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The Durability of Quantitative Host Resistance and Variability in Pathogen Virulence in the Interaction Between European Grapevine Cultivars and *Plasmopara viticola*

Lisa Heyman¹, Rebecca Höfle², Anna Kicherer², Oliver Trapp², Essaid Ait Barka³, Reinhard Töpfer² and Monica Höfte^{1*}

¹ Department of Plants and Crops, Faculty of Bioscience Engineering, Ghent University, Ghent, Belgium, ² Institute for Grapevine Breeding Geilweilerhof, Julius Kühn Institute, Federal Research Centre for Cultivated Plants, Siebeldingen, Germany, ³ RIBP-EA 4707, SFR Condorcet FR CNRS 3417, University of Reims Champagne-Ardenne, Reims, France

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Joana Figueiredo,
University of Lisbon, Portugal
Andreia Figueiredo,
University of Lisbon, Portugal

*Correspondence:

Monica Höfte
Monica.Hofte@UGent.be

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European grapevine, *Vitis vinifera*, carries no major resistances against *Plasmopara viticola*, the causal agent of grapevine downy mildew. The introgression of quantitative trait loci conferring resistance to *P. viticola* (*Rpv*) from American and Asian donor species has resulted in a range of resistant cultivars. In light of the perennial nature of grapevine and the high evolutionary potential of *P. viticola*, the durability of this quantitative resistance is an important challenge. Durability of host resistance and variability in pathogen virulence may be evaluated by describing interactions between pathogen isolates and grapevine cultivars in terms of *Rpv* loci. A set of 16 cultivars carrying different combinations of *Rpv* loci, was challenged with five *P. viticola* isolates, obtained from susceptible or *Rpv3.1*⁺ *V. vinifera* cultivars. Based on the severity of sporulation, different host and pathogen phenotypes might be distinguished, which could be related to the presence of different *Rpv* loci. The hormonal responses before and during some interactions were compared to assess the resistance mechanisms underlying *Rpv3.1*, *Rpv10*, and *Rpv12* and the infection mechanisms of the different isolates. This paper reports on the strength of some of the commonly used *Rpv* loci, single or stacked. The isolates derived from *Rpv3.1*⁺ hosts, GREPv1 and GPHPv1, were able to sporulate intensely on cultivars carrying *Rpv3.1*, without triggering necrosis. Moreover, *Rpv10* was not able to efficiently halt the development of the *Rpv3.1*-breaking isolate GPHPv1. Cultivars carrying *Rpv12*, however, were resistant to all five *P. viticola* isolates. Phytohormones might be implicated in the basal resistance against this pathogen, but during the early defense response, no significant hormonal responses to the isolates were observed. The isolate-specificity of the *Rpv3*- and *Rpv10*-mediated resistance suggests that these loci do not result in the most sustainable resistance. Furthermore, the isolate-specific behavior of the pathogen emphasizes the need for a characterization system for *P. viticola*. A standardized phenotyping assay may be used to determine

P. viticola pathogen phenotypes or measure the durability, strength, and isolate-specificity of the host quantitative resistances. The characterization of both components of the pathosystem may lead to an increased understanding of the resistance mechanisms, beneficial for a durable deployment of resistance genes.

Keywords: *Rpv*, downy mildew, resistance genes, *Vitis vinifera*, characterization, phenotyping, virulence, breakdown

INTRODUCTION

The obligatory biotrophic pathogen *Plasmopara viticola*, the causal agent of grapevine downy mildew, is considered one of the most economically important oomycetes (Kamoun et al., 2015). *Vitis vinifera*, the only grapevine species endemic in Europe, carries no resistance to this pathogen, except for a minor locus identified in Chardonnay (Bellin et al., 2009). Therefore, viticulture is heavily dependent on chemical protection. The recurrent use of high amounts of pesticides has led to the emergence of *P. viticola* strains tolerant to the most commonly applied groups of site-specific fungicides (Chen et al., 2007; Gisi et al., 2007; Blum et al., 2010; Genet and Jaworska, 2013). Breeding of new cultivars resistant to the pathogen is being explored as a promising strategy to minimize chemical control. Controlled grapevine breeding was first launched in Europe following the introduction of phylloxera and powdery and downy mildews during the mid to late 19th century (Töpfer et al., 2011). Mildew resistances are still among the first criteria in a breeding program to select prime candidates from the seedling mass, before the evaluation of their viticultural performance and wine quality.

Breeders utilize the resistance of American and Asian donor species and the closely related *Muscadinia rotundifolia* (Töpfer et al., 2011). Their genetic resistance to downy mildew is assumed to arise from gene-for-gene recognition and subsequent activation of the vine's defense (Peressotti et al., 2010). Recognition occurs when a resistance (*R*) gene and the corresponding avirulence (*Avr*) gene are present in the interacting plant and pathogen, respectively. Knowledge about *Avr* and *R* genes in the *P. viticola*/grapevine pathosystem is still limited. To our knowledge, nothing is known about the nature of the *Avr* genes in *P. viticola*, although it is likely that they encode RxLR effectors as was shown in other oomycetes (Anderson et al., 2015). *R* genes encode proteins containing a nucleotide-binding site (NBS) and leucine-rich repeats (LRR) (Zini et al., 2019). The development of molecular markers has allowed the description of 31 quantitative trait loci (QTLs) (Maul et al., 2020) where the *R*-genes are located, referred to as *Rpv* (resistance to *Plasmopara viticola*). Some of these QTLs only have a minor effect on the downy mildew resistance level. These weak QTLs, e.g., *Rpv4* in Regent (Welter et al., 2007) and *Rpv11* in Chardonnay (Bellin et al., 2009), Regent (Fischer et al., 2004), and Solaris (Schwander et al., 2012), are not particularly suited for use in breeding programs. Major QTLs, on the other hand, significantly affect the susceptibility to *P. viticola*. *Rpv3* is historically the most common major resistance locus in European resistant

cultivars (Foria et al., 2020). The *Rpv3* locus on chromosome 18 (Welter et al., 2007) originates from North-American *Vitis* sp. (Bellin et al., 2009). Peculiarly, seven conserved *Rpv3* haplotypes have been identified, named according to the UDV305-UDV737 allele sizes: *Rpv3*^{299–297} (*Rpv3.1*), *Rpv3*^{null–297} (*Rpv3.2*), *Rpv3*^{null–271} (*Rpv3.3*), *Rpv3*^{321–312}, *Rpv3*^{null–287}, *Rpv3*^{361–299}, and *Rpv3*^{299–314} (Di Gaspero et al., 2012). The most frequently occurring haplotypes *Rpv3.1* (Welter et al., 2007; Bellin et al., 2009; van Heerden et al., 2014) and *Rpv3.2* (Zyprian et al., 2016), but also *Rpv3.3* (Vezzulli et al., 2019), have been validated in segregating populations. The major QTLs *Rpv1*, *Rpv10*, and *Rpv12* are also frequently deployed in grapevine breeding. *Rpv1*, originating from the American *M. rotundifolia*, was mapped to chromosome 12 (Merdinoglu et al., 2003). It is closely associated with *Run1*, the locus conferring resistance to grapevine powdery mildew (Feechan et al., 2013). *Rpv10* and *Rpv12*, mapped on chromosome 9 (Schwander et al., 2012) and 14 (Venuti et al., 2013), respectively, are both donated by *V. amurensis*, native to Asia.

One of the most important challenges of breeding for resistance is durability. Disease resistances should preserve their effectiveness over time, even when they are employed widely in a favorable environment for the pathogen (Johnson, 1984). However, like fungicides, host genetic resistance might impose a strong directional selection for local adaptation. In grapevine, this deserves special consideration, since its perennial nature requires a vine to grow for decennia in the vineyard and the cultivar itself to be used for centuries (Töpfer et al., 2011). Moreover, *P. viticola* is suggested to have a high evolutionary potential (Gobbin et al., 2006) and the shape of its population is strongly determined by selection (Stark-Urnau et al., 2000). The breakdown of host resistance by some *P. viticola* isolates has already been reported in the resistant cultivars Bianca (Peressotti et al., 2010) and Regent (Kast et al., 2000; Delmotte et al., 2014), both carrying *Rpv3* (Welter et al., 2007; Di Gaspero et al., 2012). These isolates might be escaping specific recognition by the *Rpv3* gene product through a mutation in the corresponding *Avr* gene (Peressotti et al., 2010).

