# Anatomic and genetic factors associated with the susceptibility of grapevine cultivars to Plasmopara viticola clade aestivalis and clade riparia

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Article

# Anatomic and genetic factors associated with the susceptibility of grapevine cultivars to *Plasmopara viticola* clade *aestivalis* and clade *riparia*

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## Abstract

The susceptibility of commonly grown grapevine cultivars in the province of Quebec to *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni clades *riparia* and *aestivalis* was compared. The relationship between anatomic (density and size of stomata and type of leaf hairs) and genetic composition (percentages of *Vitis vinifera* L., *Vitis riparia* Michx. and *Vitis aestivalis* Michx.) factors of the grapevine cultivars and their susceptibility to the two clades was investigated. The grapevine cultivars were classified according to their susceptibility to each clade. The aggressiveness of the clade *riparia* was positively correlated with stomata size and negatively correlated with type of leaf hairs. However, the aggressiveness of clade *aestivalis* was positively correlated with stomata size and density, estimated percentage of *V. vinifera* ancestry, and the published downy mildew susceptibility of grapevine cultivars; and negatively correlated with estimated percentage of *V. riparia* ancestry. Furthermore, the grapevine cultivar classification showed that, for *P. viticola* clade *riparia*, 44.4%, 44.4%, and 11.1% of the grapevine cultivars were classified as minimally susceptible, moderately susceptible, and highly susceptible, respectively. Alternatively, for *P. viticola* clade *aestivalis*, 11.1%, 22.2%, and 66.7% of the grapevine cultivars were classified as minimally susceptible, moderately susceptible, and highly susceptible, respectively. Although some grapevine cultivars fell in the same susceptibility groups for both clades, 78% of grapevine cultivars were classified in different susceptibility groups. The findings of this study provide new information on grapevine and *P. viticola* interactions, and highlight the importance of knowing which clade of *P. viticola* is present so that downy mildew control measures can be adapted accordingly.

**Key words:** aggressiveness, downy mildew, comparative epidemiology

## Résumé

La sensibilité des cépages de vigne couramment cultivés au Québec à *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni clades *riparia* et *aestivalis* a été déterminée sur disques de feuilles et sur feuilles entières sur plants. Par la suite, la relation entre les caractéristiques anatomiques (taille et densité des stomates, et poils foliaires) et génétiques (pourcentages de l'héritage génétique de *Vitis vinifera* L., *Vitis riparia* Michx. et *Vitis aestivalis* Michx.) des cépages et leur sensibilité aux deux clades a été étudiées, et les cépages classés en fonction de leur sensibilité à chacun des clades. L'agressivité du clade *riparia* était positivement corrélée à la taille des stomates et négativement corrélée aux poils foliaires. Tandis que l'agressivité du clade *aestivalis* était positivement corrélée à la taille et la densité des stomates, au pourcentage estimé de l'ascendance de *V. vinifera*, et aux données publiées de sensibilité des cépages, et était négativement corrélée au pourcentage estimé de l'ascendance de *V. riparia*. Par ailleurs, les résultats issus de la classification des cépages ont montré pour ce qui est du clade *riparia* que 44,4%, 44,4% et 11,1% des cépages étaient respectivement classés peu sensibles, moyennement sensibles et très sensibles. Cependant, pour ce qui est du clade *aestivalis*, 11,1%, 22,2% et 66,7% des cépages étaient respectivement classés peu sensibles, moyennement sensibles et très sensibles. Bien que certains cépages aient été classés dans les mêmes groupes de sensibilité aux deux clades, 78% des cépages étaient classés dans des groupes de sensibilité différents. Les résultats issus de cette étude apportent des informations essentielles pour une meilleure compréhension de l'interaction entre la vigne et *P. viticola*, et pour une gestion raisonnée et durable du mildiou de la vigne.

**Mots-clés :** épidémiologie comparative, agressivité, mildiou de la vigne

## Introduction

Grapevine (*Vitis* spp.) is one of the most widely cultivated fruit plants in the world, with more than 7 million hectares of vineyards and global production of around 79 million tons in 2018 (http://faostat.fao.org). However, the most prized grapevine cultivars are often highly susceptible to diseases such as downy mildew (Dai et al. 1995; Gessler et al. 2011; Carisse 2016). Downy mildew, which is native to North America, is caused by *Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni, which is an oomycete (Berlese and De Toni 1888; Gehmann 1987; Vercesi et al. 1999; Carisse 2016). *Plasmopara viticola* infects the green leaves through stomata, which represent the main pathway of entry into the tissues. In fact, the germ tube of a zoospore clings to the substomatal cavities and forms infection vesicles and haustoria which collect nutrients (Kiefer et al. 2002; Gindro et al. 2003).

Since the discovery of this pathogen, many researchers have shown that the susceptibility of grapevine cultivars to *P. viticola* is variable (Bush and Meissner 1883; Ravaz 1914; Nysterakis 1946; Li and Doazan 1986; Yu et al. 2012; Boso et al. 2014). These researchers have concluded that the European grapevine cultivars of the *Vitis vinifera* L. species are very susceptible compared with American cultivars of the *Vitis riparia* Michx., *Vitis aestivalis* Michx., *Vitis cinerea* Engelm. ex Millardet, *Vitis labrusca* L., *Vitis rupestris* Scheele, *Vitis berlandieri*, *Vitis aestivalis* var. *lincecumii* (Buckley) Munson, and *Vitis rotundifolia* Michx. species. In addition to these observations, several studies have shown that there is variability in susceptibility between different grapevine cultivars of the same species and between interspecific hybrids of these species (Dai et al. 1995; Kortekamp et al. 1998; Unger et al. 2007; Cadle-Davidson 2008; Yu et al. 2012; Boso et al. 2014, 2016).

In the province of Quebec (eastern Canada), the most cultivated grapevine cultivars are European–American hybrid (Carisse 2016). Dubé and Turcotte (2011) and Provost and Barriault (2019) have highlighted the variability in susceptibility of European–American hybrid grapevine cultivars grown in Quebec. These researchers reported that the hybrid grapevine cultivars Frontenac Blanc, Frontenac Gris, Frontenac Noir, and Marquette are resistant, while Vidal, Hibernal, Gewurztraminer, Chancellor, and Sainte Croix are very susceptible to *P. viticola*. They also reported that the European grapevine cultivars of the *Vitis vinifera* species Cabernet franc, Cabernet Sauvignon, Chardonnay, Gamay, Merlot, Pinot Gris, Pinot Noir, Riesling, and Sauvignon Blanc grown in Quebec are very susceptible to *P. viticola*. Alonso-Villaverde et al. (2011) attributed this variability in susceptibility to the anatomical, biochemical, and genetic characteristics of the grapevine cultivars. Some cultivars have developed long leaf hairs (LHs) that reduce the wettability of the abaxial leaf surface and protect the grapevine from the penetration of *P. viticola* (Kortekamp et al. 1999; Kortekamp and Zyprian 1999). Histological studies have shown that the cell walls of some grapevine cultivars, with their complex macromolecule composition (glycoproteins, polysaccharides, and phenolic compounds) and their ability to change during a period of stress (lignification and formation of calloses), provide mechanical protections against *P. viticola* infections (Dai et al. 1995; Kortekamp and Zyprian 1999; Yu et al. 2012). Furthermore, results of several gene expression studies show that the interaction between some grapevine cultivars and *P. viticola* induced activation of pathogenesis-related (PR) proteins and enzymes involved in the production of reactive oxygen species (ROSs), calloses, lignins, hypersensitivity reactions, and phytoalexins (Kortekamp 2006; Casagrande et al. 2011; He et al. 2013; Banani et al. 2014; Yu et al. 2016; Buonassisi et al. 2017). In grapevine, susceptible cultivars are usually considered as those that do not actively respond to *P. viticola* infection by an effector-triggered-immunity (ETI)-based mechanism, while resistant cultivars are those that establish a set of ETI-based responses against the pathogen (Peressotti et al. 2010; Casagrande et al. 2011). These ETI-based responses are provided by the Rpv ("Resistance to *P. viticola*") genes of the QTL ("quantitative trait loci") regions (Merdinoglu et al. 2003; Fischer et al. 2004; Peressotti et al. 2010; Casagrande et al. 2011).

Despite the improved understanding gained from literature reports on the interaction between *P. viticola* and grapevines, it is difficult to develop a universal classification of grapevine cultivars according to their level of susceptibility to *P. viticola*. These inconsistent susceptibility ratings are observed both among genotypes of the European species, which is in general a compatible host for this pathogen, and among derivatives of interspecific hybrids, including those that have proved to have incompatible interaction with the same pathogen species. In fact, some grapevine cultivars have been classified as susceptible in some studies but as minimally susceptible or resistant in other studies. For example, the European grapevine cultivars of the *Vitis vinifera* species Cabernet Sauvignon and Chardonnay, which were both classified as susceptible by Yu et al. (2012), were classified as minimally susceptible by Boso and Kassemeyer (2008) and moderately susceptible by Dubé and Turcotte (2011). These differences in the classification of grapevine cultivars' susceptibility could be related to a difference in the aggressiveness of different *P. viticola* populations. Several studies have investigated the genetic diversity of *P. viticola* populations (Gobbin et al. 2005; Rumbou and Gessler 2006; Rouxel et al. 2012). In 2013, a study by Rouxel et al. (2013) on *P. viticola* populations showed that there are five clades of *P. viticola* (*P. viticola* clade *vinifera*, clade *riparia*, clade *aestivalis*, clade *quinquefolia*, and clade *vulpina*) and that these are specific to certain grapevine cultivars. Because of this host specificity, the prevalence of a clade in a given region depends on the grapevine cultivars grown there (Rouxel et al. 2013, 2014). Following the description of *P. viticola* clades, several studies have been undertaken to identify the prevalence of these clades in different regions of the world (Camargo et al. 2019; Hong et al. 2019; Taylor et al. 2019; Carisse et al. 2021).

In the province of Quebec, only *P. viticola* clade *aestivalis* and clade *riparia* were detected and, in general, clade *aestivalis* is detected more often than clade *riparia* (Carisse et al. 2021).

