Studies on the resistance of different developmental stages in susceptible and tolerant grapevine cultivars against the pathogen *Plasmopara viticola*

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1 Introduction

The input of pesticides by agriculture into the environment, leads to increasing pollution and threats to biodiversity. Copper, for example, is deposited in the soil as a heavy metal and cannot be biodegraded. The deposition leads to both a reduction in earthworm population and a decrease in microbial biomass. It also causes stress reactions in soil dwellers (Bünemann et al., 2006; Umweltbundesamt, 2022). Due to the toxic effect, the use of copper is regulated in the European Union to 4 kg/ha/year (Commission Implementing Regulation (EU) 2018/1981). In Germany, the regulations are stricter and are limited to an amount of 3 kg/ha/year. In organic viticulture copper is mainly used to control downy mildew, one of the most important diseases in viticulture (Töpfer et al., 2011). The causative pathogen, *Plasmopara viticola* (Berk. & M. A. Curtis) Berl. & De Toni, can lead to epidemic infestation in years with suitable warm and humid weather conditions and thus high crop losses (Figueiredo et al., 2017; Unger et al., 2007). In years of high infestation pressure, such as 2012, 2016, and 2021, limited copper levels pose enormous challenges for organic growers to adequately protect their vineyards from downy mildew (Bleyer et al., 2020). A reduction of the copper application amount in such years is not possible at all. For this reason, new strategies must be developed to secure the yield and reduce the use of pesticides to a minimum.

One possibility for this is the planting of fungus-resistant grape varieties, so-called PIWIs (German: pilzwiderstandsfähige Rebsorten). PIWIs are crosses of wild American or Asian grapevines with European cultivars. Due to coevolution of Vitis species with *P. viticola*, they have developed resistance to the pathogen. These vines carry resistance-conferring genes (Bove et al., 2019). Since *P. viticola* was not introduced into Europe until 1878 (Töpfer et al., 2011), the native grape varieties were unable to develop resistance to the pathogen and are thus highly susceptible (Eisenmann et al., 2019). The crosses of American or Asian and European grapevines combine the resistance-bearing properties of the American or Asian vines with the oenological properties of the European vines (Töpfer et al., 2011). PIWIs thus have a certain resistance potential to *P. viticola* and are therefore an excellent way of saving plant protection products for both organic and conventional viticulture (Eisenmann et al., 2019).
Nevertheless, there is a risk that the introduced resistance can be overcome by virulent pathogen strains (Ciubotaru et al., 2021). To avoid this, PIWIs must also be adequately protected. However, in this regard, the potential of the varieties to reduce pesticides should be fully exploited. For this reason, vines should be treated just enough to provide adequate protection and avoid resistance breakthrough. In addition, fungicide use should be just low enough to make the most of the varieties' resistance.

Forecasting platforms represent another helpful tool for reducing plant protection in general. These systems use computer-aided models, real-time weather data and weather forecast models to calculate the possibility of infestation of the most important vine diseases in viticulture. The required weather information is collected via weather stations which have been set up in large numbers throughout the wine-growing regions. Taking into account this data and additional phenology models for vine development, recommendations are made to the winegrowers in order to make plant protection as effective and sustainable as possible (Bleyer et al., 2008; Bleyer et al., 2020; Dubuis et al., 2019; Rossi et al., 2014). The forecasting system VitiMeteo, which has been developed by Agroscope (Changins, Switzerland) and the State Institute for Viticulture (Freiburg, Germany) refers to the calculations of the area of newly grown leaves per primary shoot for the recommendations of the treatment intervals (Bleyer at al., 2020; Bleyer et al., 2008). With regard to downy mildew, important parameters calculated and incorporated into the recommendations include the possibility of infection and the duration of the incubation period (Bleyer et al., 2008).
In view of the regulations on the use of plant protection products, such prognosis systems represent an important tool for winegrowers, especially in years with high disease pressure (Bleyer et al., 2008).