To improve durability, modern breeding focuses on pyramiding of resistance genes. For a durable deployment of resistance genes, the characterization of both components of the pathosystem is essential. On the plant's side, many studies have been mapping the *Rpv* loci but not much is known about how they convey resistance and how the resistance benefits from stacking. On the pathogen's side, more insight into the virulence variability of the pathogen is crucial. The genetic diversity of *P. viticola* populations in the field is high (Stark-Urnau et al., 2000;

Gobbin et al., 2006; Scherer and Gisi, 2006) and increases through recombination in oospores during the sexual reproduction phase. The diversity in virulence among isolates might be explained by the level of expression of RxLR effector genes (Gómez-Zeledón and Spring, 2018). Although the challenge with single-action fungicide or single-gene host resistance drives the appearance of physiological races or pathotypes (Gessler et al., 2011), the classification of the interaction between *P. viticola* and grapevine rarely focuses on the pathogen. The virulence of *P. viticola* isolates has been characterized previously and their behavior on a limited set of wild or cultivated hosts from Europe, Asia, and North-America has been described (Gómez-Zeledón et al., 2013, 2017). Performing this kind of characterization in terms of *Rpv* loci could provide a more robust idea about the durability of the *Rpv* loci.

The current study aimed to assess the durability of some of the commonly used loci conferring resistance to *P. viticola* and the influence of stacking and heritage, with regards to different isolates of *P. viticola*. At the same time, this study provides an indication of the diversity in virulence of *P. viticola* isolates in a vineyard with a high occurrence of these *Rpv* loci. We also assessed the role of phytohormones in this interaction. Phytohormones are implicated in the resistance of the donor species against *P. viticola* (Liu et al., 2016; Wang et al., 2018), but not much is known about their role in resistant cultivars. *P. viticola* isolates were collected from host cultivars from a collection in the German Pfalz region, where the presence of many cultivars carrying various resistances to *P. viticola* causes an increased selection pressure, and from the traditional French Champagne region. A set of 16 cultivars, carrying different resistance loci, was challenged with these isolates to determine host and pathogen phenotypes. The hormonal responses before and during some of these interactions were compared to assess the resistance mechanisms underlying *Rpv3.1*, *Rpv10*, and *Rpv12*.

MATERIALS AND METHODS

Plant Material

The 16 grapevine cultivars used in this work are represented in **Table 1**. A simplified pedigree showing the relationship between various cultivars is shown in **Supplementary Figure 1**. Cuttings of these 16 cultivars were rooted in Jiffy-7 peat pellets for 2 months, before transplantation in four-liter pots containing universal potting soil (type 1; Snebbout, Kaprijke, Belgium). Plants were grown in a greenhouse at 24/19°C, 16/8 h light/dark regime. To guarantee a high and continuous availability of young, healthy and uniform plant material, the susceptible cultivar Chardonnay (clone 7535) and the resistant cultivars Solaris, Regent, and Lela were also grown in tissue culture. To establish a tissue culture of the resistant cultivars, shoots from adult greenhouse plants were cut into uninodal segments and surface-sterilized for 5 min in 1% NaOCl. After three rinses, the dried nodal explants were transferred to semisolid MM medium (Martin et al., 1987). Plantlets from the four cultivars were micro-propagated as described by Ait Barka et al. (2006).

Genealogy and Pedigree Reconstruction

A simplified pedigree of the cultivars (**Supplementary Figure 1**) was reconstructed according to the data confirmed by markers reported in the *Vitis* International Variety Catalogue (Maul et al., 2020) (VIVC). Most information about the resistance traits was also found in the VIVC. Supplementary information about the *Rpv3* locus was added in accordance with Di Gaspero et al. (2012). Delmotte et al. (2014) and Zini et al. (2019) confirmed the presence of the *Rpv1* locus in Montpellier 3082-1-42 and of the *Rpv12* locus in Lela. Solaris was described to carry *Rpv10* (Schwander et al., 2012), as well as *Rpv3.3* (Possamai et al., 2020).

Pathogen Material

Infected leaves were sampled from a traditional grapevine collection in the University of Reims Champagne-Ardenne (URCA) in Reims, France, and in the breeding collection of the Julius Kühn Institute (JKI) for grapevine breeding in Siebeldingen, Germany. The URCA is located in the French Champagne region, primarily cultivated with the susceptible *V. vinifera* cv. Pinot Noir (38%), Pinot Meunier (31%), and Chardonnay (30%) (Le Comité Champagne, 2020). The German Pfalz region, where the JKI is situated, has a higher varietal diversity, still dominated by susceptible *V. vinifera* cultivars, with Riesling (25%) and Dornfelder (11.8%) on top (Statistisches Landesamt Rheinland-Pfalz, 2020). Since 2009, the fungus-tolerant variety Regent has also been covering an important part of the Pfalz viticultural area (2.2%). The grapevine collection of the JKI in Siebeldingen is particularly diverse, counting more than 3,000 wild relatives, breeding lines, and cultivars, of which more than half carry resistance genes against powdery or downy mildew.

A sterile needle was used to pick up a single sporangiophore from a lesion of the sampled field material. This single sporangiophore was transferred to a drop of sterile, distilled water on the abaxial side of a Chardonnay leaf, previously detached from an *in vitro* plantlet. Inoculated leaves were incubated on water agar (0.65%) at 16/8 h photoperiod, 22/18°C. This isolation step was repeated at least three times. In the end, five single sporangiophore isolates were obtained (**Table 2**). The cultivars from which these isolates were isolated, are highlighted in the simplified pedigree in **Supplementary Figure 1**. The isolates were preserved on infected leaves at -20°C. They were weekly propagated on leaves of Chardonnay plantlets.

Leaf Disk Assay for Phenotypic Evaluation of Host and Pathogen

The 3rd and 4th fully expanded leaves, counted from the apex of the shoot, were collected from adult greenhouse plants. In this assay, a set of 16 *V. vinifera* cultivars, carrying different resistance loci (**Table 1**), was challenged with 5 *P. viticola* isolates (**Table 2**). Leaves were punched into disks (11 mm diameter), pooled, and placed abaxial side up on water agar. Subsequently, the leaf disks were inoculated with a 20 µL drop of a *P. viticola* suspension (5×10^4 sporangia mL⁻¹). The disease progression was evaluated at 6 days post inoculation (dpi). Each disk was assigned to one of five classes based on the number of sporangiophores (**Figure 1A**). A disease severity index (DSI), ranging from 0% (no disease) to

TABLE 1 | Grapevine cultivars used for virulence screening.

Cultivar name	Variety number VIVC	Rpv	Origin	Breeder	Year of crossing
Susceptible cultivars					
Chardonnay	2455		France		
Bacchus	851		Germany	JKI	1933
Diana	3547		Germany	JKI	
Resistant cultivars					
Seyval	11558	3.2, 3.3	France	Seyve-Villard	
Villard Blanc	13081	3.1	France	Seyve-Villard	
Seyve Villard 39-639	11670	3.1	France	Seyve-Villard	
Chambourcin	2436	3.1, 3.2	France	Seyve	
Merzling	4251	3.3	Germany	WBI Freiburg	1960
Calardis Musqué	4549	3.2	Germany	JKI	1964
Regent	4572	3.1	Germany	JKI	1967
Solaris	20340	3.3, 10	Germany	WBI Freiburg	1975
Lela	14634	12	Serbia	University of Novi Sad	1977
Calardis Blanc	22828	3.1, 3.2	Germany	JKI	1993
2004-051-0001		3.1, 10	Germany	JKI	2004
2011-64-0002		1	Germany	JKI	2011
2012-114-0133		1, 3.1, 12	Germany	JKI	2012

Each registered cultivar is listed with its variety number of the *Vitis* International Variety Catalogue (VIVC) (Maul et al., 2020). If known, the major loci conferring resistance to *P. viticola*, the country of origin, breeder, and year of crossing are also listed.

TABLE 2 | Overview of the *P. viticola* isolates used in this study.

Isolate	Alias	Collection date	Origin	Host	Rpv
FCHPv1	FCH1	30/09/2016	France (URCA)	Chardonnay	None
GPNPv1	GPN1	06/10/2016	Germany (JKI)	Pinot Noir	None
GPNPv2	GPN2	06/10/2016	Germany (JKI)	Pinot Noir	None
GREPv1	GRE1	06/10/2016	Germany (JKI)	Regent	Rpv3.1
GPHPv1	GPH1	06/10/2016	Germany (JKI)	Phoenix	Rpv3.1

Their origin, original name and alias used in this work are given. Also indicated is the information about the major Rpv loci, conferring resistance to *P. viticola* in the host of origin (Maul et al., 2020).

100% (maximally diseased), was calculated as the mean disease grade divided by the maximal disease grade. The experiment was repeated twice with on average 20 disks per pathogen-cultivar combination. The average level of necrosis at the evaluation stage was estimated according to the scale in **Figure 2**.

