia, D., Antelmi, I. & Ippolito, A. (2013) Fungal species associated with a severe decline of olive in Southern Italy. J. Plant Pathol. 95, 659–668.

Nirenberg, H. (1976) Untersuchungen über die morphologische und biologische Differenzierung in der *Fusarium*-Sektion Liseola. Mitteilungen aus der Biologischen Bundesanstalt für Land- und Forstwirtschaft Berlin-Dahlem 169, 1–117.

Nordén, B., Sunhede, S. & Larsson, E. (2005) New species of *Moristroma* (*Ascomycetes*) and phylogenetic position of the genus. Mycol. Prog. 4, 325–332.

O´Donnell, K. (1993) *Fusarium* and its near relatives, pp. 225–233. In: Reynolds, D.R. and Taylor, J.W. (eds) The fungal holomorph: mitotic, meiotic and pleomorphic speciation in fungal systematics, CAB International Wallingford.

Oksanen, J., Blanchet, F.G., Friendly, M., Kindt, R., Legendre, P., McGlinn, D., Minchin, P.R., O´Hara, R.B., Simpson, G.L., Solymos, P., Stevens, M.H.H., Szoecs, E. & Wagner H. (2018) Vegan: Community ecology package. R package version 2.5-1. https://CRAN.R-project.org/package=vegan (accessed 4th December 2018).

Ollat, N., Sommer, K.J., Pool, R.M. & Clingeleffer, P.R. (1993) Quelques résultats sur la taille minimale en Australie et en France. In: Proceedings for the 6th GESCO Meeting, Reims, France, pp. 181–183.

Pancher, M., Ceol, M., Corneo, P.E., Longa, C.M.O., Yousaf, S., Pertot, I. & Campisano, A. (2012) Fungal endophytic communities in grapevines (*Vitis vinifera L.*) respond to crop management. Appl. Environ. Microbiol. 78, 4308–4317.

Peršoh, D. & Rambold, G. (2012) Lichen-associated fungi of the *Letharietum vulpinae*. Mycol. Prog. 11, 753–760.

Pertot, I., Prodorutti, D., Colombini, A. & Pasini, L. (2016) *Trichoderma atroviride* SC1 prevents *Phaeomoniella chlamydospora* and *Phaeoacremonium aleophilum* infection of grapevine plants during the grafting process in nurseries. BioControl 61, 257–267.

Pertot, I., Caffi, T., Rossi, V., Mugnai, L., Hoffmann, C., Grando, M.S., Gary, C., Lafond, D., Duso, C., Thiery, D., Mazzoni, V. & Anfora, G. (2017) A critical review of plant

protection tools for reducing pesticide use on grapevine and new perspectives for the implementation of IPM in viticulture. Crop Prot. 97, 70–84.

Pinto, C., Pinho, D., Sousa, S., Pinheiro, M., Egas, C. & Gomes, A.C. (2014) Unravelling the diversity of grapevine microbiome. PLoS One 9, e85622. doi:10.1371/journal.pone.0085622.

Pinto, C. & Gomes, A.C. (2016). *Vitis vinifera* microbiome: from basic research to technological development. BioControl 61, 243–256.

Pollock, J.G., Shepardson, E.S., Shaulis, N.J. & Crowe, D.E. (1977) Mechanical pruning of American hybrid grapevines. Trans. Am. Soc. Agric. Eng. 20, 817–821.

Pool, R.M. (1995) Predicting and controlling crops of machine and minimal pruned grapevines. In: Proceedings for the International ATW-Symposium: „Technik im Weinbau“. Kuratorium für Technik und Bauwesen in der Landwirtschaft e.V. (KTBL), Darmstadt, Germany, 11–22.

Pouzoulet, J., Pivovaroff, A.L., Santiago, L.S. & Rolshausen, P.E. (2014) Can vessel dimension explain tolerance toward fungal vascular wilt diseases in woody plants? Lessons from Dutch elm disease and esca disease in grapevine. Front. Plant Sci. 5, art. 253.