In addition, Mouafo-Tchinda et al. (2021, 2022) showed that clade *aestivalis* is more aggressive and more competitive than clade *riparia* on the Vidal grapevine cultivar. Despite these epidemiological advances, little is known about the susceptibility of the grapevine to these two clades. Yet knowledge of the level of grapevine susceptibility to these two clades is essential not only for the selection of cultivars for new plantings, but also for within-season downy mildew management decisions in regions of the world where these clades are present.

Therefore, the objectives of this study were (*i*) to determine the susceptibility of the most commonly grown grapevine cultivars in Quebec to *P. viticola* clades *riparia* and *aestivalis*, (*ii*) to investigate the relationship between anatomic and genetic factors of the grapevine cultivars and their susceptibility to *P. viticola* clades *aestivalis* and *riparia*, and (*iii*) to classify the grapevine cultivars according to their susceptibility to each clade.

## Materials and methods

### Fungal material and inoculum production

The isolates used in this study originated from the *P. viticola* collection stored in our laboratory. Sampling of infected leaves and storage of *P. viticola* were performed as described by Mouafo-Tchinda et al. (2021). Briefly, grapevine leaves with sporulating downy mildew lesions were collected at six vineyards located in Montérégie (latitude: 45°23′14.03″N; longitude: −73°06′2.76″W), Quebec, Canada. The isolates of *P. viticola* clade *riparia* were collected from diseased leaves of hybrid grapevine cultivars Vidal, Chancellor, Sainte Croix, and Saint Pépin and European grapevine cultivar Chardonnay, while those of *P. viticola* clade *aestivalis* were collected from diseased leaves of hybrid grapevine cultivars Vidal, Pinot Noir, Saint-Croix, Saint-Pépin, Vandal-cliche, and European grapevine cultivar Chardonnay. Pieces of leaves (1 $cm^2$) with sporangia (three pieces/sample) were cut and *P. viticola* clades were identified (separately for each piece) by quantitative polymerase chain reaction (qPCR), based on the ITS1 and ITS2 fragments of the genome as described by Carisse et al. (2021). Pieces of leaves with sporangia belonging to both clades were discarded, and those with sporangia belonging to a single clade were kept. These were placed in individual plastic boxes containing filter paper previously soaked in water and were maintained in the dark at room temperature (22–24 °C) overnight to stimulate the production of sporangia. The leaf pieces with sporangia were then stored at −20 °C until use. To produce inoculum, stored pieces of leaves were held successively at −4 °C for 10 min and then at room temperature (22–24 °C) for 10 min to avoid thermal shock. The *P. viticola* clade present was confirmed by qPCR as described by Carisse et al. (2021). Leaf pieces with the same *P. viticola* clade from three different vineyards were used to prepare fresh sporangia stocks. Leaf discs were placed separately in 1.5 mL Eppendorf tubes containing 1 mL sterile distilled water (one piece per tube). Subsequently, the tubes were shaken by hand for 1 min to allow the release of the sporangia. The resulting sporangia suspensions were filtered using 100 μL cell sieves (FALCON® brand). The concentration of sporangia was estimated with a hemocytometer and adjusted to $10^4$ sporangia $mL^{-1}$; the sporangia of the same clade were mixed together. Suspensions of sporangia of the same clade were used to produce fresh sporangia using the leaf disc technique as described by Mouafo-Tchinda et al. (2021).

The production of sporangia was carried out on leaf discs of the grapevine cultivar Vidal because *P. viticola* is an obligate parasite and the Vidal cultivar is susceptible to both clades (Mouafo-Tchinda et al. 2021). For this, a 50 μL (10 sporangia $mL^{-1}$) drop of sporangia was deposited on the abaxial side of leaf discs (15 mm in diameter) cut from leaves of the same physiological age (fourth to sixth leaf of the apical part of the grapevine plants aged between 8 and 10 weeks) previously placed in 9 cm Petri dishes (10 discs/dishes and 10 dishes per clade) containing water-soaked filter paper. The Petri dishes were then incubated overnight at 20 °C, 95%–100% relative humidity (RH), 12 h light/12 h dark photoperiod. Excess inoculum was removed, and the dishes were stored under the same conditions for 6 days (until sporulation). All leaf discs inoculated with the same clade were then placed in 50 mL Falcon tubes and distilled water was added at a rate of 1 mL distilled water per leaf disc. The tube was then shaken by hand for 1 min to release the fresh sporangia. The solutions were filtered through 100 μL cell sieves (FALCON®), counted with a hemocytometer, and adjusted to $10^4$ sporangia $mL^{-1}$. The solutions of fresh sporangia containing a single clade were then used as inoculum.

### Grapevine cultivar production

The leaf disc and undetached leaf materials were collected on young plants (8–10 weeks old) of 18 different grapevine cultivars. Grapevine cultivars consisted of three European grapevine cultivars *Vitis vinifera* species and 15 interspecific hybrids (Table 1). Grapevine cultivars were selected based on their differences in susceptibility to *P. viticola*, their genetic composition (average expectations based on pedigree information) resulting from interbreeding between different *Vitis* species and their prevalence in Quebec (Dubé and Turcotte 2011). Dormant bare-rooted grapevine plants of the 18 selected grapevine cultivars were transplanted into 2 L pots filled with growing substrate (PRO-MIX BX) and fertilized with 2 g/L of 10–52–10 ($N–P_2O_5–K_2O$). The plants were maintained in a greenhouse with a 16 h light/8 h dark photoperiod, 22–25 °C temperature, and 70% RH. These plants were watered every 2 days in the potting soil for a period of 8–10 weeks, which corresponds to phenological stage 5: appearance of the inflorescences.

### Evaluation of grapevine cultivars' susceptibility on leaf discs

The susceptibility of the 18 grapevine cultivars to the two clades was evaluated using the leaf disc method (Boso and Kassemeyer 2008; Yu et al. 2012). Young leaves of the same physiological age (fourth to sixth leaves from 8–10 week-old plants) were used. The leaf discs were removed using a 15 mm diameter punch and placed in Petri dishes (six leaf discs/dishes and three dishes/cultivar/clade) containing

**Table 1.** Selected grapevine cultivars' genetic composition, downy mildew susceptibility, and type of leaf hairs.

| Grapevine cultivar | Genetic composition[a] | | | DMS[a] | Types of leaf hairs and corresponding code[b] | |
|---|---|---|---|---|---|---|
| | *Vitis vinifera* (%) | *Vitis riparia* (%) | *Vitis aestivalis* (%) | | Types of leaf hairs | Code |
| Frontenac Blanc | 25.4 | 50.4 | 2.3 | 0 | Glabrous | 1 |
| Frontenac Gris | 25.4 | 50.4 | 2.3 | 0 | Glabrous | 1 |
| Frontenac Noir | 25.4 | 50.4 | 2.3 | 0 | Glabrous | 1 |
| Adalmiina | 32.0 | 10.9 | 3.3 | 1 | Felty | 4 |
| Hybernal | 65.7 | 10.1 | 4.7 | 1 | Cobwebby | 2 |
| Geisenheim 318 | 65.7 | 10.1 | 4.7 | 1 | Cobwebby | 2 |
| Saint Pepin | 39.8 | 6.3 | 13.3 | 1 | Glabrous | 1 |
| Sainte Croix | 42.2 | 9.4 | 6.6 | 2 | Glabrous | 1 |
| L'Acadie Blanc | 55.5 | 4.7 | 5.7 | 1 | Glabrous | 1 |
| Petite Perle | 41.0 | 23.6 | 3.8 | 1 | Glabrous | 1 |
| TP 1-1-12 | 29.8 | 31.7 | 4.3 | NA | NA | NA |
| Seyval Noir | 54.7 | 0.0 | 14.1 | 2 | Glabrous | 1 |
| Seyval Blanc | 54.7 | 0.0 | 14.1 | 2 | Glabrous | 1 |
| Vidal | 75.0 | 0.0 | 6.4 | 3 | Cobwebby | 2 |
| Marquette | 63.1 | 19.3 | 3.4 | 0 | Glabrous | 1 |
| Chardonnay | 100.0 | 0.0 | 0.0 | 2 | Cobwebby | 2 |
| Gamay | 100.0 | 0.0 | 0.0 | 2 | Cobwebby | 2 |
| Pinot Noir | 100.0 | 0.0 | 0.0 | 2 | Cobwebby | 2 |

[a]Genetic composition and downy mildew susceptibility (DMS) classes are from Dubé and Turcotte (2011). DMS classes: (0) resistant, (1) minimally susceptible, (2) moderately susceptible, and (3) highly susceptible. Genetic compositions are average expectations based on pedigree information.
[b]Types of leaf hairs and corresponding codes of quantification according to Swanepoel and de Villiers (1987).

water-soaked filter paper. The leaf discs were then inoculated on the underside with 50 μL of sporangia suspension ($10^4$ sporangia mL) of *P. viticola* clade *aestivalis* or clade *riparia*. The Petri dishes were then incubated overnight at 20 °C, 95%–100% RH, 12 h light/12 h dark photoperiod. The excess inoculum on leaf discs was removed, and the Petri dishes were incubated under the same conditions for 6 days. Then, five variables related to the aggressiveness of *P. viticola* (latency period, incidence, severity, sporulation efficiency, and index of aggressiveness) were measured as described by Mouafo-Tchinda et al. (2021). Briefly, Petri dishes were observed every day from the third day post inoculation (dpi) to estimate the latency period (LATLD), which was defined as the time elapsed in days from inoculation to the beginning of sporulation. Incidence of downy mildew (INCLD) was calculated as the number of leaf discs showing sporulation divided by the total number of leaf discs inoculated (six discs). The severity of downy mildew (SEVLD) was estimated by measuring the percentage of the leaf disc area with sporulation. The sporulation efficiency (SPOLD) was assessed as the number of sporangia produced per leaf disc per unit of inoculated sporangia. For each Petri dish, three leaf discs were randomly selected and inserted into a 15 mL Falcon tube. Then, 1 mL of distilled water per leaf disc was added to the tube and hand agitated for 1 min to release the sporangia. The resulting solution was counted with a hemocytometer to determine the concentration of sporangia. Finally, the index of aggressiveness (IALD) of each clade was calculated using

$$\mathrm{IA} = \ln\frac{\mathrm{INC} \times \mathrm{SPO}}{\mathrm{LAT}}$$

where IA is the index of aggressiveness of leaf discs (IALD), INC is the incidence of leaf discs (INCLD), SPO is the sporulation efficiency of leaf discs (SPOLD), and LAT is the latency period of leaf discs (LATLD). The experimental design consisted of 18 cultivars and 3 repetitions (Petri dishes) completely randomized. Each repetition consisted of six leaf discs and the experiment was conducted three times.