The aim of this work is the development of a test system that allows characterizing of different PIWIs for their susceptibility to *P. viticola*. In this work, different PIWIs were evaluated for their susceptibility to *P. viticola*. For this purpose, six different PIWIs with different resistance loci were selected and two susceptible European cultivars were used as reference.
To investigate the inflorescences/grapes, a new test system was developed in which inflorescences/grapes from potted grapevines were inoculated under optimal conditions for *P. viticola*. In parallel, the investigation was carried out on the leaves of the corresponding cultivars. One focus was the difference between young and old leaves of the vine. Another aspect of this work was to record the phenological development of the grape varieties in a vineyard in Freiburg (Germany), to highlight the differences in development over time. The data collected in this way on the susceptibility of inflorescences/clusters and leaves will be integrated into the VitiMeteo forecasting system in the long term, taking the phenological development into account, in order to expand the forecasts for PIWIs. In the future, this will allow the crop

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protection strategy to be optimally adapted to the resistance characteristics of the PIWIs.

2 Material and Methods

Plant material. To investigate the resistance level of leaves and grapes of different susceptible and resistant grape varieties the following grapevine varieties with the corresponding *Rpv* loci (*Rpv, resistance to P. viticola*) were used: Cabernet Cortis (*Rpv 3.3, Rpv 10*) (Zini et al., 2019), Calardis Blanc (*Rpv 3.1, Rpv 3.2*) (Eisenmann et al., 2019), Divico (*Rpv 3.3, Rpv 10*) (Spring, 2013), Prior (*Rpv 3.1, Rpv 3.3*) (vivc.de, 2022), Solaris (*Rpv 3.3, Rpv 10*) (Schwander et al., 2012; Vezzulli et al., 2019) and Souvignier Gris (*Rpv 3.2*) (Zini et al., 2019). *V. vinifera* cv. Mueller-Thurgau (M-TH) and Pinot Noir served as susceptible control grape varieties.
In this study, the susceptibility of the different grape varieties was investigated in the seasonal course. For this purpose, the inflorescences and grapes were inoculated at different stages of development according to the BBCH scale (Lorenz et al., 1995): BBCH 53-55 (inflorescences clearly visible/ inflorescences swelling, flowers closely pressed together), BBCH 57-59 (inflorescences fully developed, flowers separating), BBCH 65-68 (full flowering / 80 % of flower hoods fallen), BBCH 73 (berries groat-sized, bunches begin to hang), BBCH 75 (berries pea-sized, bunches hang), BBCH 77-79 (berry touch), and BBCH 81-83 (beginning of ripening, berries developing color).

Inflorescences/grapes experiments. A special test system was developed to investigate the susceptibility of the inflorescences and grapes against *P. viticola*. In this system, potted vines were placed in a climate-controlled chamber. Inoculation was carried out with a sporangia solution (40,000 sporangia / ml) directly onto the inflorescences/grapes (spray inoculated) until they were dripping wet. For the sporangia solution, freshly sporulating leaves were rinsed in desalinated water and adjusted to the desired spore count using a counting chamber. The climate-controlled chamber was then sealed. The vines were spray irrigated at 2 hourly intervals for 30 seconds each. This guaranteed high humidity and wet vine organs for optimal infection conditions. The temperature was between 18 - 24 °C. 24 hours after the first inoculation, a second inoculation with the same conditions were performed. After another 24 hours, the vines were placed back in the greenhouse. Evaluation of the disease severity was done 4 weeks after inoculation.

Leaf disc assays. In parallel, leaf discs of the same grape varieties were examined. The vines for this experiment were taken from the greenhouse. Per variant, two leaves from 3 different vines were used. For this purpose, the third leaf at the shoot base and the fifth fully unfolded leaf at the shoot tip were sampled per plant. The leaves were disinfected with 70 % ethanol and washed in distilled water. 12 leaf discs per leaf were punched out with a cork borer and placed upside down on a water agar plate (1 % w/v). Drop Inoculation was performed with 100 µl sporangia suspension (40,000 sporangia / ml) and the plates were put dark for 24 hours. After this time the sporangia droplets were removed, and the plates closed with parafilm. The sporangia solution was produced as described above. Plates were incubated for 7 days in a climate chamber with 14 hours of light and 24 °C degrees Celsius until evaluation.