Role of Phytohormones

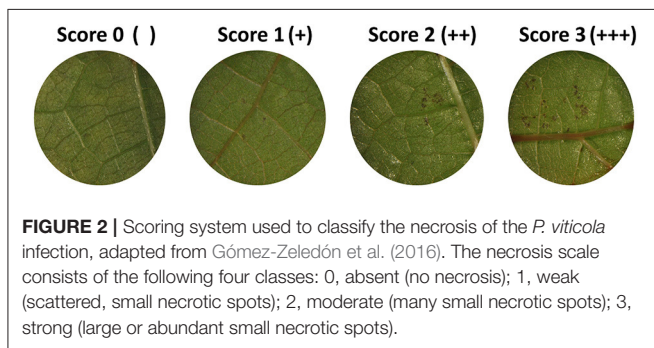
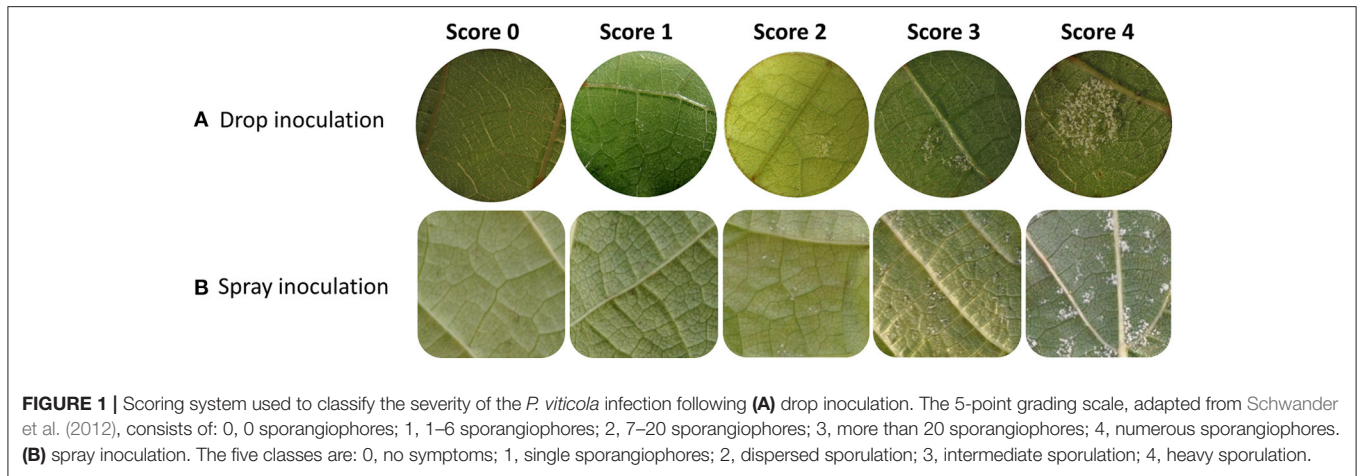
Based on the phenotyping assay, different host and pathogen phenotypes could be distinguished. The isolate-specific resistance conferred by *Rpv3.1* and *Rpv10* and the powerful resistance conferred by *Rpv12* were of particular interest. To investigate the involvement of phytohormone response before and during the first stages of the interaction, additional cross-inoculation assays were conducted using entire detached leaves or plantlets, rather than leaf disks, to reduce the influence of wounding. Regent, Solaris, and Lela were chosen to represent *Rpv3.1*⁺, *Rpv10*⁺, and *Rpv12*⁺ cultivars, respectively. Chardonnay or Diana were included as a susceptible control. They were challenged with one representative of each pathogen phenotype (GPN1, GRE1, and GPH1).

Detached Leaf Assay

To closer inspect these differential interactions, a spray inoculation was performed on detached leaves of greenhouse plants (Regent, Solaris, and Lela). Eight leaves were detached per plant and immediately placed abaxial side up on water agar (0.65%). Per plant, the leaves were sprayed pairwise with distilled water or with a sporangia suspension (5×10^4 sporangia ml⁻¹) of *P. viticola* (GPN1, GRE1, and GPH1) within 8 h after detachment. At 6, 12, and 24 h post inoculation (hpi), three replicates were collected for hormone analysis. The sporulation severity was evaluated at 8 dpi on 8 leaves per treatment. Each leaf was assigned to a 5-point grading scale for spray-inoculation (**Figure 1B**).

Plant Assay

To limit the biological variability, an additional infection assay was performed on intact plantlets of Chardonnay, Regent, Solaris, and Lela. Two-months old tissue culture plantlets were transplanted in sterilized, universal potting soil in a propagator (at 24°C, 16/8 h light/dark). After a 4-week acclimatization and



6-week growing period, all leaves were spray-inoculated with distilled water or with a sporangial suspension (5×10^4 sporangia ml^{-1}) of *P. viticola* (GPN1, GRE1, and GPH1). Per treatment, twenty young plantlets were used. They were incubated at 100% relative humidity during the first 24 h following inoculation. Five plants were sampled at 12 hpi for phytohormone analysis. The sporulation severity of each plant was evaluated at 10 dpi according to the 5-point grading scale for spray-inoculation (**Figure 1B**). At the same time, the necrosis was scored as follows: 0, no necrosis; 1, scattered small necrotic lesions; 2, many small necrotic lesions; 3, large or abundant small necrotic spots. Similar to the disease severity index, a necrosis index was calculated, ranging from 0% (no disease) to 100% (maximally diseased), as the mean necrosis grade divided by the maximal necrosis grade.

Phytohormone Analysis

The levels of abscisic acid (ABA), indole-3-acetic acid (IAA), jasmonic acid (JA), and salicylic acid (SA) were determined at the beginning of the infection and during the initial stages of infection. The basal phytohormone content was determined on leaves of adult greenhouse plants (Diana, Regent, Solaris, and Lela), with 5 replicates. The evolution of the phytohormone content during the infection was analyzed in leaves sampled from the detached leaf assay and the plant assay, with 3 and 5 replicates, respectively. For each replicate, two leaves of the same plant were

pooled, immediately frozen in liquid N_2 , and kept at -80°C until analysis. The procedure for the quantification of phytohormones is described in detail by Haeck et al. (2018) and Heyman et al. (2021).

Statistical Analysis

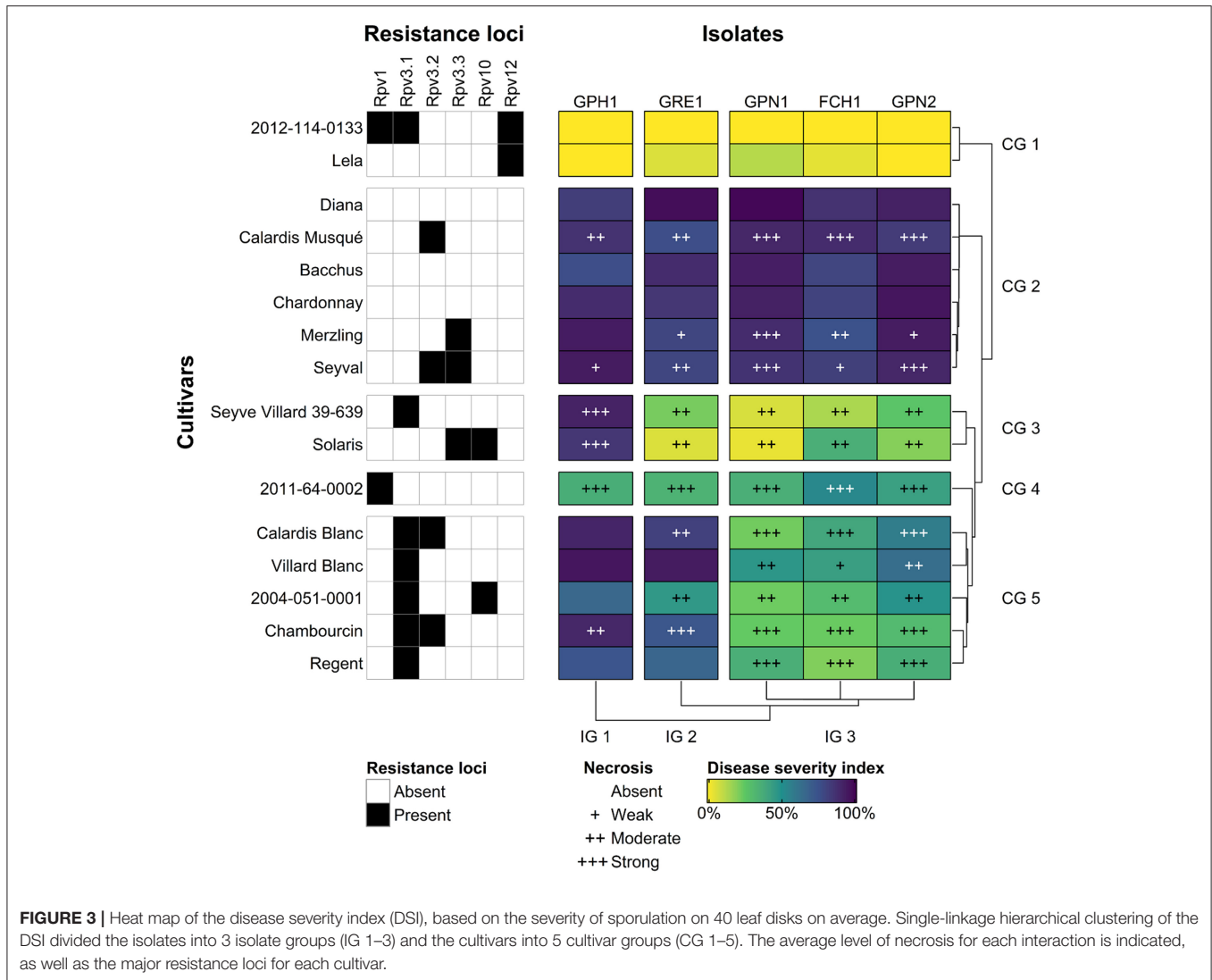
Statistical analysis was conducted using R, version 3.6.1 (R Core Team, 2019). The data were analyzed using the Kruskal-Wallis test, followed by the Dunn test ($p = 0.05$). The heat map was constructed using the ComplexHeatmap package (Gu et al., 2016).

