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Sexual Reproduction of *Plasmopara viticola* in Wine Regions of Southern Brazil, Climatic Suitability, Oospore Formation and Germination

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ABSTRACT

This study investigated the sexual reproduction of *Plasmopara viticola* in wine regions of southern Brazil. Leaves with mosaic symptoms from susceptible hosts of *Vitis vinifera* were collected from vineyards in the autumn and screened for the presence of oospores. Leaves from hosts carrying different resistance alleles were also collected in three contrasting environments in Santa Catarina State; the influence of these factors on oospore formation was assessed. Georeferenced oospore records were used in the maximum entropy ecological niche model (MaxEnt) and climatic suitability for oospore formation in southern Brazil was determined. Finally, oospore germination assays were conducted after overwintering under natural conditions in both susceptible and partially resistant hosts. Oospores were detected in all vineyards investigated. The interaction between resistance alleles and the environment influenced oospore formation. Oospores were formed in all partially resistant hosts, even those with pyramided resistance alleles. Ecological niche modelling indicated that 80.8% of southern Brazil has moderate to very high suitability for oospore formation. This is the first study to confirm *P. viticola* oospore formation in all major wine-growing regions of southern Brazil, oospore germination under local conditions in susceptible and resistant hosts, and to apply ecological niche modelling to predict climatic suitability for oospore formation. These findings expand the known distribution of the sexual cycle of *P. viticola* in the southern hemisphere confirming its sexual reproduction in southern Brazil with critical implications for grapevine breeding and disease management, as sexual reproduction promotes genetic diversity, increasing the risk of host resistance breakdown and fungicide resistance.

1 | Introduction

Plasmopara viticola is an obligate biotrophic oomycete and causes downy mildew (DM), one of the most destructive diseases of grapevine (*Vitis vinifera*) (Gessler et al. 2011; Koldenkova et al. 2022). Native to North America, the pathogen was introduced into Europe in the 1870s and subsequently spread to vineyards worldwide, including those in the southern hemisphere (Fontaine et al. 2021). The spraying of fungicides is still the

main strategy for controlling *P. viticola*, but along with the increase in production cost, there are concerns about their safety and potential adverse effects on human health and biodiversity (Merdinoglu et al. 2018; Trapp et al. 2025). A more sustainable alternative for managing the disease, both environmentally and economically, is the use of resistant grapevine varieties. Over the years, grapevine breeders have introgressed *P. viticola* resistance alleles (*Rpv*, resistance to *P. viticola*) into *V. vinifera* backgrounds to combine disease resistance with high-quality

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wine attributes (Trapp et al. 2025). The most commonly used *Rpv*-alleles in breeding programmes are *Rpv1*, *Rpv3*, *Rpv10* and *Rpv12* (Merdinoglu et al. 2003; Schwander et al. 2012; Venuti et al. 2013; Welter et al. 2007; Zyprian et al. 2016). In order to enhance resistance performance and durability, breeders are combining these resistance alleles into single genotypes (gene pyramiding).

Plasmopara viticola is a diploid pathogen with a mixed reproductive system, both sexual and asexual, with sexual reproduction occurring through haploid gametes (Burruano 2000). The sexual reproduction is heterothallic, requiring the fusion of two compatible specialised hyphae (the antheridium and oogonium) from the mating types P1 and P2 to form oospores (Wong et al. 2001). In this system the P1 mating-type locus is heterozygous (MAT-a/MAT-b), while the P2 locus is homozygous (MAT-a/MAT-a) (Dussert et al. 2020). Under field conditions, oospores play a crucial role in the pathogen's life cycle. Firstly, they are overwintering structures that allow the pathogen to survive in the field during grapevine dormancy (Vercesi et al. 1999). Secondly, oospores promote genetic recombination through meiosis, leading to new genetic combinations and increasing the pathogen's genetic diversity (Gobbin et al. 2006). Finally, oospores can germinate throughout the grapevine-growing season, continuously contributing to the primary inoculum during DM epidemics (Fedele et al. 2025; Maddalena et al. 2021).