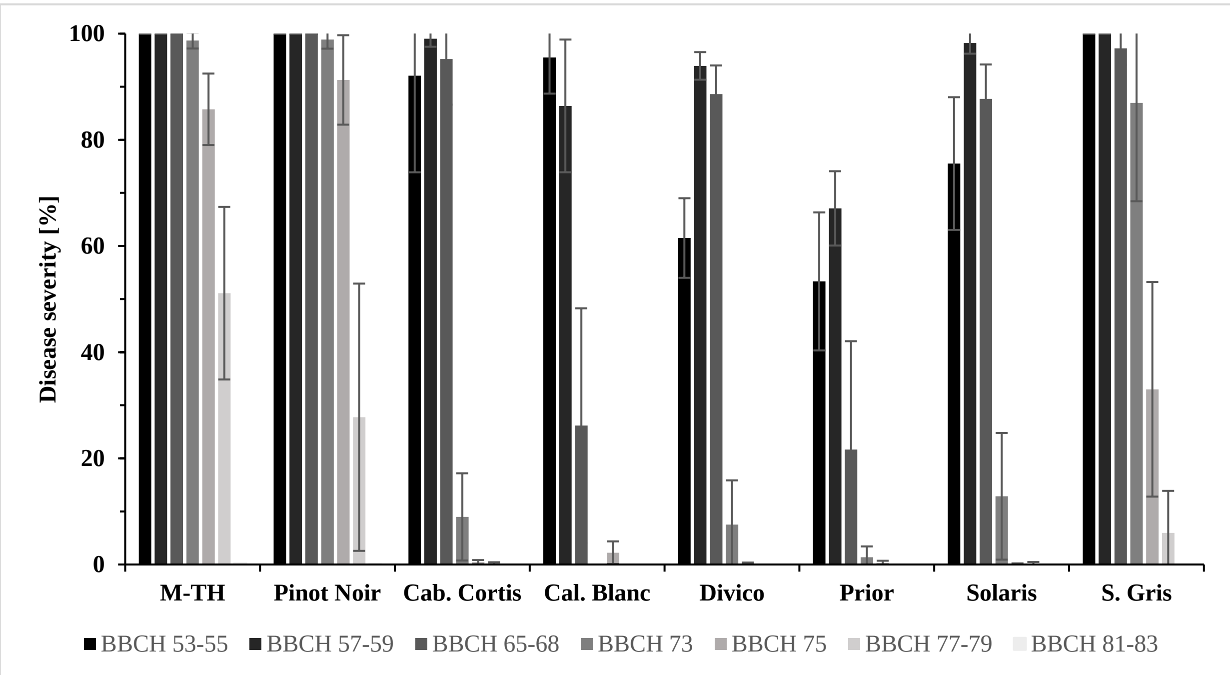

Figure 1: Results of susceptibility studies on clusters of different PIWIs to *Plasmopara viticola* of 2021. The diagram shows the disease severity according to the phenological state the inflorescences/grapes were infected. To note, if no bar is visible, this means an infestation level of 0 %.

3 Results

Susceptibility of grapes to *P. viticola* differs among PIWI cultivars. The reference grape varieties Mueller-Thurgau and Pinot Noir showed 100 % infestation until the end of flowering. With the beginning of fruit development, the infestation decreased from 86 % and 91 % (BBCH75) to 51 % and 28 % (BBCH 77). With the onset of ripening, the susceptibility of the comparative grape varieties droped rapidly to 0 % (Figure 1).