RESULTS

Phenotypic Evaluation of Leaf Disks

The level of host adaptation of the five *P. viticola* isolates to the 16 grapevine cultivars was assessed in a leaf disk assay. Leaf disks representative for each interaction are presented in **Supplementary Figure 2**. For each interaction, the severity of sporulation was evaluated (**Supplementary Figure 3**) and an index calculated. This DSI revealed substantial differences between cultivars inoculated with the same isolate (**Figure 3**). Five cultivar groups (CGs) were distinguished by cluster analysis. Moreover, the contrasting interaction of some cultivars with the different isolates uncovered heterogeneous pathogen phenotypes. The DSI clustered the isolates into three isolate groups (IGs). The *Rpv* loci present in the tested cultivars supported the grouping of isolates and cultivars based on the disease severity (**Figure 3**) to a great extent.

The highest susceptibility was observed in CG 2 (Diana, Calardis Musqué, Bacchus, Chardonnay, Merzling, and Seyval), which contained the cultivars without major resistance alleles, as well as cultivars solely carrying *Rpv3.2* and/or *Rpv3.3*. As expected, no resistance was observed in the cultivars without major resistance alleles. In cultivars carrying only *Rpv3.2* (Calardis Musqué), *Rpv3.3* (Merzling), or their combination (Seyval) a comparable, high susceptibility was observed, though combined with some necrosis. In this phenotyping assay, conducted under *in vitro* conditions, these alleles seemed to



trigger a resistance mechanism that did not efficiently influence the disease development. CG 4 (2011-64-0002) was only carrying *Rpv1*. In this breeding line, *Rpv1* merely imparted partial resistance with high necrosis, reducing the disease severity for all *P. viticola* isolates equally. In CG 1 (Lela and 2012-114-0133), showing no susceptibility, the common resistance allele was *Rpv12*. No prominent necrosis or sporulation was associated with these interactions. In Lela, this locus alone resulted in powerful resistance. In the breeding line 2012-114-0133, *Rpv12* was stacked with *Rpv1* and *Rpv3.1*, causing complete inhibition of sporulation. This conclusive resistance seemed primarily mediated by *Rpv12* since the *Rpv1* and *Rpv3.1*-mediated resistance response included noteworthy necrosis.

For the other CGs, the susceptibility showed a sharp contrast between the IGs. The cultivars in CG 5 (Calardis Blanc, Villard Blanc, 2004-051-0001, Chambourcin, and Regent) were all associated with *Rpv3.1*. Contrary to the other *Rpv3* haplotypes (*Rpv3.2* and *Rpv3.3*), *Rpv3.1* seemed to provide an efficacious

resistance response against IG 3 (GPN1, FCH1, and GPN2). Necrosis was triggered upon challenge with these isolates. IG 3 isolates had been obtained from cultivars that do not carry major *Rpv* loci. In contrast, the resistance conferred by *Rpv3.1* seemed no longer efficacious against IG 2 (GRE1) and IG 1 (GPH1). The latter isolates had been isolated from Regent and Phoenix, respectively, both carrying *Rpv3.1*. All cultivars, and Villard Blanc foremost, were heavily infected by GRE1 and GPH1. No necrosis was observed after the successful infection of single *Rpv3.1*⁺ cultivars with GRE1 and GPH1. The additional presence of the weak *Rpv3.2* in Calardis Blanc or Chambourcin did not prevent the breakdown of the *Rpv3.1*-mediated resistance by these isolates. The breeding line 2004-051-0001, carrying *Rpv10* on top of *Rpv3.1*, was less susceptible to GRE1 compared to the other cultivars in CG 5. This breeding line still showed major susceptibility to GPH1.

Leaf disks of CG 3 (Seyve Villard 39-639 and Solaris) were susceptible to GPH1 but resistant to the other isolates. Despite

its strong resistance, Seyve Villard 39-639 is currently known to carry only *Rpv3.1*. Seyve Villard 39-639 behaved remarkably similar to Solaris in this phenotypic evaluation. Solaris is known to carry *Rpv3.3* and *Rpv10*. Since *Rpv3.3* on its own did not reduce the disease severity in CG 2, *Rpv10* is expected to be its primary source of resistance against the IG 2 (GRE1) and IG 3 (GPN1, FCH1, and GPN2). GPH1 (IG 1) was able to sporulate on the *Rpv10*⁺ cultivar Solaris, though not as densely as on *Rpv3.1*⁺ cultivars (CG 5).

Disease Evaluation on Plants and Detached Leaves

The phenotyping assay showed that *Rpv12* conferred the strongest resistance, while *Rpv3.1*- and *Rpv10*-mediated resistances were isolate-specific. To further examine these interesting interactions, the phenotyping assay was extended with a detached leaf assay (Figure 4A) and plant assay (Figures 4B,C). Representatives of CG 1 (Lela), CG 3 (Solaris), and CG 5 (Regent), were challenged with representatives of IG 1 (GPH1), IG 2 (GRE1), and IG 3 (GPN1). Pathogen inoculum was used to spray-inoculate the cultivars, either via *in vitro* inoculation of detached leaves of adult greenhouse plants, or via *in planta* inoculation of acclimatized plantlets obtained through *tissue culture*. In the latter, Chardonnay (CG 2) was included as a susceptible control. The results of both assays complemented the leaf disk assay, used for phenotyping (Figure 3). In all three assays, a high disease severity was observed in the interaction of Regent (*Rpv3.1*) with the isolates GRE1 and GPH1, comparable to the interaction with cultivars carrying no major resistance alleles, like Chardonnay. Interestingly, the isolate-specificity of the interaction between Solaris (*Rpv3.3* and *Rpv10*) and GPH1 was revealed in the three assays, although the degree of sporulation varied between the assays. GPH1 sporulated less on intact Solaris plantlets compared to intact Regent or Chardonnay plantlets (Figure 4B). Both Regent and Solaris showed high resistance to GPN1 (Figures 4A,B), associated with necrosis (Figure 4C). Infections caused by GRE1 and GPH1 were associated with a reduced degree of necrosis compared to GPN1 infections, except in Solaris plants infected with GPH1, where an increased degree of necrosis was apparent (Figure 4C). None of the isolates was able to sporulate or induce necrosis in Lela (*Rpv12*). These results confirm the powerful resistance of Lela to *P. viticola* observed in the leaf disk assay.

Phytohormone Analysis

To assess the involvement of phytohormones in the defense underlying *Rpv3.1*, *Rpv10*, and *Rpv12*, the hormonal responses before and during some specific interactions were compared. The isolate-specific behavior of *Rpv3.1* and *Rpv10*, in particular, allowed to examine the mechanisms underlying these resistances in more detail. The phytohormone content before and during the first stages of the interaction with the different pathogen phenotypes IG 1 (GPH1), IG 2 (GRE1), and IG 3 (GPN1) was determined. The basal differences in hormone content were determined in non-infected leaves of susceptible cultivars (Diana and Chardonnay, representing CG 2) and resistant cultivars (Lela, Solaris, and Regent as representatives of CG 1, CG

3, and CG 5, respectively) (Table 3). These leaves had been collected in three conditions in which *P. viticola* can easily infect. Uninfected leaves were collected from young plantlets (in the plant assay) or from adult greenhouse plants. Leaves from adult plants were sampled either immediately following detachment from the plant, or after 8 h of detachment on water agar (in the detached leaf assay). To assess the phytohormone content during the early stages of the interaction between CG 1, CG 3, and CG 5 (Lela, Solaris, and Regent respectively) with IG 1, IG 2, and IG 3 (isolates GPH1, GRE1, and GPN1 respectively), the levels of ABA, IAA, SA, and JA were determined in leaves from the detached leaf assay (at 6, 12, and 24 hpi; Supplementary Figure 4) and plant assay (at 12 hpi; Supplementary Figure 5). In the latter, Chardonnay (CG 2) was included as a susceptible control.