Pouzoulet, J., Scudiero, E., Schiavon, M. & Rolshausen, P.E. (2017) Xylem vessel diameter affects the compartmentalization of the vascular pathogen *Phaeomoniella chlamydospora* in grapevine. Front. Plant Sci. 8, art. 1442.

Poyet, M., Le Roux, V., Gibert, P., Meirland, A., Prévost, G., Eslin, P. & Chabrerie, O. (2015) The wide potential trophic niche of the asiatic fruit fly *Drosophila suzukii*: The key of its invasion success in temperate Europe? PLoS One 10, e0142785.

Quaglia, M., Covarelli, L. & Zazzerini, A. (2009) Epidemiological survey on esca disease in Umbria, central Italy. Phytopathol. Mediterr. 48, 84–91.

R Core Team (2013). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL http://www.R-project.org/ (accessed 4th December 2018)

R Studio Team (2016) RStudio: Integrated development for R. RStudio Inc Boston MA URL http://www.rstudio.com/ (accessed 4th December 2018).

Rayner, R.W. (1970) A mycological colour chart. Commonwealth Mycological Institute Kew UK.

Romanazzi, G., Murolo, S., Pizzichini, L. & Nardi, S. (2009). Esca in young and mature vineyards, and molecular diagnosis of the associated fungi. Eur. J. Plant. Pathol. 125, 277–290.

Romero, A.I. & Samuels, G.J. (1991) Studies on xylophilous fungi from Argentina: VI. *Ascomycotina* on *Eucalyptus viminalis* (*Myrtaceae*). Sydowia 43, 228–248.

Rossman, A.Y., Schoch, C.L., Farr, D.F., Nishijima, N., Keith, L. & Coenaga, R. (2010) *Dolabra nepheliae* on rambutan and lychee represents a novel lineage of phytopathogenic *Eurotiomycetes*. Mycoscience 51, 300–309.

Rouzes, R., Delbac, L., Ravidat, M.L. & Thiéry, D. (2012) First occurrence of *Drosophila suzukii* in the Sauternes vineyards. OENO One 46, 145–147.

Saguez, J., Lasnier, J., & Vincent C. (2013) First record of *Drosophila suzukii* in Quebec vineyards. OENO One 47, 69–72.

Sánchez Márquez, S., Bills, G.F. & Zabalgogeazcoa, I. (2011) Fungal species diversity in juvenile and adult leaves of *Eucalyptus globulus* from plantations affected by *Mycosphaerella* leaf disease. Ann. Appl. Biol. 158: 177–187.

Schmid, J. & Schultz, H.R. (2000) Influence of two training systems and irrigation on water consumption of grapevines in the field. Acta Hortic. 537, 587–595.

Schultz, H.R., Kraml, S. & Werwitzke, U. (1999) Distribution of glycosides, including flavour precursors in berries from Minimally Pruned (MP) and Vertical Shoot Positioned grapevines (VSP). Gesco: Groupe d' Etude des Systèmes de Conduite de la Vigne = Study group for vine training systems: Marsala, Italy, 6.6.-12.6.2000, pp. 271–280.

Senanayake, I.C., Maharachchikumbura, S.S.N., Hyde, K.D., Bhat, J.D., Jones, E.B.G., McKenzie, E.H.C., Dai, D.Q., Dagaranagama, D.A., Dayarathne, M.C., Goonasekara, I.D., Konta, S., Li, W.J., Shang, Q.J., Stadler, M., Wijayawardene, N.N., Xiao, Y.P., Norphanphoun, C., Li, Q., Liu, X.Z., Bahkali, A.H., Kang, J.C., Wang, Y., Wen, T.C., Wendt, L., Xu, J.C. & Camporesi, E. (2015) Towards unraveling relationships in *Xylariomycetidae* (*Sordariomycetes*). Fungal Divers. 73, 73–144.