Heterothallic pathogens with mixed reproductive systems (such as *P. viticola*) have greater evolutionary potential; this increases the likelihood of each pathogen overcoming host resistance mechanisms and developing resistance to fungicides used for their control (McDonald and Linde 2002). Erosion of genetic resistance has been reported in Europe for *Rpv3*, *Rpv10* and *Rpv12*, three of the four main resistance genes used worldwide in grapevine breeding programmes (Delmas et al. 2016; Delmotte et al. 2014; Heyman et al. 2021; Paineau et al. 2022, 2024; Peressotti et al. 2010; Possamai et al. 2020; Wingerter et al. 2021). Even more concerning are the reports about pathogen adaptation to resistance gene pyramids, such as *Rpv3.1 + Rpv10*, *Rpv3.1 + Rpv12* (Paineau et al. 2022; Wingerter et al. 2021). Similarly, there are many studies reporting the resistance of *P. viticola* isolates to fungicidal molecules such as quinone outside inhibitors (QoIs), carboxylic acid amides (CAAs), quinone inside inhibitors (QiIs) and quinone inside-outside inhibitors (QioIs) (Massi et al. 2021). Although *P. viticola* has been studied worldwide, the research on the sexual phase is concentrated in the northern hemisphere, particularly in Europe. Currently, major research priorities include advancing sustainable grapevine DM management and deepening our understanding of the sexual phase in the life cycle of *P. viticola* (Burruano 2000; Delbac et al. 2019; Dussert et al. 2020; Dvorak et al. 2025; Fedele et al. 2025; Gobbin et al. 2006; Maddalena et al. 2021; Vercesi et al. 1999; Wong et al. 2001). Building upon this knowledge, researchers are also exploring innovative approaches to disrupt the process of sexual reproduction (Poeydebat et al. 2022; Fournier et al. 2025).

In the southern hemisphere, studies on the sexual reproduction of *P. viticola* are scarce. A study conducted in Western Australia

detected oospores in only 1 of 16 vineyards analysed, suggesting that their formation is rare (Killigrew et al. 2005). In southern Africa, new genotypes appeared steadily during the grapevine-growing season, representing 12%–74% of the pathogen population contributing to the epidemic, providing strong genetic and epidemiological evidence that oospores are formed (Koopman et al. 2007). In São Paulo State (SP), Brazil, *P. viticola* populations are formed by a few clonal genotypes of *P. viticola*, indicating that DM epidemics result from multiple clonal infections and that the reproduction of the pathogen in this region is predominantly asexual (Camargo et al. 2019). On the other hand, another study reported that *P. viticola* populations collected in Rio Grande do Sul (RS), Brazil, presented greater genetic diversity than those from SP. The authors suggested that sexual reproduction may occur under the environmental conditions of RS, although oospore formation was not investigated (Santos et al. 2020).

According to climate data from a 30-year historical series, the southern region of Brazil experiences mean daily temperatures between 17°C and 25°C and annual precipitation exceeding 1250 mm during the grapevine-growing season (Wrege et al. 2012). These climatic conditions are highly conducive to the asexual development of *P. viticola*. As a result, numerous secondary infection cycles occur within a single grapevine season if the disease is not effectively managed (Zanghelini et al. 2019). However, during autumn and winter, mean minimum temperatures range from 9°C to 17°C and temperatures frequently fall below freezing during this period (Wrege et al. 2012). These conditions induce natural dormancy in grapevines and limit the asexual reproduction of *P. viticola* (Mouafo-Tchinda et al. 2020; Williams et al. 2007). *P. viticola* oospore formation was recently confirmed in the highland region of Santa Catarina (SC), which is the coldest region of Brazil (Bitencourt et al. 2021). However, information on the occurrence and suitability of climatic conditions for oospore formation in the other main wine-producing regions of southern Brazil remains limited.

In Brazil, viticulture has economic, cultural and oenotourism importance. In 2024 the total vineyard area reached 83,000 ha, representing a 1.5% increase compared to 2022 and marking the third consecutive year of expansion (OIV 2024). Simultaneously, national wine production totalled 3.6 million hectolitres, reflecting a 12.1% increase over the previous year and a 31.4% increase compared to the 5-year mean (Protas et al. 2024; OIV 2024). The southern region stands out as the main producer, accounting for 58.1% of the country's grape production and leading the production of fine wines (Protas et al. 2024). Furthermore, the southern region has 13 registered Geographical Indications (GIs) for wines, which reinforce the recognition of the quality, authenticity and distinctive characteristics of local products in both national and international markets (MAPA 2025). However, DM is the most important viticultural disease in the region, compromising grape productivity and threatening the long-term economic and environmental sustainability of the sector (Zanghelini et al. 2019).

To develop more efficient, sustainable and long-lasting disease management strategies, it is crucial to understand the sexual reproduction of *P. viticola*. These studies are incipient in the

southern hemisphere where many famous wine regions are located worldwide. This study aimed to contribute to the mitigation of this lack of information by investigating the sexual phase of *P. viticola* in southern Brazil. The main objectives of the study were (i) to evaluate the occurrence and determine climatic suitability for the formation of *P. viticola* oospores in the main wine grape-producing regions of southern Brazil; (ii) to assess the influence of resistance alleles and environmental factors on the formation of *P. viticola* oospores; and (iii) to confirm the germination of *P. viticola* oospores formed in both susceptible and resistant hosts.