## Evaluation of grapevine cultivars' susceptibility on undetached leaves

In addition to the leaf disc assays, the susceptibility of the same 18 grapevine cultivars was assessed on undetached leaves as described by Boso et al. (2014). Inoculation solutions produced as described above were used to inoculate 10 leaves per plants (8–10-week-old plants). The leaves were inoculated as a uniform layer of fine droplets using an airbrush (Model LR 115950; Aztek AC100 Air Compressor). Briefly, 10 mL of inoculum at a concentration of $10^4$ sporangia $mL^{-1}$ was sprayed on the adaxial and abaxial surfaces of the 10 youngest fully expanded leaves (0.5 mL/side) of one shoot per grapevine. Immediately after inoculation, the inoculated plants were covered with plastic bags for 24 h to maintain a high RH around

the leaves and kept in a greenhouse (16 h light/8 h dark photoperiod, 22–25 °C temperature, and 70% RH). The plants were watered every 2 days on the potting soil for 10 days until sporulation occurred on inoculated leaves. Incidence (INCLL) was calculated as the proportion of inoculated leaves showing sporulation, and the severity (SEVLL) was estimated using a 0–4 scale (0 = 0%, 1 = 1%–10%, 2 = 11%–25%, 3 = 26%–50%, and 4 = >50%). The rating scale scores were converted into % severity by using the scale obtained by averaging the percentages defined for each score (0 = 0%, 1 = 5.5%, 2 = 18%, 3 = 38%, and 4 = 75.5%). The completely randomized experimental design consisted of 18 grapevine cultivars and 3 replicates.

## Measurement of grapevine cultivars' stomata size and density

Stomata density (SD) and size of the 18 grapevine cultivars were measured using the protocol described by Nicolas et al. (2018). On the abaxial side of the leaves, thin layers of shiny nail polish were applied and left to dry for at least 5 min, after which the dry layers of varnish were delicately removed and deposited on glass slides. The slides were observed under an optical microscope (Zeiss Imager M1) with the 40× objective and then photographed. For each grapevine cultivar, SD (number of stomata per $mm^2$ of leaf area) and stomata size (SS) ($\mu m^2$) were counted and measured from the photos and analyzed using ImageJ software (developed by Wayne Rasband in 1987, https://imagej.net/Wayne_Rasband). A minimum of six images were taken and analyzed for each grapevine cultivar (three plants × two leaves per plant).

## Data analysis

To compare grapevine cultivar susceptibility to *P. viticola* clade *riparia* and clade *aestivalis*, three sets of variables were used. The first set consisted of variables related to the aggressiveness of the two clades measured in this study (latency period, incidence, severity, sporulation efficiency, and index of aggressiveness), the second set consisted of the variables related to the cultivars themselves measured in this study (stomata density and size), and the third set consisted of variables obtained from cultivar descriptions (genetic composition, reported downy mildew susceptibility (DMS) class, and type of LH) (Swanepoel and de Villiers 1987; Dubé and Turcotte 2011). These variables are presented in Table 1. Because several variables related to the aggressiveness of *P. viticola* and to the anatomy and genetic composition of the grapevine cultivars were collected, both univariate and multivariate analysis were conducted.

Univariate analysis (analysis of variance (ANOVA), Student's *t* test and Tukey's test, Pearson's correlation *r*) was performed to compare the susceptibility of the grapevine cultivars to both clades of *P. viticola*. ANOVA (alpha = 0.05) was used to determine whether there was a significant effect of the grapevine cultivar, of clades, and of the interaction between cultivar and clade on each of the variables of aggressiveness (latency period, incidence, severity, sporulation efficiency, and index of aggressiveness). ANOVA was also used to determine whether there were significant differences in stomata size and density among grapevine cultivars. Subsequently, one-way Student's *t* tests (alpha = 0.05) were performed to compare the susceptibility of each grapevine cultivar to the two clades. Then, the percentages of grapevine cultivars for which aggressiveness variables for calculated for clade *aestivalis* were higher or lower (latency period) than those for clade *riparia*.

Tukey's tests (pairwise comparison) at the threshold of significance (alpha = 0.05) were used to determine whether there was a significant difference in stomata size and density among grapevine cultivars. Correlations (Pearson's correlation *r*) between the variables of aggressiveness of the two clades, the anatomical characteristics (size, density of stomata, and type LHs) of the grapevine cultivars, the genetic composition (percentages of *Vitis vinifera*, *V. riparia*, and *V aestivalis*) of the interspecific grapevine cultivars, and the published classification of DMS of grapevine cultivars (Table 1) were used to measure the degree of association between these variables.

Multivariate analyses (principal component analysis (PCA) and hierarchical ascendant classification (HAC)) were performed to find the variables of grapevine cultivar susceptibility, making it possible to identify variability in the susceptibility profiles of the grapevine cultivars and to group the grapevine cultivars based on their susceptibility to the two clades. For the PCA, the data for each grapevine cultivar were first centered and reduced to homogenize all variables involved. The PCA was performed using Factoshiny package version 2.2 (Vaissie et al. 2020) of the statistical analysis software R. For the construction of the principal components, all variables were first used as active variables (contributing to the construction of the principal components) and the contributing percentage of each variable for the construction of principal components was determined. Then, the variables with the highest percentages of contribution were used as active variables for the construction of the new principal components, and the variables with the lowest percentages of contribution were used as illustrative variables (not contributing to the construction of the principal components). Therefore, in this study, the variables of aggressiveness were used as active variables and the other variables were used as illustrative variables. After the PCA, the active variables were used to perform the HAC using the Euclidean distance. This involved grouping the grapevine cultivars according to their similarities expressed as their susceptibility to each clade. The HAC was performed using Factoshiny package version 2.2 (Vaissie et al. 2020). All the statistical analyses in this study were performed with R software version 3.6.3.

# Results

## Grapevine cultivars' susceptibility on leaf discs

On leaf discs, clade ($P < 0.020$) and grapevine cultivar ($P < 0.001$) had a significant effect on all variables of aggressiveness; however, clade had little effect on incidence ($P = 0.132$) (Table 2). There was a significant interaction between the clade and the grapevine cultivar ($P < 0.001$). For some of the grapevine cultivars, the variables of

**Table 2.** Analysis of variance for the effect of grapevine cultivars and clades of *Plasmopara viticola* on aggressiveness variables measured on leaf discs and undetached leaves.

| | Variables of aggressiveness | Factors | Degrees of freedom | Mean square | *F* value | *P* value |
|---|---|---|---|---|---|---|
| Leaf disc | | Grapevine cultivar | 17 | 4.571 | 5.708 | <0.001 |
| | Latency | Clade | 1 | 16.446 | 20.539 | <0.001 |
| | | Grapevine cultivar∗clade | 17 | 1.493 | 1.865 | 0.018 |
| | | Grapevine cultivar | 17 | 0.348 | 10.866 | <0.001 |
| | Incidence | Clade | 1 | 0.073 | 2.280 | 0.132 |
| | | Grapevine cultivar∗clade | 17 | 0.103 | 3.227 | <0.001 |
| | | Grapevine cultivar | 17 | 1886.800 | 10.985 | <0.001 |
| | Severity | Clade | 1 | 2025.100 | 11.791 | <0.001 |
| | | Grapevine cultivar∗clade | 17 | 435.600 | 2.536 | <0.001 |
| | | Grapevine cultivar | 17 | 26 044.000 | 15.651 | <0.001 |
| | Sporulation efficiency | Clade | 1 | 161 505.000 | 97.055 | <0.001 |
| | | Grapevine cultivar∗clades | 17 | 5153.000 | 3.097 | <0.001 |
| | | Grapevine cultivar | 17 | 12.205 | 20.979 | <0.001 |
| | Index of Aggressiveness | Clade | 1 | 31.410 | 53.991 | <0.001 |
| | | Grapevine cultivar∗clades | 17 | 1.076 | 1.849 | 0.020 |
| Undetached leaves | Incidence | Grapevine cultivar | 17 | 0.539 | 28.401 | <0.001 |
| | | Clade | 1 | 1.224 | 64.492 | <0.001 |
| | | Grapevine cultivar∗clades | 17 | 0.148 | 7.787 | <0.001 |
| | Severity | Grapevine cultivar | 17 | 75.180 | 49.438 | <0.001 |
| | | Clade | 1 | 147.450 | 96.966 | <0.001 |
| | | Grapevine cultivar∗clades | 17 | 13.850 | 9.108 | <0.001 |
| Stomata | Size | Grapevine cultivar | 17 | 38 423.000 | 12.040 | <0.001 |
| | Density | Grapevine cultivar | 17 | 6537.000 | 9.958 | <0.001 |

aggressiveness of *P. viticola* clade *aestivalis* were significantly higher ($P \leq 0.05$) than those of the clade *riparia*, whereas the opposite was not observed (Figs. 1–3). For the cultivar Sainte Croix, for example, the incidence, sporulation efficiency, and index of aggressiveness for *P. viticola* clade *aestivalis* were 0.96, 124.15, and 3.10, respectively, compared with 0.83, 37.33, and 1.40 for the clade *riparia* (Figs. 1B, 2B, and 3). Overall, for 28% of the grapevine cultivars (Petite Perle, Seyval Noir, Chardonnay, Gamay, and Pinot Noir), the latency period of *P. viticola* clade *riparia* was significantly longer ($P \leq 0.05$) than the latency period of clade *aestivalis* (Fig. 1A). In terms of incidence, only 17% of the grapevine cultivars (Adalmiina, Sainte Croix, and Pinot Noir) showed a higher incidence ($P \leq 0.05$) when inoculated with the clade *aestivalis* compared with the clade *riparia* (Fig. 1B). For 28% of the grapevine cultivars (Adalmiina, Petite Perle, Seyval Noir, Vidal, and Pinot Noir), severity was significantly higher ($P \leq 0.05$) on cultivars inoculated with the clade *aestivalis* compared with the clade *riparia* (Fig. 2A). Based on sporulation efficiency and index of aggressiveness, 56% of the grapevine cultivars (Adalmiina, Saint Pepin, Sainte Croix, Petite Perle, TP 1-1-12, Seyval Noir, Vidal, Chardonnay, Gamay, and Pinot Noir) had a significantly higher sporulation efficiency ($P \leq 0.05$) and index of aggressiveness ($P \leq 0.05$) when inoculated with the clade *aestivalis* than with the clade *riparia* (Figs. 2B and 3).