Serra, S., Mannoni, M.A. & Ligios, V. (2008) Studies on the susceptibility of pruning wounds to infection by fungi involved in grapevine wood diseases in Italy. Phytopathol. Mediterr. 47, 234–246.

Siegfried, W. & Sacchelli, M. (2005) Blattflächenbezogene Dosierung von Pflanzenschutzmitteln im Rebanbau. Schweizer Zeitschrift für Obst- und Weinbau, 4, 13–16.

Sivanesan, A., Rajak, R.C. & Gupta, R.C. (1988) *Thaxterina,* a new tubeufiaceous genus with multispored asci from India. Trans. Br. Mycol. Soc. 90, 662–665.

Sommer, K.J., Clingeleffer, P.R. & Ollat, N. (1993) Effects of minimal pruning on grapevine canopy development, physiology and cropping level in both cool and warm climates. Vitic. Enol. Sci. 48, 135–139.

Sommer, K.J. (1995) Mechanized pruning in Australia. In: Proceedings for the International ATW-Symposium: „Technik im Weinbau“. Kuratorium für Technik und Bauwesen in der Landwirtschaft e.V. (KTBL), Darmstadt, Germany, 23–50.

Sosnowski, M.R., Creaser, M.L., Wicks, T.J., Lardner, R. & Scott, E.S. (2008) Protection of grapevine pruning wounds from infection by *Eutypa lata*. Aust. J. Grape Wine Res. 14, 134–142.

Sosnowski, M.R., Loschiavo, A.P. & Wicks, T.J. (2013) Evaluating treatments and spray application for the protection of grapevine pruning wounds from infection by *Eutypa lata*. Plant Dis. 97, 1599–1604.

Steel, C.C., Blackman, J.W. & Schmidtke, L.M. (2013) Grapevine Bunch Rots: Impacts on wine composition, quality, and potential procedures for the removal of wine faults. J. Agric. Food Chem. 61, 5189–5206.

Surico, G., Marchi, G., Braccini, P. & Mugnai, L. (2000) Epidemiology of esca in some vineyards in Tuscany (Italy). Phytopathol. Mediterr. 39, 190–205.

Surico, G., Mugnai, L. & Marchi, G. (2006) Older and more recent observations on esca: a critical review. Phytopathol. Mediterr. 45, S68–S86.

Surico, G. (2009) Towards a redefinition of the diseases within the esca complex of grapevine. Phytopathol. Mediterr. 48, 5–10.

Taylor, D.L. & Houston, S. (2011) A bioinformatics pipeline for sequence-based analyses of fungal biodiversity. Methods Mol. Biol. 722, 141–155

Tillett, D. & Neilan, B.A. (2000) Xanthogenate nucleic acid isolation from cultured and environmental cyanobacteria. J. Phycol. 36, 251–258.

Tochen, S., Woltz, J.M., Dalton, D.T., Lee, J.C., Wiman, N.G. & Walton, V.M. (2016). Humidity affects populations of *Drosophila suzukii* (Diptera: *Drosophilidae*) in blueberry. J. Appl. Entomol. 140, 47–57.

Travadon, R., Lawrence, D. P., Rooney-Latham, S., Gubler, W. D., Wilcox, W. F., Rolshausen, P. E. & Baumgartner, K. (2015) *Cadophora* species associated with wood-decay of grapevine in North America. Fungal Biol. 119, 53–66.

Travadon, R., Lecomte, P., Diarra, B., Lawrence, D.P., Renault, D., Ojeda, H., Rey, P. & Baumgartner, K. (2016) Grapevine pruning system and cultivars influence the diversity of wood-colonizing fungi. Fungal Ecol. 24, 82–93.

Trouillas, F., Úrbez-Torres, J.R. & Gubler, W.D. (2010) Diversity of diatrypaceous fungi associated with grapevine canker diseases in California. Mycologia 102, 319–336.