2 | Materials and Methods

2.1 | Oospore Formation of *P. viticola* in Southern Brazil

Leaf samples were collected from commercial vineyards located in Paraná (PR), SC and RS States, in the main wine grape-producing regions of southern Brazil, which represent different edaphoclimatic conditions (Figure S1). The leaves were collected from susceptible *V. vinifera* varieties naturally infected by *P. viticola*. Information regarding hosts, collection dates, vineyard locations and meteorological stations is provided in Table S1.

All samples were collected and stored according to the method described by Bitencourt et al. (2021). Ten leaves from the upper third of shoots showing typical DM mosaic symptoms (Figure S2) were collected from each susceptible host/vineyard during the autumns of 2021 and 2022. From each leaf, a 12 mm diameter disc was excised from an area showing mosaic symptoms and fixed in FAA (formaldehyde, acetic acid and 70% ethanol in the proportion 5:2:93) for 48 h, then stored in containers with 70% ethanol at room temperature. For microscopic observation, each disc was placed on a glass slide, mounted with a drop of distilled water, and covered with a coverslip. The 10 discs per host/location were examined for the presence of oospores using a BX-60 light microscope (Olympus), and images were captured using a DP73 digital camera (Olympus) connected to the microscope.

The mean density (oospores mm⁻²) and mean diameter (μm) of *P. viticola* oospores in leaf tissue were measured. To assess oospore density, five fixed 12 mm diameter leaf discs showing mosaic symptoms of DM were examined under the BX-60 light microscope at 100× magnification. Within a defined area of 363,538 μm², oospores were counted using ImageJ software and the mean oospore density per square millimetre was calculated. To evaluate the diameter of the oospores in the leaf disc, 10 discs previously fixed were analysed using the BX-60 light microscope at 200× magnification. The diameter of 10 randomly selected oospores from each leaf disc was measured using cellSens software (Olympus), totalling 100 oospores per host/vineyard.

The oospore density and diameter from the main wine grape-producing regions of southern Brazil were compared using generalised linear models (GLMs). Density was modelled using a quasi-Poisson distribution, and diameter was modelled using a gamma distribution, both with a logarithmic link function

(Zuur et al. 2009). Model assumptions were assessed through graphical residual analysis, inspection of half-normal plots, and overdispersion and zero inflation assessments. The significance of fixed effects was tested using deviance analysis, with a χ^2 test applied to the gamma model fitted to mean oospore diameter and an *F*-test based on quasi-likelihood used for the quasi-Poisson model fitted to oospore density. Pairwise comparisons among varieties of *V. vinifera* were performed using Tukey-adjusted post hoc tests based on estimated marginal means (EMMs) (Searle et al. 1980). Analyses were performed in the R statistical software (R Core Team 2023) and the packages used were *stats* v. 4.3.2 (R Core Team 2023), *hnp* v. 1.2-6 (Patriota and Young 2020), *performance* v. 0.13.0 (Lüdtke et al. 2021), *emmeans* v. 1.8.9 (Lenth 2023), *multcomp* v. 1.4-24 (Hothorn et al. 2008) and *agricolae* v. 1.3-7 (de Mendiburu 2023).

2.2 | Influence of Resistance Alleles and Environmental Conditions on Oospore Formation

To evaluate the combined effect of resistance alleles and environmental conditions on *P. viticola* oospore formation under natural field conditions, leaf samples were collected from experimental vineyards in three distinct regions of SC State. Samples were collected from a panel of hosts of *V. vinifera* containing different combinations of *Rpv* alleles, as well as from the susceptible *V. vinifera* variety Sauvignon Blanc and the interspecific hybrid Isabella (*Vitis labrusca* × *V. vinifera*). Details regarding the hosts, *Rpv* alleles, locations and sampling dates are provided in Table S2. From this point onward, the host carrying *Rpv* alleles will be considered to be resistant hosts, although most of them did not have complete resistance to the pathogen.