Basal Phytohormone Content

In non-infected leaves, the hormone levels differed depending on the conditions and cultivar (Table 3). Although the levels of the phytohormones IAA, ABA, and SA were much lower in the young plantlets than in the adult greenhouse plants, prior or after detachment, the trends were generally similar. Of the resistant cultivars, Regent and Lela tended toward the highest and lowest basal levels of ABA, respectively. Solaris showed high basal levels of SA and JA. The JA levels might have peaked with detachment since the JA content was particularly high in detached leaves of Solaris. However, the detachment of the leaf caused the levels of JA to decrease strongly over time in Solaris (Supplementary Figure 4). ABA, IAA, and SA levels remained fairly constant during this detachment period.

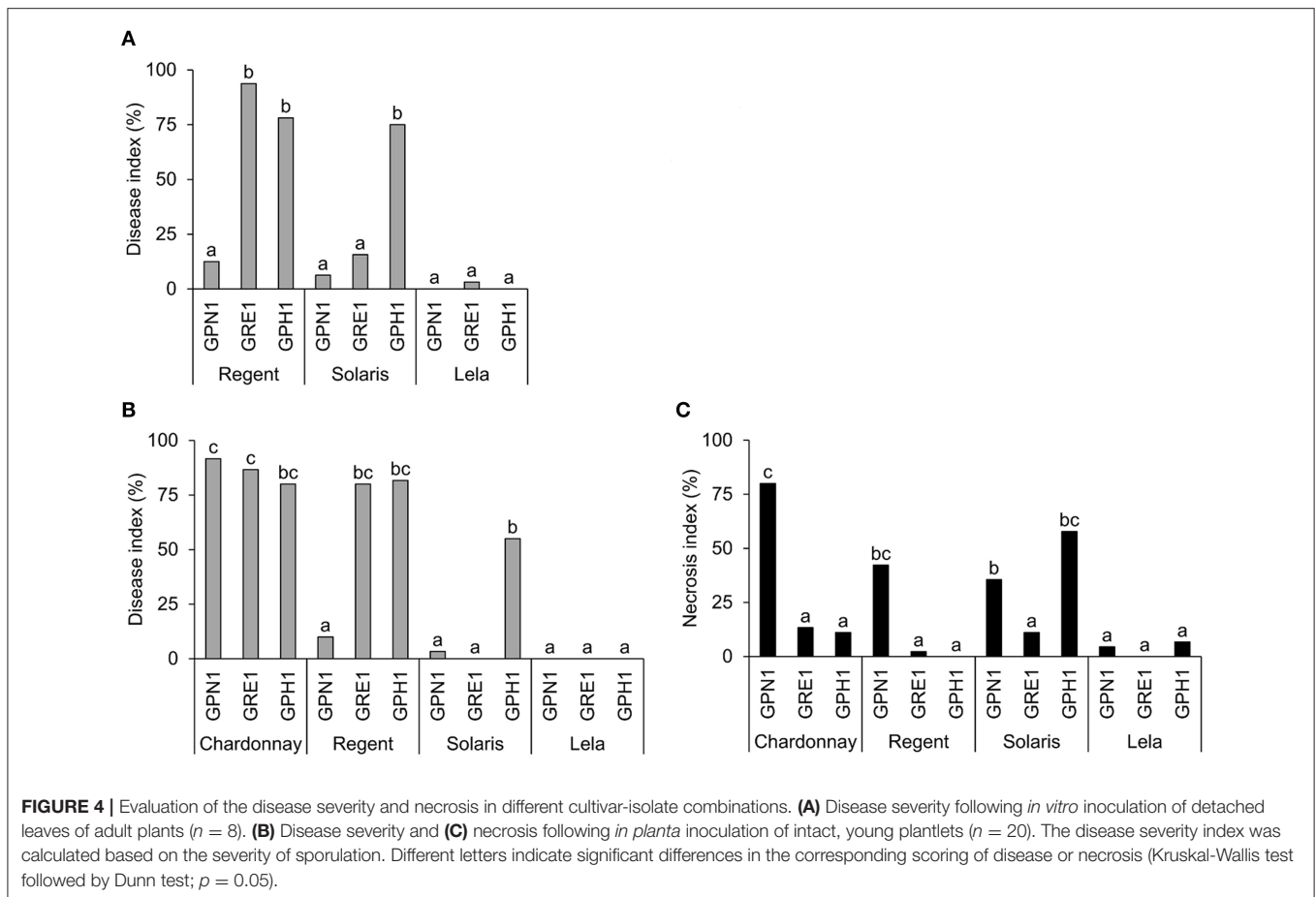
Hormonal Responses to *P. viticola*

To investigate the hormone response in the *Rpv3.1*, *Rpv10*, and *Rpv12* resistance mechanism, the phytohormone content of the resistant cultivars Regent, Solaris, and Lela was analyzed during their interaction with the isolates GPN1, GRE1, and GPH1. The analysis was first performed on leaves from the detached leaf assay, at 6, 12, and 24 hpi (Supplementary Figure 4). IAA seemed to accumulate in Regent and Solaris following inoculation with GPN1 and GRE1. However, if hormonal differences between interactions with different isolates existed, they were masked by the large biological variability. Some fluctuations were observed, not only between cultivars interacting with the same isolate but also between isolates interacting with the same cultivar. To reduce the biological variability, the phytohormone content was also measured in leaves from the plant assay at 12 hpi (Supplementary Figure 5). In the end, no obvious trends could be found when comparing the interactions of a cultivar with different isolates across both experiments.

DISCUSSION

Linking Phenotype to QTL

A set of 16 cultivars carrying different *Rpv* loci was challenged with five *P. viticola* isolates to assess the durability of some of the commonly used *Rpv* loci and the influence of stacking and



heritage. We were able to distinguish different host phenotypes based on the severity of sporulation. The phenotyping assay was able to efficiently group the cultivars according to their susceptibility to the isolates. The level of necrosis proved to add valuable information to the assay. The specific symptomatologies could be related to the presence of different *Rpv* loci. However, carriers of the same resistance allele also exhibited slightly varying degrees of resistance, as shown for *Rpv3.1*. Differences in historic use and genetic background may be the cause of these variations in phenotypes (Sánchez-Mora et al., 2017).

Regarding the isolates obtained from susceptible *V. vinifera* cultivars (GPN1, GPN2, and FCH1), the resistances derived from the Asian *V. amurensis*, *Rpv12* and *Rpv10*, were extremely efficient in restricting the sporulation. A steady resistance was observed for *Rpv3.1*, derived from North-American *V. rupestris*. However, cultivars carrying *Rpv12* and *Rpv10* were much more effective in restricting pathogen growth than those carrying *Rpv3.1*, in accordance with Schwander et al. (2012), Venuti et al. (2013) and Zini et al. (2019). *Rpv1*, originating from *M. rotundifolia*, only conferred an intermediate resistance, lower than the degree of resistance provided by *Rpv3.1*, as reported by Eibach et al. (2007) and Sánchez-Mora et al. (2017). Finally, we observed that *Rpv3.2* and *Rpv3.3*, also derived from North-American *Vitis* species, were unable to reduce sporulation despite

evoking necrosis. Although *Rpv3.2* was confirmed to provide a less prominent resistance than *Rpv3.1*, some studies have found that *Rpv3.2* and *Rpv3.3* can cause mid-level resistance (Zyprian et al., 2016; Foria et al., 2018; Vezzulli et al., 2019). The observed necrosis indicates that effector-triggered immunity (ETI) was activated in all *Rpv3* haplotypes but, under the highly conducive, *in vitro* conditions, its intensity may have been insufficient to restrict pathogen growth (Foria et al., 2018).