Trouillas, F.P., Pitt, W.M., Sosnowski, M.R., Huang, R., Peduto, F., Loschiavo, A., Savocchia, S., Scott, E.S. & Gubler, W.D. (2011) Taxonomy and DNA phylogeny of *Diatrypaceae* associated with *Vitis vinifera* and other woody plants in Australia. Fungal Divers. 49, 203–223.

Úrbez-Torres, J.R. (2011) The status of *Botryosphaeriaceae* species infecting grapevines. Phytopathol. Mediterr. 50 (Supplement), S5–S45.

Úrbez-Torres, J.R., Peduto, F., Striegler, R.K., Urrea-Romero, K.E., Rupe, J.C., Cartwright, R.D. & Gubler, W.D. (2012) Characterization of fungal pathogens associated with grapevine trunk diseases in Arkansas and Missouri. Fungal Divers. 52, 169–189.

Úrbez-Torres, J.R., Peduto, F., Vossen, P.M. & Gubler, W. (2013a) Olive twig and branch dieback: Etiology incidence and distribution in California. Plant Dis. 97, 231–244.

Úrbez-Torres, J. R., Peduto, F., Smith, R. J. & Gubler, W. D. (2013b) Phomopsis dieback: A grapevine trunk disease caused by *Phomopsis viticola* in California. Plant Dis. 97, 1571–1579.

Úrbez-Torres, J.R., Haag, P., Bowen, P., Lowery, T. & O'Gorman, D. (2015) Development of a DNA-macroarray for the detection and identification of fungal pathogens causing decline of young grapevines. Phytopathology 105, 1373–1388.

Unger, S., Büche, C., Boso, S. & Kassemeyer, H.H. (2007) The course of colonization of two different *Vitis* genotypes by *Plasmopara viticola* indicates compatible and incompatible host-pathogen interactions. Phytopathology 97, 780–786.

Váczy, K.Z. (2017) First report of *Seimatosporium vitis* associated with Grapevine Trunk Disease symptoms in Hungary. Plant Dis. 101, 253.

Vail, M.E. & Marois, J.J. (1991) Grape cluster architecture and the susceptibility of berries to *Botrytis cinerea*. Phytopathology 81, 188–191.

Van Kan, J.A.L. (2006) Licensed to kill: the lifestyle of a necrotrophic plant pathogen. Trends Plant Sci. 11, 247–253.

Van Niekerk, J.M., Calitz, F.J., Halleen, F. & Fourie, P.H. (2010) Temporal spore dispersal patterns of grapevine trunk pathogens in South Africa. Eur. J. Plant Pathol. 127, 375–390.

Vasquez S.J., Gubler, W.D. & Leavitt, G.M. (2007). Economic loss in California's table grape vineyards due to measles. Phytopathol. Mediterr. 46, 118.

Walg, O. (2011a) Umstellung auf Minimalschnitt im Spalier, Teil I: Was ist zu beachten? Das Deutsche Weinmagazin. 25/26, 14–16.

Walg, O. (2011b) Minimalschnitt in Spalier, Teil II: Erziehung mit Zukunft? Das Deutsche Weinmagazin. 12, 12–17.

Walg, O. (2012a) Neue Erkenntnisse und Ergebnisse: Minimalschnitt im Spalier. Das Deutsche Weinmagazin. 11, 30–33.

Walg, O. (2012b) Minimalschnitt im Spalier: Ausdünnen mit dem Vollernter. Das Deutsche Weinmagazin. 13, 12–15.

Walg, O. (2012c) Minimalschnitt im Spalier: Arbeiten im Frühjahr. Das Deutsche Weinmagazin. 8, 32–33.

Walg, O. (2013) Intensiv oder extensiv: Erziehungssysteme im Vergleich. Das Deutsche Weinmagazin. 17/18, 50–53.

Walg, O. (2015) Minimalschnitt im Spalier – Umstellung, Pflege und Neuanlage. Das Deutsche Weinmagazin. 6, 26–29.

Weyand, K.W. & Schultz, H.R. (2006a) Light interception, gas exchange and carbon balance of different canopy zones of minimally and cane-pruned field grown Riesling grapevines. Vitis 45, 105–114.