Surprisingly, the S. Gris variety showed great similarities in the level of resistance on the grapes compared to the two reference varieties (Figure 1). The resistance of the variety started only one developmental stage earlier. The first strong decrease in susceptibility was observed at the stage BBCH 75 with 33 % infestation level. One stage earlier, at BBCH 73, the susceptibility was still almost 90 %. In further development, the grape variety showed almost no susceptibility to *P. viticola*. While all other investigated grapevine varieties carry two different resistance loci, S. Gris has only one resistance loci. In addition, it should be mentioned at this point that S. Gris vines showed significantly less infestation on the grapes than Pinot Noir in an untreated field trial in 2021. While grape infestation was 94 % for Pinot Noir, S. Gris showed only 25 % infestation (data not shown). Why the PIWI cultivars performed significantly worse in test system could possibly be due to the conditions chosen. A very high inoculum concentration was deliberately chosen to best show the differences between the cultivars. It is unlikely that such a high sporangia concentration would occur in nature. It is important to note that the infestation levels found can therefore not be directly transferred to viticultural practice.

Looking at the grape varieties Solaris, Divico and Cabernet Cortis (Figure 1), which all carry the resistance loci *Rpv 3.3* and *Rpv 10*, great parallels can be seen between these cultivar. Until flower set, these cultivars were highly susceptible and recorded a large yield loss. With fruit set, BBCH 73, these cultivars showed a strong level of resistance. As of BBCH 75, all 3 varieties showed no more infestation.

The two varieties Prior and Calardis Blanc showed a similar resistance pattern. Increased resistance was already seen at flowering, BBCH 65-68 (disease severity: Prior 22 %, Calardis Blanc: 26 %). From BBCH 75 onwards, both varieties showed almost no infestation.

Leaves of all PIWI cultivars show high resistance to *P. viticola*. Leaf disc assays were carried out to investigate the susceptibility of leaves of different PIWIs. The focus was mainly on the differences in the susceptibilities of young (shoot tip) and old (grape zone) leaves.

The reference grape variety Mueller-Thurgau showed a clear difference between young and old leaves. The infestation intensity decreased by 26 % from 73 % to 47 % (Figure 2). Lower infestation levels of older leaves were also observed in PIWIs. The highest infestation of young leaves was observed in the S. Gris variety (7.5 %). This decreases in leaves from the grape zone and droped to 3.8 %. The other PIWIs also showed an infestation, but it is very low throughout. The lowest infestation was found in the Calardis Blanc variety with 0.13 % on young leaves. Looking at the grape zone, the values of older leaves were even smaller with 0.04 % and 0.05 % for Solaris, Divico and Calardis Blanc (Figure 2).

The difference in susceptibility between young and old leaves in Mueller-Thurgau can possibly be attributed to ontogenetic resistance. Studies have shown that the low susceptibility of leaves to other diseases, such as powdery mildew, is due to ontogenetic resistance mechanisms (Ficke et al., 2003; Ficke et al., 2004). Whether the slight difference in infestation levels between young and old leaves of PIWIs is also due to ontogenetic resistance cannot be conclusively determined at this time. Further investigations, e.g. microscopic analysis, are required for this purpose.

Ontogentic resistance mechanisms that prevent grape infection with *P. viticola*, for example, have been described in susceptible and resistant cultivars (Gindro et al., 2012; Kennelly et al., 2005). Conversion of stomata to lenticels on susceptible grape cultivars was shown by Kennelly et al. (2005) for Riesling and Chardonnay. Depending on climatic conditions and the respective grape variety, lenticels and thus ontogenetic resistance could be observed one to six weeks after flowering. For the variety Solaris it could be shown that in stage BBCH 53 still open stomata could be found, whereas the stages BBCH 69 and 75 showed only lenticels or closed appearing stomata (Gindro et al., 2012). The transformed stomata can no longer be infected by *P. viticola* because entry into the host occurs through the stomata (Gindro et al., 2012). However, the resistance of PIWI cultivars cannot be attributed to stomata changes alone. Rather, defense responses, for example, through the formation of stilbenes or hypersensitive reactions, are crucial (Eisenmann et al., 2019; Gindro et al., 2012; Heyman et al., 2021).