## Susceptibility of grapevine cultivars on undetached leaves

The observations made on undetached leaves also showed that there was a significant effect of clade ($P < 0.001$) grapevine cultivar ($P < 0.001$) and of the interaction between clade and cultivar ($P < 0.001$) on incidence and severity (Table 2). For 39% of grapevine cultivars, incidence and severity were significantly higher ($P \leq 0.05$) on cultivars that had been inoculated with the clade *aestivalis* than on those that had been inoculated with the clade *riparia* (Figs. 4A and 4B). Based on incidence, the grapevine cultivars concerned were Geisenheim 318, Saint Pepin, Sainte Croix, L'Acadie Blanc, TP 1-1-12, Seyval Noir, and Chardonnay (Fig. 4A), and for severity, the grapevine cultivars were Geisenheim 318, Saint Pepin, Sainte Croix, L'Acadie Blanc, Seyval Noir, Vidal, and TP 1-1-12 (Fig. 4B).

## Stomata size and density of grapevine cultivars

There was a significant effect ($P < 0.001$) of grapevine cultivar on SS and SD (Table 2). For the 18 grapevine cultivars, SS varied between 148 and 248 $\mu m^2$ and SD varied between 130 and 255 stomata $mm^{-2}$. The pairwise comparison showed that SS and density of most of the grapevine cultivars did not vary significantly. However, some grapevine cultivars such as Gamay, Marquette, and Seyval Noir had significantly larger

**Fig. 1.** Latency period (A) and incidence (B) of *Plasmopara viticola* clades *riparia* and *aestivalis* on the leaf discs of selected grapevine cultivars. Error bars represent the standard deviation of the mean (STD) of the latency period and of the incidence. For each grapevine cultivar, the values of bars with different letters are significantly different according to one-way Student's *t* tests ($P \leq 0.05$). dpi, day post infection.

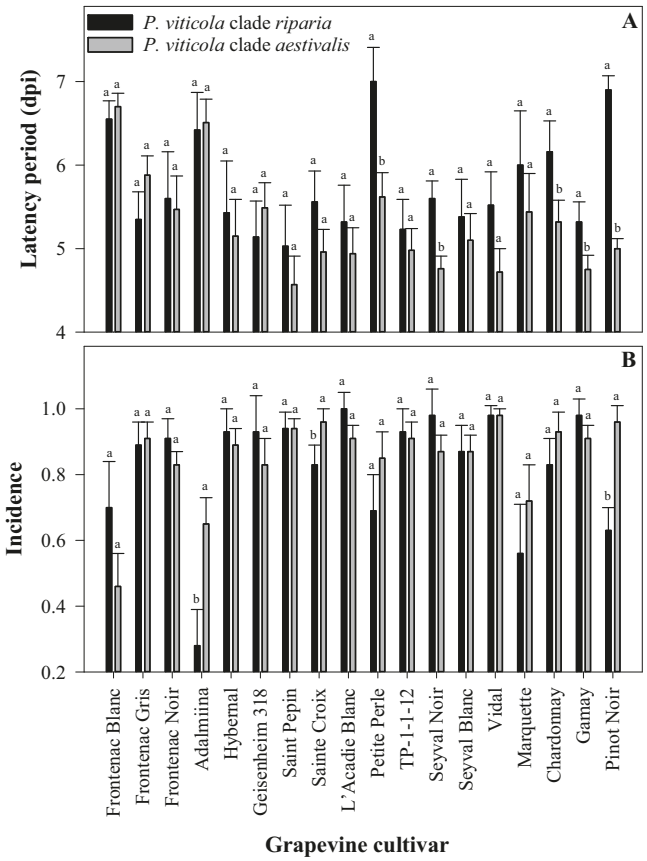
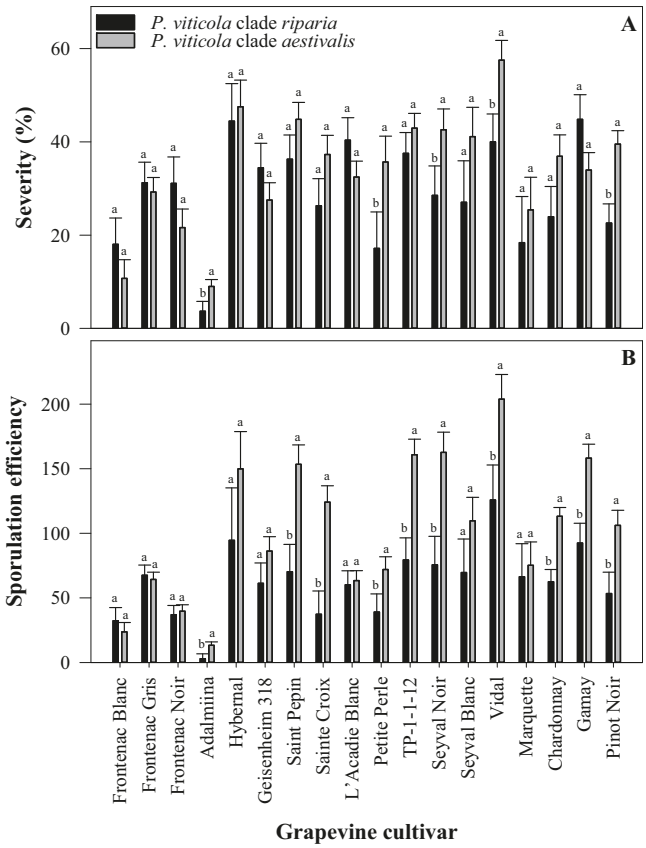



**Fig. 2.** Severity (A) and sporulation efficiency (B) of *Plasmopara viticola* clades *riparia* and *aestivalis* on the leaf discs of selected grapevine cultivars. Error bars represent the standard deviation of the mean (STD) of severity and sporulation efficiency. For each grapevine cultivar, the values of bars with different letters are significantly different according to one-way Student's *t* tests ($P \leq 0.05$).




SSs ($P \leq 0.05$) in comparison with Frontenac Blanc, Adalmiina, TP 1-1-12, and Chardonnay (Fig. 5A). In addition, Vidal and Saint Pepin had significantly higher SD ($P \leq 0.05$) than Frontenac Blanc, Marquette, and Pinot Noir (Fig. 5B).

## Correlation between aggressiveness variables and grapevine cultivar characteristics

For both clades, there was a positive and significant correlation between the incidence of downy mildew observed on leaf discs and on undetached leaves, with a correlation coefficient of $r = 0.50$ ($P = 0.034$) and $r = 0.63$ ($P = 0.005$) for clades *riparia* and *aestivalis*, respectively (Table 3). Similarly, the correlation between the severity measured on leaf discs and on undetached leaves was positive and significant, with a correlation coefficient of $r = 0.62$ ($P = 0.006$) and $r = 0.67$ ($P = 0.002$), for the clades *riparia* and *aestivalis*, respectively (Table 3).

The correlation analysis between the variables of aggressiveness and the anatomical characteristics of the grapevine cultivars showed that, for *P. viticola* clade *riparia,* there was a positive and significant correlation between SS and sporula-

**Fig. 3.** Index of aggressiveness of *Plasmopara viticola* clades *riparia* and *aestivalis* on the leaf discs of selected grapevine cultivars. Error bars represent the standard deviation of the mean (STD) of the index of aggressiveness. For each grapevine cultivar, the values of bars with different letters are significantly different according to one-way Student's *t* tests ($P \leq 0.05$).

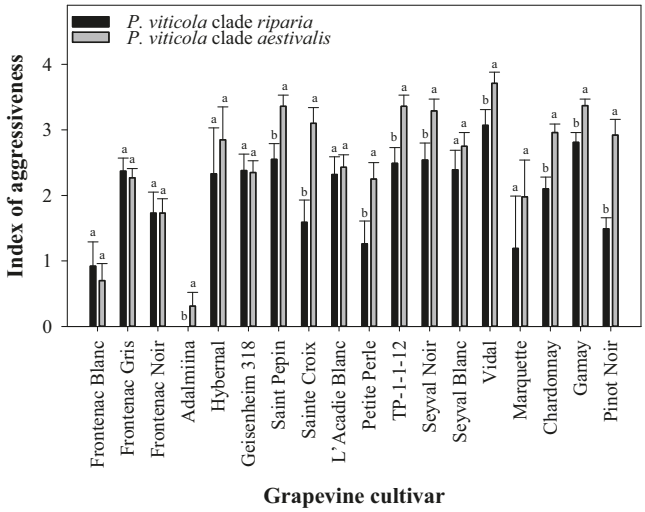


**Fig. 4.** Incidence (A) and severity (B) of *Plasmopara viticola* clades *riparia* and *aestivalis* on the undetached leaves of selected grapevine cultivars. Error bars represent the standard deviation of the mean (STD) of the incidence and severity. For each grapevine cultivar, the values of bars with different letters are significantly different according to one-way Student's *t* tests ($P \leq 0.05$).