Samples were collected and stored as described in Section 2.1. Leaf discs were screened for the presence of oospores with minor methodological modifications. For this experiment, from 10 leaves of each host/location up to two discs per leaf were analysed, aiming to measure 100 oospores (10 per leaf) per host/location (the number of oospores varied among host/location). For all evaluated samples, data corresponding to the number of discs with and without oospores were analysed using a GLM with binomial distribution and logit link function. Fixed effects (location, genotype and their interaction) were tested via deviance analysis (χ^2 test). Model assumptions and post hoc comparisons followed the procedures described in Section 2.1. Results are expressed as the percentage of oospore incidence (IO) to facilitate interpretation. For leaf discs containing oospores, the mean oospore density (oospores mm⁻²) and mean oospore diameter (μm) were measured for each host × location combination. Oospore density was measured as described in Section 2.1. Oospore diameter was measured using 10–20 leaf discs. On each disc, up to 10 randomly selected oospores were measured, totalling up to 100 oospores per host × location combination. Both oospore diameter and density datasets were analysed using the same statistical procedures described in Section 2.1.

The results for the three variables were summarised by presenting mean values and the corresponding statistical differences among the evaluated factors. In addition, heatmaps were used to provide an integrated visualisation of host, environmental and host × environment interaction effects.

2.3 | Climatic Data of the Sample Collection Locations

Mean temperature (°C) and precipitation (ppt; mm) were recorded for the 30 days preceding the sample collection dates. Climatic data was obtained from the meteorological stations closest to the vineyards where the samples were collected, operated by INMET, EPAGRI/CIRAM and/or AgriTempo/RS.

2.4 | Climatic Suitability for *P. viticola* Oospore Formation in Southern Brazil

The climatic suitability for *P. viticola* oospore formation across the southern Brazilian region was conducted using ecological niche modelling, employing the maximum entropy model MaxEnt (Elith et al. 2011; Phillips et al. 2006). This approach allowed the extrapolation of local data to a regional scale and the identification of the main climatic drivers of oospore formation in a subtropical climate region in the southern hemisphere.

Historical bioclimatic data were obtained from the TerraClimate database, with a spatial resolution of approximately $1/24^\circ$ (~4 spatial resolution) (Abatzoglou et al. 2018), for the months of March, April and May from 2017 to 2022, which encompass the period of time that oospore occurrence was recorded in the studied area. The extracted climatic variables included ppt, potential evapotranspiration (pet), actual evapotranspiration (aet), maximum temperature ($t_{\max}$), minimum temperature ($t_{\min}$) and vapour pressure deficit (vpd). All variables were obtained as monthly data. From $t_{\max}$ and $t_{\min}$, mean temperature [$t_{\text{med}} = (t_{\max} + t_{\min})/2$] and thermal amplitude ($t_{\text{amp}} = t_{\max} - t_{\min}$) were calculated. For modelling purposes, t_{med} , t_{amp} and ppt were selected as predictor variables based primarily on their biological relevance, complemented by Pearson correlation analysis ($r < 0.7$) and variance inflation factor (VIF < 5), to minimise multicollinearity among climatic covariates (Dormann et al. 2013; Tesfamariam et al. 2022). The selected variables were converted into raster layers and stacked into a multilayer raster object to serve as environmental predictors in the ecological niche modelling using *raster* v. 3.6-32 package (Hijmans 2023).

Georeferenced records of *P. viticola* oospores were obtained from previously collected oospore density data and their respective geographic coordinates, as described in Sections 2.1 and 2.2 ($n = 10$). To reduce spatial redundancy in the modelling process, only one sampling point (BG1; Table S1) was used to represent the municipality of Bento Gonçalves (RS), due to the proximity of two samples collected in that area. To complement the spatial characterisation of *P. viticola* occurrence, two additional records reported by Bitencourt et al. (2021) were included, corresponding to the municipalities of São Joaquim (SC; $28^\circ 16' 28.0''$ S, $49^\circ 55' 53.1''$ W; 1415 m.a.s.l.) and Curitiba (SC; $27^\circ 16' 15.7''$ S, $50^\circ 37' 54.5''$ W; 987 m.a.s.l.). In total, 12 occurrence records were considered for modelling, which currently represent the only documented records of *P. viticola* oospores in South America.

Modelling was performed in the R statistical software (R Core Team 2023) using the MaxEnt algorithm (ENMeval v. 2.0.5 and *maxnet* v. 0.1.4 packages), with leave-one-out jackknife cross-validation and a total of 155 background points were randomly

generated within the study area (Elith et al. 2011; Muscarella et al. 2014; Pearson et al. 2007; Phillips 2021; Phillips et al. 2006). Different regularisation parameters (RM = 1 to 3, with a step of 0.5) and linear and quadratic feature classes were tested to avoid overfitting (Ikeda and Osawa 2020). The selection of the most suitable model was based on the lowest corrected Akaike information criterion (AICc) value (Fu et al. 2024).