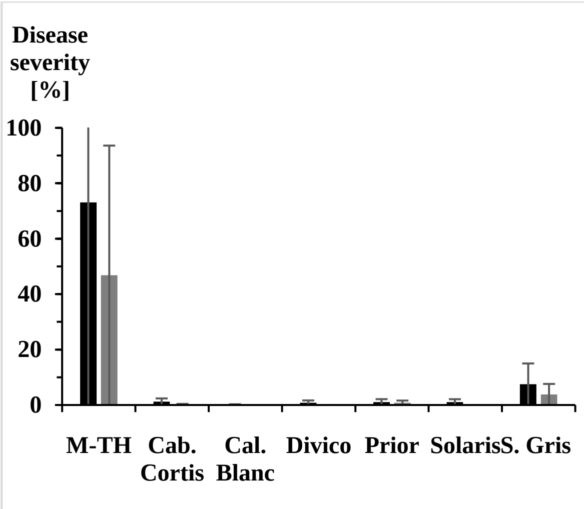

Figure 2: Results of susceptibility studies on young and old leaves of different PIWIs to *Plasmopara viticola*. Diagram shows the disease severity of leaves from the shoot tip and the shoot base. To note, if no bar is visible, this means an infestation level of 0 %. Black bars: shoot tip; grey bars: grape zone.

4 Conclusions

In this work, PIWIs were shown to have higher resistance to *P. viticola* than standard cultivars, especially on the leaves. This gives a great potential to reduce fungicide use in viticulture in the long term.

In addition, inflorescence and grape experiments have shown that PIWI cultivars have different levels of resistance at different stages of development. PIWI cultivars with two resistance loci have higher resistance than S. Girs, which carries only one resistance locus. Surprisingly, cultivars with the same resistance loci have comparable resistance during different developmental stages. For example, Cabernet Cortis, Divico, and Solaris, all carrying *Rpv 3.3* and *Rpv 10*, showed comparable infestation levels. It is difficult to say whether these findings can be applied to other PIWI cultivars with the same *Rpv* loci combination, as certainly not all resistance loci have been fully identified in all PIWIs. Furthermore, there seem to be qualitative differences in one and the same resistance locus. Heyman et al. (2021) demonstrated for *Rpv 3.1* that cultivars carrying this locus show a different strength of resistance response. Thus, it is difficult to make a simple statement about the resistance or susceptibility of PIWIs in general but a simplified classification of PIWIs is necessary to incorporate the data into VitiMeteo in the most useful way.

For PIWI cultivars to be usefully incorporated into the VitiMeteo forecasting model, the phenological development of the cultivars should also be taken into account, as it has been shown that the resistance level correlates with the phenological development stage of the vine. As phenology monitoring showed in 2020, the BBCH 57 stage was reached by the different cultivars within a 12-day period (Solaris: 29 April; Divico: 05 May; S. Gris: 11 May) (data not shown). The BBCH 65 stage was reached by Solaris and Divico (13 May) seven days before S. Gris, Prior, Cabernet Cortis, and Calardis Blanc (20 May). The cultivars also reached the bunch closure stage (BBCH 79) in a period of 14 days (Solaris: 23 June; S. Gris, Prior: 29 June; Calardis Blanc: 07 July) (data not shown). Classification of PIWIs into early- and late-flowering varieties would further fine tune the forecasting model.

It is important to determine the right time for plant protection for each variety in order to reduce the use of plant protection products and to protect the vine as efficiently as possible.

Although grapes of PIWIs show under normal field conditions little susceptibility to *P. viticola*, especially at later stages of development, to avoid resistance overcoming by *P. viticola*, these grape varieties still need to be subjected to some plant protection

at the beginning of the fruit development.

With the help of the data presented and the VitiMeteo "*Plasmopara*" forecasting model, the aim is to give winegrowers the most optimal plant protection strategy for sustainable and future-oriented organic viticulture.

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