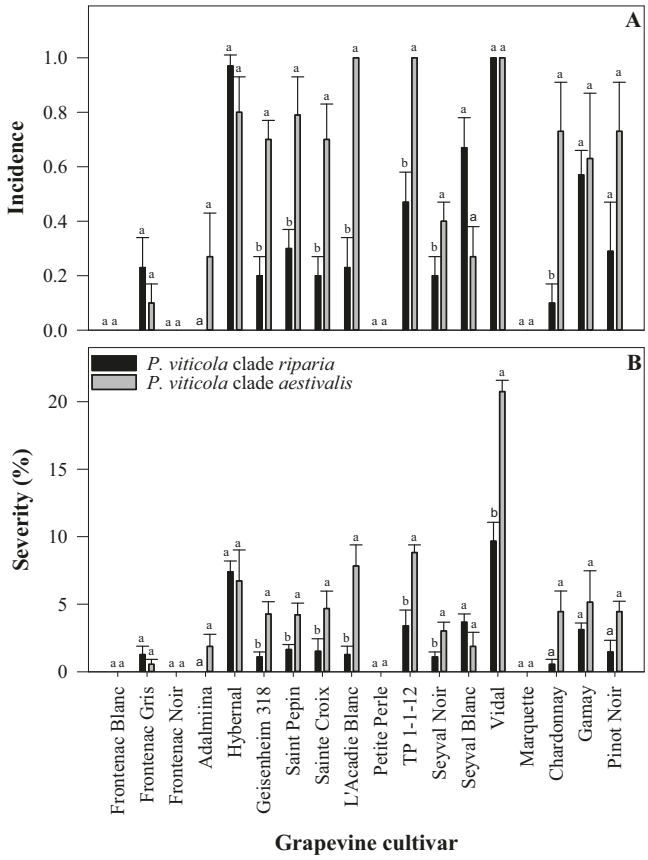
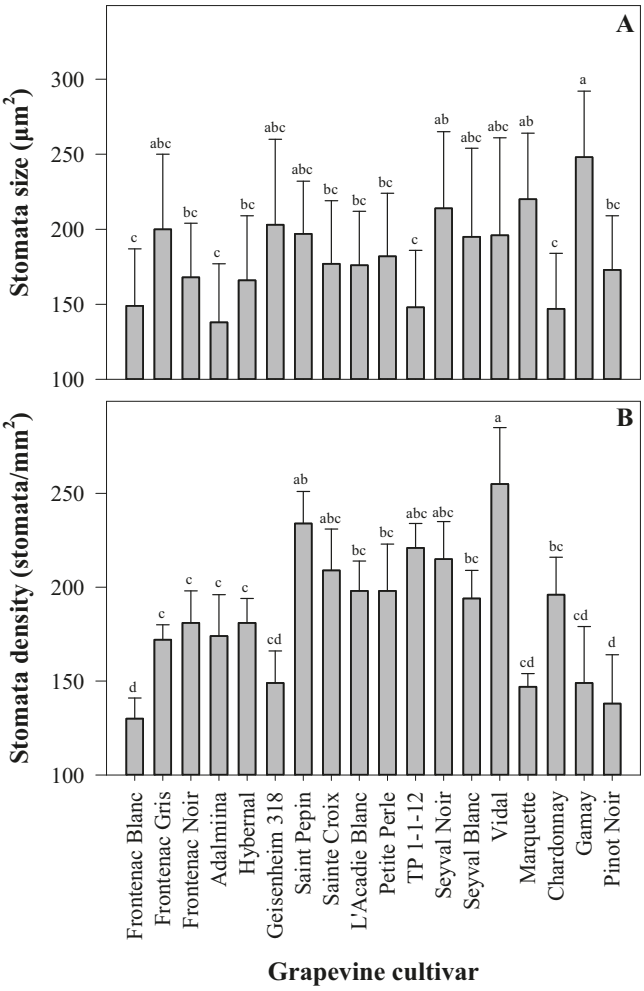



**Fig. 5.** Stomata size (A) and density (B) of the selected grapevine cultivars. Error bars represent the standard deviation of the mean (STD) of the stomatal sizes and densities. For each stomatal variable, the values of bars with different letters are significantly different according to Tukey's test ($P \leq 0.05$).




tion efficiency and between SS and index of aggressiveness, with a correlation coefficient of $r = 0.50$ ($P = 0.034$) and $r = 0.51$ ($P = 0.032$), respectively (Table 3). There were no significant correlations between SD and each of the variables of aggressiveness (Table 3). However, for *P. viticola* clade *aestivalis*, there was a significant negative correlation between SS and latency period ($r = -0.48$; $P = 0.045$; Table 3). A significant negative correlation ($r = -0.55$; $P = 0.018$) was found between SD and latency period, whereas a significant positive correlation was observed between SD and incidence ($r = 0.55$; $P = 0.017$), severity ($r = 0.67$; $P = 0.002$), sporulation efficiency ($r = 0.59$; $P = 0.01$), and index of aggressiveness ($r = 0.56$; $P = 0.016$) (Table 3). For type of LHs, the only significant correlation observed was between LHs and incidence ($r = -0.52$; $P = 0.027$) for *P. viticola* clade *riparia* (Table 3).

For *P. viticola* clade *riparia*, there was no significant correlation between the genetic percentages of *V. vinifera*, *V. riparia*, and *V. aestivalis* ancestry and each of the variables of aggressiveness (Table 3). However, for the clade *aestivalis*, there was a significant negative correlation between the genetic percentage of *V. vinifera* ancestry and latency period ($r = -0.47$; $P = 0.05$), and a significant positive correlation between the genetic percentage of *V. vinifera* ancestry and index of aggressiveness ($r = 0.49$; $P = 0.04$; Table 3). For the genetic percentage of *V. riparia* ancestry, there was a significant correlation with all variables of aggressiveness, with a correlation coefficient of $r = 0.61$ ($P = 0.007$), $r = -0.49$ ($P = 0.041$), $r = -0.53$ ($P = 0.023$), $r = -0.56$ ($P = 0.017$), and $r = -0.53$ ($P = 0.024$) for the latency period, incidence, severity, sporulation efficiency, and index of aggressiveness, respectively (Table 3). However, there was no significant correlation between the genetic percentage of *V. aestivalis* ancestry and each of the variables of aggressiveness (Table 3).

Finally, for *P. viticola* clade *riparia*, the correlations between reported DMS and all variables of aggressiveness (Table 3) were not significant. In contrast, for clade *aestivalis*, there was a significant correlation between the DMS and all variables of aggressiveness, with correlation coefficients of $r = -0.62$

**Table 3.** Coefficient of correlation between pairs of variables for both clades of *Plasmopara viticola* and for 18 grapevine cultivar characteristics.

| Pair of variables[a] | | *P. viticola* clade *riparia* | | *P. viticola* clade *aestivalis* | |
|---|---|---|---|---|---|
| Variable 1 | Variable 2 | *r* | *P* | *r* | *P* |
| INCLL | INCLD | **0.50** | **0.034** | **0.63** | **0.005** |
| SEVLL | SEVLD | **0.62** | **0.006** | **0.67** | **0.002** |
| Stomata size | LATLD | −0.38 | 0.120 | **−0.48** | **0.045** |
| | INCLD | 0.38 | 0.120 | 0.29 | 0.210 |
| | SEVLD | 0.39 | 0.100 | 0.27 | 0.270 |
| | SPOLD | **0.50** | **0.034** | 0.39 | 0.110 |
| | IALD | **0.51** | **0.032** | 0.44 | 0.068 |
| Stomata density | LATLD | −0.38 | 0.110 | **−0.55** | **0.018** |
| | INCLD | 0.43 | 0.077 | **0.55** | **0.017** |
| | SEVLD | 0.31 | 0.200 | **0.67** | **0.002** |
| | SPOLD | 0.40 | 0.100 | **0.59** | **0.010** |
| | IALD | 0.44 | 0.065 | **0.56** | **0.016** |
| Leaf hairs | LATLD | 0.24 | 0.330 | 0.29 | 0.240 |
| | INCLD | **−0.52** | **0.027** | −0.15 | 0.560 |
| | SEVLD | −0.29 | 0.250 | −0.23 | 0.360 |
| | SPOLD | −0.18 | 0.480 | −0.12 | 0.650 |
| | IALD | −0.41 | 0.090 | −0.31 | 0.220 |
| *V. vinifera* (%) | LATLD | 0.09 | 0.720 | **−0.47** | **0.050** |
| | INCLD | 0.13 | 0.590 | 0.42 | 0.082 |
| | SEVLD | 0.26 | 0.300 | 0.40 | 0.097 |
| | SPOLD | 0.46 | 0.054 | 0.45 | 0.061 |
| | IALD | 0.33 | 0.180 | **0.49** | **0.040** |
| *V. riparia* (%) | LATLD | 0.11 | 0.680 | **0.61** | **0.007** |
| | INCLD | −0.11 | 0.670 | **−0.49** | **0.041** |
| | SEVLD | −0.18 | 0.450 | **−0.53** | **0.023** |
| | SPOLD | −0.36 | 0.150 | **−0.56** | **0.017** |
| | IALD | −0.27 | 0.270 | **−0.53** | **0.024** |
| *V. aestivalis* (%) | LATLD | −0.39 | 0.110 | −0.37 | 0.130 |
| | INCLD | 0.27 | 0.290 | 0.15 | 0.550 |
| | SEVLD | 0.08 | 0.750 | 0.41 | 0.093 |
| | SPOLD | 0.21 | 0.410 | 0.40 | 0.098 |
| | IALD | 0.29 | 0.240 | 0.32 | 0.200 |
| DMS | LATLD | −0.08 | 0.770 | **−0.62** | **0.007** |
| | INCLD | 0.27 | 0.290 | **0.59** | **0.012** |
| | SEVLD | 0.25 | 0.330 | **0.69** | **0.002** |
| | SPOLD | 0.47 | 0.054 | **0.74** | **0.001** |
| | IALD | 0.42 | 0.092 | **0.69** | **0.002** |