Weyand, K.W. & Schultz, H.R. (2006b) Long-term dynamics of nitrogen and carbohydrate reserves in woody parts of minimally and severely pruned Riesling vines in a cool climate. Am. J. Enol. Vitic. 57, 172–182.

White, T.J., Bruns, T., Lee, S. & Taylor, J.W. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis MA, Gelfand DH, Sninsky JJ,

White TJ (eds) PCR protocols: a guide to methods and applications, pp. 315–322. Academic Press, Inc., New York.

White, C.L., Halleen, F. & Mostert, L. (2011) Symptoms and fungi associated with esca in South African vineyards. Phytopathol. Mediterr. 50 (Supplement), S236–S246.

Wickham, H. (2009) ggplot2: Elegant graphics for data analysis. Springer, New York.

Wilcox, W.F., Gubler, W.D. & Uyemoto, J.K. (2015a) Compendium of grapevine diseases, disorders, and Pests (2nd edition) – Botrytis Bunch Rot and Blight. 39–45. APS Press, Minnesota. ISBN: 978-0-89054-479-2.

Wilcox, W.F., Gubler, W.D. & Uyemoto, J.K. (2015b) Compendium of grapevine diseases, disorders, and Pests (2nd edition) – Powdery Mildew. 75–83. APS Press, Minnesota. ISBN: 978-0-89054-479-2.

Wilcox, W.F., Gubler, W.D. & Uyemoto, J.K. (2015c) Compendium of grapevine diseases, disorders, and Pests (2nd edition) – Downy Mildew. 46–52. APS Press, Minnesota. ISBN: 978-0-89054-479-2.

Williamson, B., Tudzynski, B., Tudzynski, P. & Van Kan, J.A.L. (2007) *Botrytis cinerea*: the cause of grey mould disease. Mol. Plant Pathol. 8, 561–580.

Wolf, T.K., Dry, P.R., Iland, P.G., Botting, D., Dick, J., Kennedy, U. & Ristic, R. (2003) Response of Shiraz grapevines to five different training systems in the Barossa Valley. Aust. J. Grape Wine Res. 9, 82–95.

Zendler, D., Schneider, P., Töpfer, R. & Zyprian, E. (2017) Fine mapping of Ren3 reveals two loci mediating hypersensitive response against *Erysiphe necator* in grapevine. Euphytica 213, 68.

Zhang, Y., Crous, P.W., Schoch, C.L. & Hyde, K.D. (2012) *Pleosporales*. Fungal Divers. 53, 1–221.

Summary

The novel grapevine training system “semi minimal pruned hedge” (SMPH), enables farmers to reduce their production costs by replacing the time and labour intensive winter pruning, usually done by hand, with a mechanical pruning approach. Due to its economic benefits, SMPH became more popular in Germany over the last decade and the number of SMPH vineyards is still increasing. However, due to the lack of extended monitoring in the field, some cultivation aspects of SMPH are poorly understood. One important issue is related to the incidence of fungal grapevine diseases and the respective protection of the vines. Therefore, it was the aim of this study to investigate on a long term basis (2015–2018) the influence of the pruning system on the fungal community of grapevine, with special regard to phytopathogenic fungi.

One part of this study compared the canopy architecture and microclimate of SMPH and “vertical shoot positioning” (VSP) trained vines. The incidence of major fungal grapevine diseases, i.e. Downy Mildew (DM), Powdery Mildew (PM) and Botrytis Bunch Rot (BR) was surveyed over four consecutive years. Within this time frame it was noted that SMPH trained vines were more susceptible to DM and PM compared to VSP trained vines. It is assumed that the comparatively slower drying process of SMPH canopies produces a microclimate that favours growth and spread of these diseases. By contrast, SMPH bunches were less susceptible to BR compared to VSP bunches, which is probably linked to the more loose bunch architecture.