When isolates derived from *Rpv3.1* carriers (GPH1 and GRE1) were also taken into account, it became apparent that *Rpv12* and *Rpv1* provided durable levels of resistance. The *Rpv3.1* and *Rpv10*-mediated resistances, however, appeared to be isolate-specific. *Rpv3.1* cultivars showed high susceptibility to GPH1 and GRE1. GPH1 was also able to infect cultivars carrying *Rpv10*. The sporulation on cultivars not carrying *Rpv* loci was comparable between all isolates, indicating that all isolates have a similar aggressiveness. This suggests that the isolates GRE1 and GPH1 were not simply selected for a higher aggressiveness, but for a higher virulence particularly on carriers of *Rpv3.1* and *Rpv10*, without apparent fitness costs. While *Rpv10* could efficiently restrict the development of GRE1, this QTL did not halt the spread of isolate GPH1. This suggests that the loci *Rpv3.1* and *Rpv10*, single or stacked, might not result in a sustainable resistance. A more durable stacking partner for *Rpv3.1* or *Rpv10*

TABLE 3 | Basal phytohormone levels in leaves of different cultivars, according to the sampling condition.

Condition	Cultivar	ABA	IAA	JA	SA
Detached leaves (<i>n</i> = 3)	Regent	494.07 ± 42.82 (b)	12.13 ± 1.33 (a)	2.68 ± 1.67 (ab)	489.39 ± 41.52 (a)
	Solaris	294.85 ± 78.85 (ab)	10.74 ± 2.08 (a)	15.47 ± 7.41 (b)	1,219.72 ± 713.15 (a)
	Lela	78.88 ± 12.2 (a)	13.71 ± 2.54 (a)	0.13 ± 0.22 (a)	423.17 ± 112.95 (a)
Adult plants (<i>n</i> = 5)	Diana	217.44 ± 32.45 (a)	12.32 ± 0.84 (a)	2.59 ± 0.79 (bc)	139.81 ± 15.41 (a)
	Regent	586.55 ± 101.92 (c)	23.16 ± 17.69 (a)	1.23 ± 0.66 (a)	218.77 ± 129.75 (a)
	Solaris	360.18 ± 53.25 (bc)	13.07 ± 1.13 (a)	6.99 ± 3.37 (c)	1,156.5 ± 488.06 (b)
Young plantlets (<i>n</i> = 5)	Lela	239.23 ± 58.02 (ab)	13.61 ± 1.21 (a)	1.78 ± 0.19 (ab)	229.79 ± 162.59 (a)
	Chardonnay	445.17 ± 177.33 (b)	3.61 ± 0.3 (a)	2.54 ± 1.34 (a)	53.63 ± 9.21 (a)
	Regent	130.19 ± 35.47 (ab)	6.02 ± 0.72 (a)	1.32 ± 0.43 (a)	64.08 ± 16.81 (a)
	Solaris	96.57 ± 40.58 (a)	4.03 ± 0.87 (a)	2.39 ± 2.24 (a)	115.53 ± 33.67 (a)
	Lela	65.12 ± 67.7 (a)	7.3 ± 9.23 (a)	4.11 ± 2.99 (a)	198.3 ± 300.75 (a)

Either the leaves had been detached from adult greenhouse plants on water agar for 8 h, or they were sampled directly from adult greenhouse plants or young plantlets. The mean and standard deviation of *n* observations are shown. Each observation existed of two pooled leaves from the same plant. Different letters indicate significant differences between the cultivars (Kruskal-Wallis followed by Dunn test; *p* = 0.05).

may be found in *Rpv12* or *Rpv1* since both QTLs were shown to efficiently inhibit a *P. viticola* population that breaks through *Rpv3*-mediated resistance (Calonnec et al., 2013; Venuti et al., 2013).

Isolate-Specific Interaction With *Rpv3.1* and *Rpv10*

The heavy sporulation and absence of a hypersensitive response (HR) indicated that isolates GRE1 and GPH1, derived from *Rpv3.1*⁺ cultivars, were able to overcome *Rpv3.1*-mediated resistance. The breakdown of the resistance conferred by *Rpv3.1* was first described by Peressotti et al. (2010). The inter-isolate variation in the *Rpv3.1* resistance was attributed to a putative mutation in the avirulence factor, through which these isolates escape recognition by the *Rpv3.1* locus and fail to trigger HR and resistance on *Rpv3.1* carriers (Peressotti et al., 2010). This mutation is suggested to be facilitated by the narrow recognition capabilities of the *Rpv3* gene product (Casagrande et al., 2011), allowing *P. viticola* to adapt in a couple of years (Delmotte et al., 2014). The large spatial scale at which these *avrRpv3.1*⁻ isolates are found (Delmotte et al., 2014) and the restricted gene flow in *P. viticola* populations in Europe (Gobbin et al., 2006; Fontaine et al., 2013) suggested that this fast adaptation occurred at multiple sites independently. Moreover, genetic evidence showed these *avrRpv3.1*⁻ isolates had indeed adapted from the European gene pool and did not segregate into a separate phylogenetic group (Peressotti et al., 2010; Delmotte et al., 2014).

Despite the fast and multiregional adaptation of European *P. viticola* to *Rpv3.1*, *P. viticola* isolates overcoming other *Rpv* genes in the grapevine-downy mildew interaction have not specifically been reported. However, Li et al. (2015) identified a Chinese *P. viticola* isolate able to break down the resistance of *V. amurensis*. We demonstrated that only isolate GPH1 can sporulate heavily on cultivars carrying *Rpv10*. The idea that specific isolates have gained virulence against *Rpv10*, is supported by the instability of the *Rpv10* resistance of cultivars across studies, using different *P. viticola* isolates or field mixtures. Although many studies have found *Rpv10*⁺ vines to be remarkably resistant to *P. viticola*

(Boso and Kassemeyer, 2008; Vezzulli et al., 2018), other studies do not entirely confirm this foliar resistance (Gómez-Zeledón et al., 2013; Possamai et al., 2020). Moreover, in Cabernet Cortis (*Rpv3.3*, *Rpv10*), offspring of Solaris, isolates of *P. viticola* have shown varying levels of sporulation (Gómez-Zeledón et al., 2013). The behavior of field isolate 1136 described by Gómez-Zeledón et al. (2013), sporulating profusely on Regent and Cabernet Cortis, is phenotypically similar to that of our isolate GPH1.

Interestingly, both GPH1 and 1136 were derived from *Rpv3.1*⁺ cultivars. The adaptation to *Rpv10* might have been inherited or developed during an earlier encounter with an *Rpv10*⁺ cultivar, but it also possible that, in adapting to *Rpv3.1*, these isolates simultaneously gained virulence on *Rpv10*⁺ cultivars (so-called cross-virulence). This is supported by our observation that GPH1 sporulated more profusely on *Rpv3.1*⁺ cultivars than on *Rpv10*⁺ cultivars. The necrotic response to GPH1 in Solaris was retained, yet this could also be attributed to the *Rpv3.3*-mediated resistance. Cross-virulence would also imply that the adaptation of GPH1 to overcome *Rpv3.1*-mediated resistance, is substantially different from the adaptation of GRE1. Gómez-Zeledón et al. (2013) identified an isolate like GRE1, isolate 1137, capable to break through the resistance of Regent (*Rpv3.1*) but with reduced sporulation on the *Rpv10*⁺ cultivar. Field isolate 1136 may be more efficiently adapted to *Rpv3.1* compared to isolate 1137, because it sporulated more profusely on *V. rupestris* (Gómez-Zeledón et al., 2013), the donor of *Rpv3.1* (Di Gaspero et al., 2012). After all, the intensity of the *Rpv*-dependent defense is assumed to be highest in wild-type genetic backgrounds and decreases with an increasing number of backcross generations (Foria et al., 2018).

It is not unlikely that plant pathogens overcoming one resistance source, can gain virulence on another, even when the resistance loci are distinct and the isolates have never been exposed to that resistance source. It is possible that the presence of one QTL might have selected for genetic adaptations linked to genes responsible for the development of virulence on other QTLs. A similar observation of cross-virulence was made by Eisenmann et al. (2019), who suggested

Rpv3.1 and *Rpv3.2* may recognize the same effector. The most obvious explanation for cross-virulence would be the two QTLs sharing similarities in resistance mechanism. From what is known, there are many similarities between *Rpv3.1* and *Rpv10*-mediated resistance. The early colonization of *Rpv3.1*⁺ cultivars (Eisenmann et al., 2019) and Solaris or the *Rpv10* wild-type *V. amurensis* (Boso and Kassemeyer, 2008; Jürges et al., 2009) does not differ from cultivars carrying no major resistance genes. The *Rpv3.1*-mediated resistance has been traced to a TIR-NB-LRR (Toll/interleukin-1 receptor-nucleotide binding-leucine-rich repeats, also known as *TNL*) gene pair, which triggers pathogen-induced necrosis when transiently co-expressed (Foria et al., 2020). The necrotic response occurred from 32 hpi (Bellin et al., 2009; Casagrande et al., 2011; Eisenmann et al., 2019; Vezzulli et al., 2019) and hinders the pathogen development in the mesophyll air spaces from 48 hpi (Foria et al., 2018; Eisenmann et al., 2019). Accumulation of peroxidase (Kortekamp et al., 1998) and induction of the biosynthesis of fungitoxic stilbenes (Eisenmann et al., 2019) have been implicated in this resistance. Stilbenoids were also implicated in the *Rpv3.3*-mediated resistance (Malacarne et al., 2011; Vezzulli et al., 2019). Similarly, the resistance of Solaris has been linked to a necrotic response (Gindro et al., 2003; Boso and Kassemeyer, 2008) and the induction of fungi-toxic stilbenes (Pezet et al., 2004; Alonso-Villaverde et al., 2011), as well as the fast synthesis of callose in infected stomata at 7 hpi (Gindro et al., 2003). Isolate GPH1 may have developed a strategy to circumvent or suppress the *Rpv3.1* defense, which might promote its virulence on *Rpv10*⁺ cultivars. To examine whether this is an issue of cross-virulence, or rather the independent development of virulence on *Rpv3.1* and *Rpv10*, single *Rpv10* carriers and *Rpv3.1*-breaking isolates without prior exposure to *Rpv10*⁺ would be necessary.