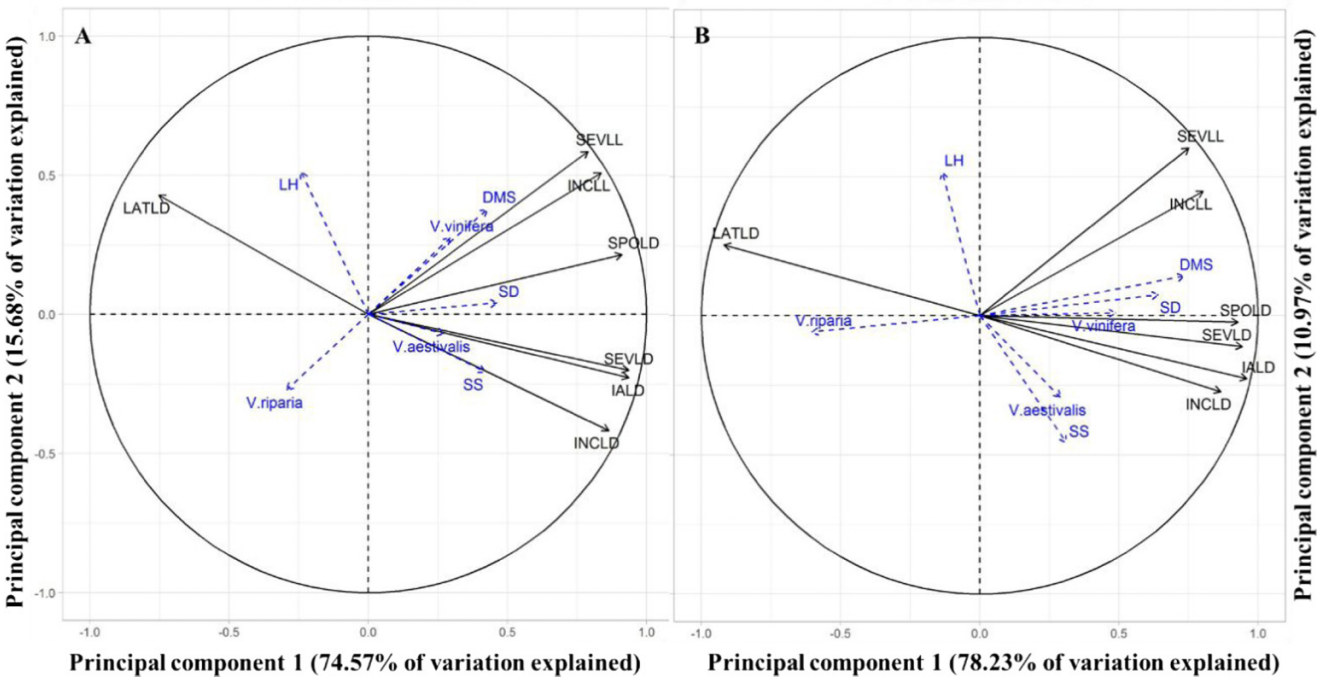
[a]Variables: latency period on leaf discs (LATLD), incidence on leaf discs (INCLD), severity on leaf discs (SEVLD), sporulation efficiency on leaf discs (SPOLD), index of aggressiveness on leaf discs (IALD), incidence on living leaves (INCLL) (undetached leaves), severity on living leaves (SEVLL) (undetached leaves), and downy mildew susceptibility (DMS). *r* is the Pearson correlation coefficient. The pairs of variables with *P* values $\leq 0.05$ were significantly correlated.

($P = 0.007$), $r = 0.59$ ($P = 0.012$), $r = 0.69$ ($P = 0.002$), $r = 0.74$ ($P = 0.001$), and $r = 0.69$ ($P = 0.002$) for the latency period, incidence, severity, sporulation efficiency, and index of aggressiveness, respectively (Table 3).

## Principal component analysis

The results of the PCA for the two clades using aggressiveness data, grapevine characteristics (SS, SD, type of LHs), reported DMS, and genetic percentages of *V. vinifera*, *V. riparia*, and *V. aestivalis* of the grapevine cultivars are presented in Figs. 6 and 7 and in Table 4. For *P. viticola* clade *riparia*, 90.25% of the variability in the data was represented by the first two principal components of the variable factor map (biplot), with 74.57% and 15.68% for the first and second principal components of the biplot, respectively (Figs. 6A and 7A). For *P. viticola* clade *aestivalis*, 89.20% of the variability in

**Fig. 6.** Variable factor maps of the principal component analysis (PCA) with all selected variables. Active or contributing variables are shown in black and illustrative variables in blue. (A) *Plasmopara viticola* clade *riparia*; (B) *Plasmopara viticola* clade *aestivalis*. Variables used to construct this biplot were latency period on leaf discs (LATLD), incidence on leaf discs (INCLD), severity on leaf discs (SEVLD), sporulation efficiency on leaf discs (SPOLD), index of aggressiveness on leaf discs (IALD), incidence on living leaves (INCLL) (undetached leaves), severity on living leaves (SEVLL) (undetached leaves), stomata size (SS), stomata density (SD), leaf hairs (LH), and downy mildew susceptibility (DMS). Longer vectors represent the variables that are best represented by the two principal components, while small vectors represent the variables that are not well represented by these principal components. Variables with small angles between vectors and vectors in the opposite direction are positively and negatively correlated, respectively.

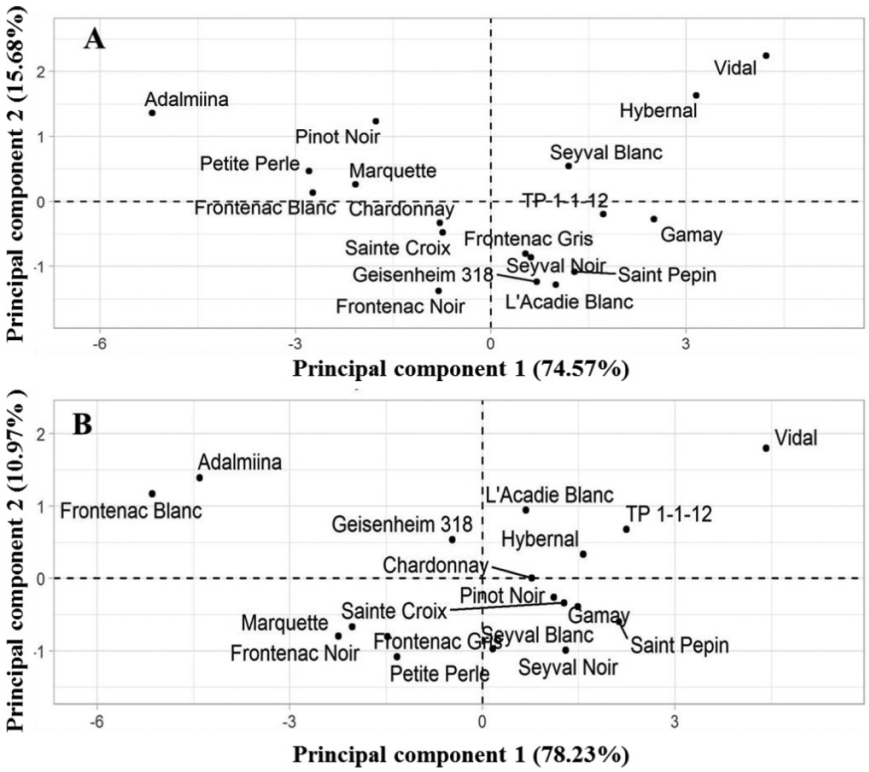



the data was represented by the first two components, with 78.23% and 10.97% for the first and second principal components, respectively (Figs. 6B and 7B). The analysis presented in Table 4 and Fig. 6 shows that all variables of aggressiveness (LATLD, INCLD, SEVLD, SPOLD, IALD, SEVLL, and INCLL) made a contribution to the construction of the first principal component, which ranged from 10.31% to 16.81%, and that the correlation between these variables and the first principal component varied between 0.56 and 0.92. However, only the variables SEVLL and INCLL contributed to the construction of the second principal component, with contributions of 23.51%–47.51%, and in this case the correlation with the principal component varied between 0.20 and 0.37 (Table 4). For the illustrative variables, only DMS, genetic percentage of *V. riparia,* genetic percentage of *V. vinifera*, and SD for the first component and LH for the second component had a good affinity (between 0.22 and 0.54) with the principal components of the biplot (Table 4; Fig. 6). Figures 6 and 7 show that for the clade *riparia*, Vidal and Hybernal grapevine cultivars have the highest INCLD, SEVLD, SPOLD, IALD, SEVLL, SS, SD, and DMS, while Adalmiina has the longest LATLD (Figs. 6A and 7A). For clade *aestivalis*, the Vidal grapevine cultivar has the highest INCLD, SEVLD, SPOLD, IALD, SEVLL, DMS, SD, and genetic percentage of *V. vinifera* ancestry, while the Adalmiina and Frontenac Blanc grapevine cultivars had the longest LATLD and the highest percentage of *V. riparia* (Figs. 6B and 7B).

## Classification of grapevine cultivars

For each clade taken separately, the HAC of grapevine cultivars, based on the variables that made the greatest contribution to the construction of the principal components of PCA, allowed the cultivars to be divided into three classes of susceptibility (Fig. 8). The following variables contributed to the construction of the first principal components, for clade *riparia* and clade *aestivalis*, respectively: SEVLD with a contribution of 16.78% and 15.26%, IALD with 16.78% and 16.81%, SPOLD with 15.91% and 15.72%, INCLD with 14.33% and 13.63%, LATLD with 10.84% and 15.45%, INCLL with 13.42% and 11.79%, and SEVLL with 11.96% and 10.31% (Table 4). SEVLL (31.32% and 47.51%) and INCLL (23.51% and 26.03%) contributed to the construction of the second component for clade *riparia* and clade *aestivalis*, respectively (Table 4). The nearby values of these contribution percentages show that each of these variables can be used to classify the grapevine cultivars according to their susceptibility to each *P. viticola* clade. However, in this study, to capture variations among cultivars as much as possible, all variables of aggressiveness (SEVLD, IALD, SPOLD, INCLD, LATLD, INCLL, and SEVLL) were considered for the HAC (Fig. 8). Figure 8 shows that 44.4%

**Fig. 7.** Individual (grapevine cultivars) factors map of the principal component analysis (PCA) performed using the centered and reduced data of the selected variables. (A) *Plasmopara viticola* clade *riparia* and (B) *Plasmopara viticola* clade *aestivalis*.




and 11.1% of the grapevine cultivars are classified as minimally susceptible (black), 44.4% and 22.2% are classified as moderately susceptible (red), and 11.1% and 66.7% are classified as highly susceptible (green) to clades *riparia* and *aestivalis*, respectively (Fig. 8). In fact, for *P. viticola* clade *riparia*, Class 1 (black rectangle) considered as minimally susceptible is characterized by long LATLD and low INCLD, IALD, SEVLD, SPOLD, SEVLL, and INCLL. Class 1 includes Frontenac Blanc, Adalmiina, Marquette, Chardonnay, Petite Perle, Frontenac Noir, Sainte Croix, and Pinot Noir (Fig. 8A). Class 2 (in red rectangle), classified as moderately susceptible is characterized by high SEVLD, INCLD, IALD, and short LATLD. Class 2 includes Saint Pepin, TP 1-1-12, Seyval Noir, Geisenheim 318, L'Acadie Blanc, Seyval Blanc, Gamay, and Frontenac Gris (Fig. 8A). Class 3 (in green rectangle), considered highly susceptible, is characterized by high SEVLL, INCLL, and SPOLD. Class 3 includes Hybernal and Vidal (Fig. 8A).