An analysis of the temporal development of the fungal community in healthy grapevine branches covering two months to eight years old branches revealed that fast growing fungi like *Alternaria* spp., *Aureobasidium pullulans*, *Botrytis cinerea*, *Cladosporium* spp., *Diaporthe* spp., *Epicoccum nigrum*, *Penicillium* spp., and *Truncatella angustata* are characteristic for young branches (two months to one year old). However, in older branches (> one year) the fungal community is more characterized by grapevine trunk disease (GTD) associated fungi,

with *Cadophora luteo-olivacea*, *Diplodia seriata* and *Eutypa lata* as predominant species. These data show that grapevines with older branches, as found in SMPH trained vines, are more threatened by GTDs than grapevines with only annual branches, as in VSP trained vines.

In four consecutive years a monitoring for Esca related foliar symptoms was performed in twelve vineyards each subdivided in SMPH and VSP trained sections to investigate the influence of the training system on symptom occurrence. Significant differences between the two training systems regarding the incidence of Esca foliar symptoms were found in three out of the four years. However, overall the results are inconsistent. While in 2016 SMPH trained vines expressed more symptoms than VSP trained vines, the opposite was the case in 2017 and 2018.

Spore traps were placed in a vineyard subdivided into SMPH and VSP trained sections to monitor the incidence of the Esca associated fungus *Phaeomoniella chlamydospora* over the year. Besides *P. chlamydospora* also other species of the *Phaeomoniellales* were isolated, including one known species, *Neophaeomoniella zymoides*, and six unknown species, which were newly described. The number of fungal strains and species of the *Phaeomoniellales* tend to be higher for spore traps attached to SMPH trained vines. A pathogenicity test made with potted plants in the greenhouse revealed that of the eight isolated species of the *Phaeomoniellales* only *P. chlamydospora* was able to induce significant lesions in the wood.

Data collected in this study shall help to developing a plant protection regime adapted to SMPH.

Zusammenfassung

„Minimalschnitt im Spalier“ (MMS) ist ein neues Reben-Erziehungssystem, das den Winzern die Möglichkeit eröffnet, Einsparungen bei den Produktionskoste vorzunehmen. Bei diesem Erziehungssystem wird der zeit- und arbeitsaufwändige Winterschnitt, der normalerweise per Hand erfolgt, maschinell, mit Hilfe eines Laubschneiders, durchgeführt. Aufgrund dieses ökonomischen Vorteils erlebte der MMS eine erhöhte Popularität unter den Winzern in den letzten Jahren, Tendenz steigend. Jedoch fehlen wichtige Informationen zu einige Kultivierungsaspekten, da es kaum detaillierte Feldstudien zu diesem Erziehungssystem gibt. Einer dieser Aspekte betrifft das Vorkommen von pilzlichen Krankheiten und den entsprechenden Schutz der Reben. Aus diesem Grund war es Ziel dieser Arbeit, basierend auf Langzeitversuchen (2015–2018), den Einfluss des Schnittsystems auf die Pilzgemeinschaft der Rebe zu untersuchen, mit besonderem Hinblick auf phytopathogene Pilze.

Ein Teil dieser Arbeit verglich die Laubwandarchitektur und das Mikroklima von MMS-Reben mit Reben in der klassischen Bogenerziehung (BE). Zudem wurde das Auftreten der wichtigsten Pilzkrankheiten, nämlich Falscher Mehltau (FM), Echter Mehltau (EM) und Graufäule (GF), vier Jahre hintereinander in den beiden Erziehungssystemen untersucht. In diesem Zeitraum wurde festgestellt, dass MMS-Reben im Vergleich zu BE-Reben anfälliger sind gegenüber FM und EM. Es wird vermutet, dass das vergleichsweise langsamere Abtrocknen der MSS-Laubwand ein Mikroklima erzeugt, welches das Wachstum und die Verbreitung der Krankheiten begünstigt. GF betreffend waren MMS-Trauben weniger befallen als BE-Trauben, was vermutlich mit der lockeren Traubenarchitektur in MMS-Reben zusammenhängt.