Phytohormones Responses in *Rpv3.1*, *Rpv10*, and *Rpv12*

Many studies have compared defense responses between susceptible and wild or cultivated resistant grapevine species (Gindro et al., 2003; Kortekamp and Zyprian, 2003; Polesani et al., 2010; Liu et al., 2015; Figueiredo et al., 2017; Lazazzara et al., 2018; Fröbel et al., 2019). The interaction between *P. viticola* and a grapevine cultivar has not often been described from the perspective of virulent and avirulent strains. The availability of *Rpv3.1*-breaking isolates allows examining the *Rpv3.1* resistance mechanism in more detail. Previous studies have reported that phytohormones play a role in the resistance of the donor species against *P. viticola*. In response to the successful infection of *P. viticola*, only small fluctuations in ABA, JA, and SA have been observed in *V. vinifera* cultivars (Liu et al., 2016; Wang et al., 2018). However, in incompatible interactions between *P. viticola* and *V. amurensis* (Liu et al., 2016) or *M. rotundifolia* (Liu et al., 2016; Wang et al., 2018), SA, JA, and ABA were found to accumulate during the first 12 h following inoculation. The discovery of the resistance-breaking isolate ZJ-1-1 allowed to also describe a compatible interaction in *V. amurensis* (Li et al., 2015). In the compatible interaction,

the fast surges in JA and SA upon pathogen inoculation were no longer observed. However, not much is known about the role of phytohormones in resistant *V. vinifera* cultivars. It has been suggested that in Regent (*Rpv3.1*), an incompatible *P. viticola* interaction triggers an early increase in α -linolenic acid and subsequent biosynthesis of JA, which is converted to jasmonic acid-isoleucine (JA-Ile) within 12 hpi (Guerreiro et al., 2016). The authors recorded peaks of endogenous JA and SA levels in response to unsuccessful *P. viticola* infections at 6 and 12 hpi.

To investigate the role of phytohormones in the *Rpv3.1*, *Rpv10*, and *Rpv12*-mediated resistance, we determined the content of ABA, IAA, JA, and SA in grapevine leaves before and during differential interactions. However, we were unable to detect isolate-specific hormonal responses during the interaction of detached leaves or intact plants with Regent, Solaris, or Lela. This is in accordance with Casagrande et al. (2011), who did not observe significant differences in the expression of genes involved in the SA, ET, and JA signaling pathway in *Rpv3*⁺/*Rpv3*⁻ cultivars in response to *avrRpv3*⁺/*avrRpv3*⁻ isolates. The hormonal responses might have been concealed by the large biological variability or by some aborted infections, which often occur in compatible interactions. Moreover, it is possible that sampling the whole leaf diluted the early response beyond detection. On the other hand, it may well be that no significant hormonal responses occurred within these early stages of infection. It is possible that the plant's early defenses were primarily activated by the pathogen-specific signaling transmission pathway, instead of the phytohormone-mediated pathway. There are some indications for the occurrence of a secondary defense mechanism. In *V. riparia*, a dramatic increase in JA was observed only at 48 hpi (Polesani et al., 2010) and in *Rpv12* carriers, genes related to SA synthesis and signaling were only differentially expressed from 48 hpi (Chitarrini et al., 2020). Moreover, the first infection steps of *Rpv3.1*⁺ cultivars (Eisenmann et al., 2019) and Solaris (Busam et al., 1997; Boso and Kassemeyer, 2008) are essentially the same as in the susceptible *V. vinifera*, even though gene expression studies show that the defenses are induced at the first interaction between host cells and pathogen hyphae (Polesani et al., 2010; Eisenmann et al., 2019). The delay and restriction of invasive pathogen growth by the *Rpv3.1*-mediated defense initiates only from 24 hpi and is effective before 48 hpi (Eisenmann et al., 2019). Therefore, it may be worthwhile to track the hormonal changes over a longer period after inoculation.

While we were unable to establish what hormonal responses are triggered during the resistance mechanism of Regent, Solaris, and Lela, we were able to distinguish some basal hormonal differences between cultivars. The relative contents observed were similar in all three conditions in which *P. viticola* can easily infect (on adult plants, on detached leaves, and on young plantlets). Given the important influence of these hormones on disease development, the levels of hormones basally present in the resistant cultivars might be associated with their basal resistance. Of the resistant cultivars, Regent tended toward the highest basal levels of ABA. Wang et al. (2018) concluded ABA in particular is

an important positive regulator of defense against *P. viticola*, because ABA was found to regulate stilbene accumulation. Solaris, on the other hand, showed high basal levels of SA and JA. SA and especially JA are hypothesized to be involved in the defense responses against *P. viticola* (Guerreiro et al., 2016). Moreover, exogenous JA has been shown to protect grapevine leaf disks against *P. viticola* through callose deposition (Hamiduzzaman et al., 2005). However, the role of the phytohormone pathway in the resistance to *P. viticola* remains to be elucidated.

The Use of a Standardized Phenotyping Assay for Grapevine Cultivars

The deployment of various combinations of *R*-loci decreases the chances of the pathogen overcoming the genetic barriers. Pyramiding *R*-loci is crucial in delaying the erosion of laboriously introgressed resistance loci, yet a higher overall disease resistance is a great bonus. However, estimating disease resistance based on the QTLs is not straightforward. Stacking might result in higher disease resistance, as observed for *Rpv12*, *Rpv1*, and *Rpv3.1* in 2012-114-0133, resulting in absence of sporulation. For a higher disease resistance, the strength of the resistances is of great importance. For example, we did not observe a clear additive effect in stacking with the weaker QTLs *Rpv3.2* or *Rpv3.3*. Still, the resistance resulting from pyramiding seems to be strongly reliant on the genetic background. Despite the strong resistance provided by both *Rpv10* and *Rpv3.1*, stacking of *Rpv10* and *Rpv3.1* in 2004-051-0001 resulted in a reduced resistance compared to its parent Solaris (*Rpv10* and *Rpv3.3*). Schwander et al. (2012) did demonstrate an additive effect of *Rpv10* and *Rpv3.1* on the degree of resistance, but both *Rpv3*- and *Rpv10*-dependent responses have been demonstrated to attenuate with backcrossing (Foria et al., 2018; Possamai et al., 2020). Progeny of Solaris carrying only *Rpv10* has even been shown to exhibit a disease severity similar to vines not carrying *Rpv* loci (Possamai et al., 2020). Although the availability of molecular markers facilitates the screening of QTL carriers (Eibach et al., 2007), the presence of the QTLs is insufficient to predict the strength of the disease resistance. A general phenotyping assay using *Rpv*-breaking isolates would allow easy classification of resistant phenotypes. Most phenotyping studies focus only on the characterization of the susceptibility of European cultivars and resistant cultivars against a field population of *P. viticola* (Boso and Kassemeyer, 2008; Boso et al., 2014). When performed against a characterized set of isolates, the phenotyping of resistant cultivars would become more informative and would provide sufficient power to discriminate partial and isolate-specific resistance. We observed a large variability between cultivars, not only in sporulation, but also in necrosis. Ideally, the assay would combine a measure for sporulation with necrosis, as proposed by Gómez-Zeledón et al. (2017). The phenotyping assay can also be applied as an empirical method for screening of interesting genetic resources. Seyve Villard 39-639 is currently only known to carry *Rpv3.1*. However, its strong resistance and isolate-specific susceptibility in our phenotyping assay implies another factor might be

influencing the resistance to *P. viticola*, which was not inherited by Calardis Blanc.