For *P. viticola* clade *aestivalis*, class 1 (minimally susceptible), which includes Frontenac Blanc and Adalmiina, is characterized by high LATLD and low INCLD, IALD, SEVLD, SPOLD, and SS (Fig. 8B). Class 2 (moderately susceptible), which includes Petite Perle, Marquette, Frontenac Noir, and Frontenac Gris, is characterized by high genetic percentages of *V. riparia* ancestry and low INCLL and DMS (Fig. 8B). Class 3 (highly susceptible) includes Hybernal, Geisenheim 318, Saint Pepin, Sainte Croix, L'Acadie Blanc, TP 1-1-12, Seyval Noir, Seyval Blanc, Vidal, Chardonnay, Gamay, and Pinot Noir. Class 3 is characterized by high INCLL, IALD, SPOLD, DMS, SEVLD, INCLD, genetic percentages of *V. vinifera* ancestry and SEVLL, and short LATLD and low genetic percentages of *V. riparia* ancestry (Fig. 8B).

Careful observation of the distribution of grapevine cultivars in different susceptibility groups shows that only 4 of the 18 grapevine cultivars (22%) were classified in the same susceptibility groups for both clades. These were Vidal and Hybernal in the highly susceptible group and Frontenac Blanc and Adalmiina in the minimally susceptible group (Fig. 8).

## Discussion

Following the description in 2013 of five clades of *P. viticola* and reports of the presence of *P. viticola* clade *riparia* and clade *aestivalis* in Quebec (eastern Canada), Mouafo-Tchinda et al. (2021, 2022) reported that clade *aestivalis* was more aggressive and more competitive than clade *riparia*. In Quebec, the clade *aestivalis* is more prevalent than the clade *riparia* (Carisse et al. 2021). Hence, in this study, our objectives were to determine the susceptibility of the most commonly grown grapevine cultivars in the province of Quebec to *P. viticola* clades *riparia* and *aestivalis,* to investigate the relationship between variables related to the grapevine cultivars and their susceptibility, and to classify the grapevine cultivars according to their susceptibility to each clade.

Several studies have reported differences in grapevine cultivar susceptibility to *P. viticola* (Staudt and Kassemeyer 1995; Kortekamp et al. 1998; Unger et al. 2007; Yu et al. 2012; Boso et al. 2014). However, because most of these studies were conducted before the different clades were described, in all

**Table 4.** Contribution of the selected variables to the construction of the first and second principal components, and affinity of these variables with the components (PCA).

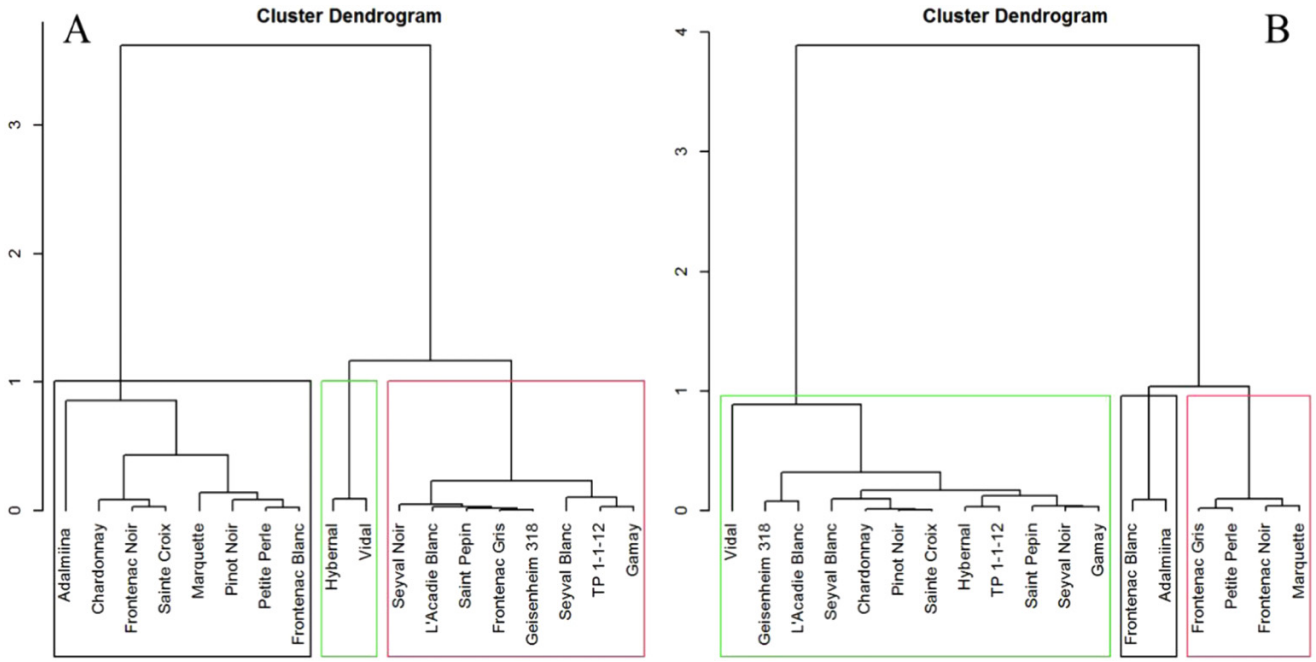
| | | | PC 1[b] | | | PC 2[b] | | |
|---|---|---|---|---|---|---|---|---|
| | Variables[a] | *P. viticola* clades | Coord | Ctr | $Cos^2$ | Coord | Ctr | $Cos^2$ |
| Active variables | LATLD | *riparia* | −0.752 | 10.842 | 0.566 | 0.428 | 16.707 | 0.183 |
| | | *aestivalis* | −0.919 | 15.427 | 0.845 | 0.255 | 8.440 | 0.065 |
| | INCLD | *riparia* | 0.865 | 14.326 | 0.748 | −0.418 | 15.880 | 0.174 |
| | | *aestivalis* | 0.866 | 13.694 | 0.750 | −0.272 | 9.666 | 0.074 |
| | SEVLD | *riparia* | 0.936 | 16.777 | 0.876 | −0.201 | 3.682 | 0.040 |
| | | *aestivalis* | 0.944 | 16.259 | 0.890 | −0.110 | 1.585 | 0.012 |
| | SPOLD | *riparia* | 0.911 | 15.908 | 0.830 | 0.215 | 4.200 | 0.046 |
| | | *aestivalis* | 0.928 | 15.721 | 0.861 | −0.023 | 0.069 | 0.001 |
| | IALD | *riparia* | 0.936 | 16.776 | 0.876 | −0.227 | 4.701 | 0.052 |
| | | *aestivalis* | 0.959 | 16.809 | 0.920 | −0.227 | 6.695 | 0.051 |
| | INCLL | *riparia* | 0.837 | 13.415 | 0.700 | 0.508 | 23.505 | 0.258 |
| | | *aestivalis* | 0.803 | 11.785 | 0.645 | 0.447 | 26.034 | 0.200 |
| | SEVLL | *riparia* | 0.790 | 11.955 | 0.624 | 0.586 | 31.324 | 0.344 |
| | | *aestivalis* | 0.751 | 10.306 | 0.564 | 0.604 | 47.511 | 0.365 |
| Illustrative variables | SS | *riparia* | 0.420 | – | 0.177 | −0.207 | – | 0.043 |
| | | *aestivalis* | 0.311 | – | 0.097 | −0.457 | – | 0.209 |
| | SD | *riparia* | 0.463 | – | 0.215 | 0.041 | – | 0.002 |
| | | *aestivalis* | 0.643 | – | 0.413 | 0.075 | – | 0.006 |
| | LH | *riparia* | −0.242 | – | 0.058 | 0.512 | – | 0.262 |
| | | *aestivalis* | −0.135 | – | 0.018 | 0.516 | – | 0.267 |
| | *V. vinifera* (%) | *riparia* | 0.294 | – | 0.087 | 0.276 | – | 0.076 |
| | | *aestivalis* | 0.489 | – | 0.239 | 0.010 | – | 0.000 |
| | *V. riparia* (%) | *riparia* | −0.293 | – | 0.086 | −0.271 | – | 0.073 |
| | | *aestivalis* | −0.604 | – | 0.365 | −0.058 | – | 0.003 |
| | *V. aestivalis* (%) | *riparia* | 0.272 | – | 0.074 | −0.069 | – | 0.005 |
| | | *aestivalis* | 0.290 | – | 0.084 | −0.293 | – | 0.086 |
| | DMS | *riparia* | 0.427 | – | 0.183 | 0.378 | – | 0.143 |
| | | *aestivalis* | 0.735 | – | 0.540 | 0.143 | – | 0.020 |

[a]Variables: latency period on leaf discs (LATLD), incidence on leaf discs (INCLD), severity on leaf discs (SEVLD), sporulation efficiency on leaf discs (SPOLD), index of aggressiveness on leaf discs (IALD), incidence on living leaves (INCLL) (undetached leaves), severity on living leaves (SEVLL) (undetached leaves), stomata size (SS), stomata density (SD), leaf hairs (LHs), and downy mildew susceptibility (DMS).
[b]The parameters are principal component 1 (PC 1), principal component 2 (PC 2), and vector coordinates or coordinates of the variables (Coord). Contribution (Ctr) is the contribution percentages of the variables for the construction of the principal components, and square cosine ($Cos^2$) is the quality of representation or the affinity or correlation of the variables with the principal components.

these studies, the reported susceptibility depends on the clades of *P. viticola* that were present or inoculated. The results of our study reveal that there is a difference in the susceptibility of grapevine cultivars to the two *P. viticola* clades. This variability in susceptibility may be due to anatomical, biochemical, or genetic factors of grapevine cultivars, on the one hand, and to differences in the aggressiveness of the clades, on the other hand. Similar studies conducted with other pathogens have shown that variability in cultivar susceptibility was related to cultivar characteristics and to the pathogen's genotype aggressiveness (Garrett and Mundt 1999; Jaunet and Wang 1999; Santos et al. 2018; Young et al. 2018). Young et al. (2018) assessed the aggressiveness of *Phytophthora infestans* genotypes on different potato cultivars and found that there were significant differences in the latency period, incidence, and severity of late blight between *P. infestans* genotypes and potato cultivars. According to Garrett et al. (2009), variability in disease response to host diversity could also be explained by functional divergence of hosts, season length, and environmental conduciveness.