Eine Analyse der zeitlichen Entwicklung der Pilzgemeinschaft in äußerlich gesunden Rebtriebe, von zwei Monate bis acht Jahre, ergab, dass schnell wachsende Pilze wie *Alternaria* spp., *Aureobasidium pullulans*, *Botrytis cinerea*, *Cladosporium* spp., *Diaporthe* spp.,

Epicoccum nigrum, *Penicillium* spp. und *Truncatella angustata* charakteristisch sind für junge Triebe (zwei Monate bis ein Jahr). In älteren Trieben (> ein Jahr) allerdings ist die Pilzgemeinschaft mehr durch grapevine trunk disease (GTD)-assoziierte Pilze geprägt, mit *Cadophora luteo-olivacea*, *Diplodia seriata* und *Eutypa lata* als vorherrschende Arten. Diese Daten zeigen, dass Reben mit älteren Trieben, wie bei MSS-Reben zu finden, stärker durch GTDs gefährdet sind als BE-Reben mit ausschließlich einjährigen Trieben.

In vier aufeinanderfolgenden Jahren wurde das Vorkommen von externen Esca-Blattsymptomen in zwölf Weinbergen, unterteilt in MMS und BE, untersucht, um herauszufinden, ob und inwieweit, dass Schnittsystem das Auftreten der Blattsymptome beeinflusst. Signifikante Unterschiede zwischen den zwei Erziehungssystemen hinsichtlich der Häufigkeit von Esca-Blattsymptomen wurden in drei von vier Jahren gefunden, jedoch sind die Ergebnisse widersprüchlich. Während in 2016 MMS-Reben häufiger Blattsymptome zeigten als BE-Reben, war es in den Jahren 2017 und 2018 das Gegenteil der Fall.

Sporenfallen wurden in einem Weinberg an Trieben von MMS- und BE-Reben befestigt, um das Vorkommen des mit Esca assoziierten Pilzes *Phaeomoniella chlamydospora* im Jahresverlauf zu erfassen. Neben *P. chlamydospora* wurden dabei auch andere Arten der *Phaeomoniellales* isoliert; eine bereits bekannte Art, *Neophaeomoniella zymoides*, und sechs unbekannte Arten, die neu beschrieben wurden. Die Anzahl an Pilzstämmen und Arten von *Phaeomoniellales* war tendenziell höher für Sporenfallen, die an Ästen von MMS-Reben befestigt waren. Ein Pathogenitätstest mit Topfpflanzen im Gewächshaus ergab, dass von den acht isolierten Arten der *Phaeomoniellales* nur *P. chlamydospora* signifikante Läsionen im Holz verursachen kann.

Die Daten, welche in dieser Arbeit generiert wurden, sollen helfen, ein an MMS-Reben angepasstes Pflanzenschutzregime zu entwickeln.

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Publikationen:

Kraus, C., Voegele, R.T. & Fischer, M. (2018) Temporal development of the culturable, endophytic fungal community in healthy grapevine branches and occurrence of GTD associated fungi. Microbial Ecology DOI: https://doi.org/10.1007/s00248-018-1280-3

Kraus, C., Pennington, T., Herzog, K., Hecht, A., Fischer, M., Voegele, R.T., Hoffmann, C., Töpfer, T. & Kicherer, A. (2018) Effects of canopy architecture and microclimate on grapevine health in two training systems. Vitis 57, 53–60.

Pennington, T., **Kraus, C.**, Alakina, E., Entling, M.H. & Hoffmann, C. (2017) Minimal pruning and reduced plant protection promote predatory mites in grapevine. Insects 8, 86; doi: 10.3390/insects8030086.

Fischer, M., Schneider, P., **Kraus, C.**, Molnar, M., Dubois, C., D´Aguiar, D. & Haag, N. (2016) Grapevine trunk disease in German viticulture: occurrence of lesser known fungi and first report of *Phaeoacremonium viticola* and *P. fraxinopennsylvanicum*. Vitis 55, 145–156.

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