The Use of a Standardized Pathotyping System for *P. viticola*

While the host population is relatively well-described, at least in terms of parentage and QTLs, many aspects of the pathogen population remain undisclosed. The existence of different pathotypes could offer an explanation for the strong variability of downy mildew resistance, observed between years (Savary et al., 2009; Calonnec et al., 2013; Vezzulli et al., 2018; Zini et al., 2019) and between studies (Lisek, 2010; Pavloušek, 2012; Vezzulli et al., 2018; Zini et al., 2019). Contrary to our results, for example, the resistance of Lela (*Rpv12*) was reported only to be mid-level, below *Rpv3.1* resistance (Pavloušek, 2012), while Seyval and Regent were found highly resistant (Lisek, 2010; Vezzulli et al., 2018). Especially for partially resistant genotypes, the pathogen strain is of great importance for the resistance outcome (Possamai et al., 2020). However, many resistance studies have been using field mixtures, yearly collected in the field. The determination of pathotypes and the use of genetically homogeneous *P. viticola* material would allow comparing studies in time and space and future-proofing these results for the dynamic pathogen population. The increasing deployment of *Rpv*-loci and the ability of *P. viticola* to recombine each year could facilitate the breakdown of *Rpv*-mediated resistances.

The diversity of *P. viticola* has mainly been studied with regards to population studies and fungicide resistance, for which genotyping was facilitated by molecular markers, such as RAPD (Stark-Urnau et al., 2000), microsatellites (Gobbin et al., 2003; Delmotte et al., 2006; Rouxel et al., 2012), AFLP (Scherer and Gisi, 2006) and SNP (Chen et al., 2007; Delmotte et al., 2011). However, these molecular markers have not been designed to describe the pathotypes prevalent in current populations (Gessler et al., 2011). Pathotypes are often outlined by a unique pattern of reactions on different resistant cultivars (Crute and Johnson, 1976). Although described for many other downy mildews, such as *Plasmopara halstedii* (Trojanová et al., 2017), a uniform bioassay-based differentiation of pathotypes has not yet been established for *P. viticola*. The perennial nature of grapevine complicates the conduction of this assay and its practical use, since crop rotation in response to the findings is hard. The pathotyping system is also strongly dependent on the use of single isolates, experimental conditions, and fitness of plant and pathogen. Even so, a global characterization system of standardized host genotypes, using a nomenclature system as defined by Limpert and Müller (1994), could be very useful for those interested in *P. viticola* pathotypes and for plant breeding programs in particular. Moreover, an identification of the prevalent races within a local, regional, or large-scale population using a small set of differential cultivars could be an important advisory tool. Finally, such a system could be paramount to understanding how a pathotype is overcoming the resistance of its host. A pathotyping system and an increased understanding of the

pathosystem might be necessary for the reliable, durable control of the pathogen.

CONCLUSION

To evaluate the durability of some of the commonly used *Rpv* loci, the influence of stacking, and the virulence diversity of *P. viticola*, a set of 16 grapevine cultivars, carrying different *Rpv* loci, was challenged with five *P. viticola* isolates. The cultivars and isolates were grouped according to the evaluation of the severity of sporulation. The level of necrosis provided additional, valuable information about the activation of the resistance mechanism. To a great extent, the specific symptomatologies could be related to the presence of major *Rpv* loci. Pyramiding of resistances did not always result in durable or increased disease resistance. More than the presence of QTLs, this phenotyping assay can predict the strength of the host resistance to *P. viticola*. When performed against a characterized set of isolates, a phenotyping assay based on sporulation and necrosis would provide sufficient power to discriminate partial and isolate-specific resistances in cultivars. However, this requires *P. viticola* isolates to be characterized. Conversely, using standardized host genotypes, such a bio-assay could also be employed to identify pathotypes of *P. viticola*. Currently, no uniform characterization system has been established for *P. viticola*. The determination of pathotypes would allow comparing studies of this pathosystem in time and space. Moreover, the characterization of pathotypes could serve as an advisory tool informing about the prevalent races and help to assess how the host's resistance is overcome. An increased understanding of both members of the pathosystem is necessary for the reliable, durable control of the pathogen.

An efficient resistance was observed for *Rpv12*, *Rpv10*, and *Rpv3.1* against the isolates obtained from susceptible *V. vinifera* cultivars. *Rpv1* only conferred partial resistance and *Rpv3.2* and *Rpv3.3* failed to restrict sporulation. The sporulation and necrosis on *Rpv3.1*⁺ and *Rpv10*⁺ hosts, however, were found to be isolate-specific. The isolates derived from *Rpv3.1*⁺ hosts, GRE1 and GPH1, were able to sporulate intensely on cultivars carrying *Rpv3.1*, without triggering necrosis. Moreover, *Rpv10* was not able to efficiently halt the development of the *Rpv3.1*-breaking isolate GPH1. This isolate could have developed an adaptation to *Rpv10*, during an earlier encounter with a *Rpv10*⁺ cultivar, or might have gained virulence on *Rpv10*⁺ cultivars because of its adaptation to *Rpv3.1*. This suggests that the loci *Rpv3* and *Rpv10*, single or stacked, do not result in the most sustainable resistance. To assess the resistance mechanisms underlying *Rpv3.1*, *Rpv10*, and *Rpv12* and the infection mechanisms of the different isolates, the hormonal responses in differential interactions were compared. Phytohormones are known to play a role in resistance to *P. viticola* and might be implicated in the basal resistance of a cultivar against this pathogen. In the defense response, however, the role of phytohormones remains to be elucidated. No significant differences were observed in the levels of ABA, IAA, JA or SA levels in response to the isolates during the first 24 hpi. Nevertheless, this does not preclude their involvement in the defense response.

The knowledge gained in this paper may help breeders assess the durability of resistance in their varieties and to communicate recommendations for effective plant protection measurements to the wine growers. Furthermore, it may help grapevine breeders choose the most suitable parents for their crossings in order to develop new varieties with pyramided resistances against a broad range of *P. viticola* isolates.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in **Supplementary Table 1**. Further inquiries can be directed to the corresponding author.

AUTHOR CONTRIBUTIONS

LH, RH, AK, RT, and MH contributed to the conceptualization and design of the study. Resources were provided by RH, AK, EA, RT, and MH. LH conducted the experiments and formal analysis. LH wrote the first draft of the manuscript, supervised by MH. EA, RT, and MH contributed to the funding acquisition. All authors contributed to manuscript revision, read, and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fagro.2021.684023/full#supplementary-material>

Supplementary Figure 1 | Simplified pedigree showing the biological relationship between the different cultivars used in this study (in gray boxes). The cultivars of origin of the *P. viticola* isolates are indicated with a bold outline. Full and dashed lines mark direct and distant progeny, respectively. For resistant cultivars, the ancestral species that are known to be crossed with *V. vinifera* are shown on the left. If none are indicated, the cultivar is expected to belong to *V. vinifera* subsp. *vinifera*. The presence of the major loci conferring resistance to *P. viticola* (*Rpv*) carried by the cultivars used in this study is indicated underneath the corresponding cultivar or species group. The overview of the resistance traits and the species of origin is limited to the information currently available.

Supplementary Figure 2 | Overview of the symptoms observed in the phenotyping assay. One representative leaf disk is shown for each cultivar-isolate interaction.

Supplementary Figure 3 | Disease severity caused by five different isolates of *P. viticola* on a set of cultivars. For each interaction, leaf disks were assigned to disease classes ranging from 0 (no sporulation) to 4 (numerous sporangiophores). Different letters indicate significant differences in the interaction between isolates with the same cultivar (Kruskal-Wallis followed by Dunn test; $p = 0.05$).

Supplementary Figure 4 | Course of the levels of ABA, IAA, SA, and JA in detached leaves of the resistant cultivars Regent, Solaris, and Lela during the interaction with *P. viticola*. Sampling occurred 6, 12, and 24 h after spray-inoculation with water or with the isolates GPN1, GRE1 or GPH1. No

significant differences were found in the interaction between isolates with the same cultivar at the same time point (Kruskal-Wallis followed by Dunn test; $p = 0.05$).

Supplementary Figure 5 | Levels of ABA, IAA, SA, and JA in young plantlets of Chardonnay and the resistant cultivars Regent, Solaris, and Lela during the interaction with *P. viticola*. Sampling occurred 12 h after spray-inoculation with

water or with the isolates GPN1, GRE1 or GPH1. Different letters indicate significant differences in the interaction between isolates with the same cultivar (Kruskal-Wallis followed by Dunn test; $p = 0.05$). When statistical differences are absent, no letters are shown.

Supplementary Table 1 | Overview of the data presented in this study.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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