Variables of aggressiveness have been widely used as important indicators of cultivar susceptibility (Dai et al. 1995; Jaunet and Wang 1999; Santos et al. 2018). In fact, in this study, these variables (SEVLD, IALD, SPOLD, INCLD, LATLD,

**Fig. 8.** Hierarchical ascendant classification tree of the grapevine cultivars performed using the centred and reduced data of variables that made the greatest contribution to the construction of the principal components of PCA. Cluster 1 (in black) represents minimally susceptible grapevine cultivars, cluster 2 (in red), moderately susceptible grapevine cultivars, and cluster 3 (in green) highly susceptible grapevine cultivars for (A) *Plasmopara viticola* clade *riparia* and (B) *Plasmopara viticola* clade *aestivalis*.




INCLL, and SEVLL) were robust enough to allow the classification of grapevine cultivars according to their susceptibility to each clade. Several studies have also used some of these variables to classify grapevine cultivars' susceptibility to *P. viticola* (Staudt and Kassemeyer 1995; Boso and Kassemeyer 2008; Yu et al. 2012; Boso et al. 2014). In our study, hierarchical ascendent classification was used to group grapevine cultivars in three classes: minimally susceptible, moderately susceptible, and highly susceptible. This difference in susceptibility of the grapevine cultivars can be associated with the difference in synthesis of PR proteins (Kortekamp 2006), calloses (Gindro et al. 2006), phytoalexins (Kortekamp 2006), etc. For example, Gindro et al. (2006) associate the minimally susceptibility (or resistance) of some grapevine cultivars with the synthesis of high concentrations of toxic stilbenes. Although some grapevine cultivars are found in the same groups of susceptibility to clade *riparia* and clade *aestivalis*, this study confirms that the susceptibility of grapevine cultivars to both clades is different. For example, some European grapevine cultivars such as Chardonnay and Pinot Noir, which were classified by Dubé and Turcotte (2011) as moderately susceptible, were both classified as highly susceptible to clade *aestivalis* and as moderately susceptible and minimally susceptible to clade *riparia,* respectively. Pinot Noir was classified as moderately susceptible to *P. viticola* by Boso and Kassemeyer (2008), and Chardonnay was classified as susceptible (highly susceptible in our context) to *P. viticola* by Yu et al. (2012).

The variability in susceptibility of grapevine cultivars could be explained by the difference in SS and density. Stomata constitute one of the major pathways for pathogen penetration in plants (Swanepoel and de Villiers 1987; Nicolas et al. 2018). Based on the roles of stomata in the infection process, grapevine cultivars with higher SD and size may be more susceptible to both clades of *P. viticola*. In fact, Nicolas et al. (2018) reported that there was a relationship between SD and susceptibility of lettuce cultivars to *Xanthomonas campestris*, while there was no influence of SS on cultivar susceptibility. In the present study, the aggressiveness of the clade *riparia* was positively correlated with SS but was not significantly correlated with SD. The aggressiveness of clade *aestivalis* was positively correlated with SS (expressed as negative correlation with the latency period) and SD. These results contradict those reported by Boso et al. (2010) showing that the density of stomata had no influence on the susceptibility of grapevine cultivars. However, taking into account these results and the finding reported by Rouxel et al. (2013) that sporangia size was larger for clade *riparia* than for clade *aestivalis*, additional research is required to investigate the relationship between the size of sporangia and the size of stomata during the process of infection.

Besides SD and size, the type of LHs may also play a key role in the susceptibility or resistance of grapevine cultivars to *P. viticola* (Kortekamp and Zyprian 1999; Boso et al. 2010). The lack of or a limited number of LHs does not necessarily contribute to the susceptibility of grapevine cultivars; however, in some grapevine cultivars, the type (number and size) of LHs seems to reduce the susceptibility of grapevine cultivars to *P. viticola* (Kortekamp et al. 1999; Kortekamp and Zyprian 1999). We suspect that the low aggressiveness of the two clades on the Adalmiina cultivar is closely related to the cottony aspect of its LHs. Several studies have reported that LHs play an important role in the resistance of grapevine cultivars against

*P. viticola* (Swanepoel and de Villiers 1987; Kortekamp and Zyprian 1999; Boso et al. 2010).

Considering that most grapevine cultivars grown in eastern Canada are interspecific hybrids, it was essential to assess the correlation between the genetic composition (genetic percentages of *V. vinifera*, *V. riparia*, and *V. aestivalis*) of cultivars and the aggressiveness of the two clades. However, no correlation was observed between aggressiveness of the clade *riparia* and the genetic percentages of *V. vinifera*, *V. riparia*, or *V. aestivalis* ancestry. In contrast, for the clade *aestivalis*, there was no significant correlation between the aggressiveness and the genetic percentage of *V. aestivalis* ancestry. However, there was a positive and significant correlation between the genetic percentage of *V. vinifera* and the aggressiveness of clade *aestivalis*, and a negative and significant correlation between the genetic percentage of *V. riparia* and the aggressiveness of clade *aestivalis*. These results suggest that the genetic percentages of *V. vinifera*, *V. riparia*, and *V. aestivalis* ancestry do not influence the susceptibility of grapevine cultivars to clade *riparia*. Although the genetic percentage of *V. aestivalis* in the grapevine cultivars did not influence the susceptibility of grapevine cultivars to clade *aestivalis,* the genetic percentage of *V. vinifera* was positively associated with the susceptibility of grapevine cultivars, and the genetic percentage of *V. riparia* was negatively associated with the susceptibility of grapevine cultivars. Several authors (Boubals 1959; Staudt and Kassemeyer 1995; Yu et al. 2012; Boso et al. 2014) have reported that *V. vinifera* grapevine cultivars were very susceptible, *V. aestivalis* grapevine cultivars were partially susceptible, and *V. riparia* grapevine cultivars were resistant. These results refute the assertion of Rouxel et al. (2013) that clade specificity exists for certain grapevine cultivars.

There was a significant and positive correlation between the incidence and severity observed on leaf discs (in the laboratory) and on undetached leaves (in the greenhouse). This result suggests that both the leaf disc and undetached leaf methods are robust enough to be used in assessing grapevine cultivar susceptibility. Our observations are in accordance with those of several researchers (Brown et al. 1999; Kortekamp and Zyprian 2003; Sotolar and Vachün 2005; Boso et al. 2014) who reported a significant correlation between grapevine cultivar susceptibility results obtained in the laboratory, greenhouse, and field.

This study showed that there is no significant correlation between the published classifications of grapevine cultivars according to their DMS (Dubé and Turcotte 2011) and the variables of aggressiveness of the clade *riparia*. However, for clade *aestivalis*, the published classifications of grapevine cultivars according to DMS susceptibility (Dubé and Turcotte 2011) show a positive and significant correlation with the aggressiveness. This result may be explained by the fact that clade *aestivalis* is the most common clade of *P. viticola* in eastern Canada and in other regions of the world. Research on the prevalence of different *P. viticola* clades in different regions of the world has shown that clade *aestivalis* is one of the two most prevalent *P. viticola* clades. This prevalence has been found in eastern Canada (Carisse et al. 2021), in the US states of Florida and Georgia (Hong et al. 2019), in Australia (Taylor et al. 2019), and in Sao Paulo, Brazil (Camargo et al. 2019). Taking this into account, it can be assumed that the *P. viticola* clade used by Dubé and Turcotte (2011) to classify grapevine cultivars according to their DMS was clade *aestivalis*. Therefore, the susceptibility of grapevine cultivars grown in vineyards infected with *P. viticola* clade *aestivalis* could be represented by the published grapevine cultivars classifications of DMS. With regard to the susceptibility of grapevine cultivars to the clade *riparia*, however, it would be wise to perform a new classification or to apply the classification obtained in this study.

## Conclusion

The results of this study show that the susceptibility of grapevine cultivars to *P. viticola* clade *riparia* and clade *aestivalis* is different regardless of whether the host genotype has a pure European ancestry or an American–European mixed ancestry. Some groups of grapevine cultivars were found to be minimally susceptible, others moderately susceptible, and others highly susceptible to each of the two clades. Susceptibility to clade *riparia* was associated with SS and type of LHs, whereas susceptibility to clade *aestivalis* was associated with SS and density, and with genetic percentage of *V. vinifera* and *V. riparia* ancestry. Overall, grapevine cultivars were more susceptible to clade *aestivalis* than to clade *riparia*. The results of this study highlight the importance of knowing which clade of *P. viticola* is present in vineyards to support the sustainable management of downy mildew. Future research should evaluate gene expression involved in the defense mechanisms of grapevine cultivars. Furthermore, to improve understanding of the epidemiology of these two clades of *P. viticola* in eastern Canada, it would be useful to evaluate ontogenic resistance to each of the two clades as well as the susceptibility of other grapevine organs such as flowers and berries.

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