The significance of the model was assessed using a null model test, applying 1000 random permutations of the presence points to evaluate whether the predicted models performed significantly better than random expectations (Raes and ter Steege 2007). Model accuracy was evaluated using the area under the curve (AUC) of the receiver operating characteristic (ROC) curve, the true skill statistic (TSS), sensitivity, specificity, and the Boyce Index (Boyce et al. 2002; Hirzel et al. 2006; Ikeda and Osawa 2020). The relative importance of each predictor variable was evaluated by permuting its values across presence and background points and calculating the resulting decrease in AUC, with greater reductions indicating higher model dependence (Cohen 2023). The values were then normalised to express the percentage contribution of each variable (Ikeda and Osawa 2020).

The prediction results were generated using the MaxEnt model with the logistic output format, which returns the probability of occurrence and, in this context, represents the climatic suitability for *P. viticola* oospore formation (Phillips et al. 2017). Suitability was calculated for each pixel in the study area, generating continuous values ranging from 0 (unsuitable) to 1 (highly suitable) (Fu et al. 2024; Ikeda and Osawa 2020). These values were interpolated onto a map of southern Brazil at a spatial resolution of approximately 4 km, enabling the identification of critical areas with a higher risk of pathogen sexual reproduction occurrence.

In addition to the mean suitability map for the March–May period, individual suitability maps were generated for each of these months. Descriptive statistics of mean climatic suitability, including minimum and maximum thresholds, were calculated based on the number of valid pixels. The same approach was applied to estimate the relative proportion of each suitability class, defined according to thresholds proposed by Cohen (2023): low (0–0.2), moderate (> 0.2–0.4), high (> 0.4–0.6) and very high (> 0.6–1.0). Additionally, municipal boundary polygons were reprojected onto the map to calculate the mean, standard deviation and corresponding classes of climatic suitability at the municipal level.

2.5 | Germination of *P. viticola* Oospores in the Highland Region of Santa Catarina

In order to confirm that *P. viticola* oospores germinate under the natural environmental conditions in southern Brazil, leaves naturally infected with *P. viticola* were collected in the autumn of 2024 in two vineyards in Curitiba, SC. Oospore formation in both vineyards had already been confirmed in a previous study by Bitencourt et al. (2021). Leaves of the susceptible cultivar Cabernet Sauvignon were collected from a commercial vineyard, while leaves from hosts carrying different combinations of

resistance alleles were collected from an experimental vineyard of the Federal University of Santa Catarina. Detailed information regarding collection dates, hosts, resistance alleles and sampling locations is provided in Table S3.

For all samples, oospore-rich leaf areas were selected using a BX-60 light microscope using 100× magnification, and 12-mm diameter leaf discs were carefully excised. The leaf discs were placed in five nylon bags with 100 µm pores, each containing 20 discs (Vercesi et al. 2010). The nylon bags were overwintered in the field under a layer of 5 cm of sand on the soil surface, close to an experimental vineyard located at the Agricultural Experimental Station of the Federal University of Santa Catarina, Campus of Curitiba (27°16'58"S, 50°35'04"W; 1000 m a.s.l.). The substrate was covered with an anti-hail net to secure the samples during the overwintering period (Maddalena et al. 2021; Vercesi et al. 2010). The samples were maintained in that condition until the germination assays. During this period, climatic data on temperature (°C) and precipitation (mm) were collected from the meteorological station UFSC Curitiba 1026, located next to the overwintered samples.

Germination tests were performed at the grapevine sprouting stage (16 September 2024). For each sample, the nylon bags were collected from the field, taken to the laboratory and analysed using protocols adapted from Vercesi et al. (2010) and Maddalena et al. (2021). The leaf discs were macerated with a pellet pestle in a microtube containing 1.5 mL of sterile distilled water (SDW) to extract the oospores. The oospore suspension was filtered through a 75 µm sieve and added to 47 mm Petri dishes (Millipore) containing 1% water agar. For each sample, five Petri dishes were prepared and 40 µL of the suspension containing 20 oospores was added to each plate, totalling 100 oospores evaluated per nylon bag. The samples were incubated at 20°C with a 12-h light photoperiod. Every day, 100 µL SDW was added to the samples. The number of germinated oospores was examined daily using a BX-60 microscope for 14 days after incubation (dai), and the percentage of oospore germination was calculated.

Germination data (counts of germinated and non-germinated oospores) were analysed using a GLM with binomial distribution and logit link function. Fixed effects were tested via deviance analysis (χ^2 test). Model assumptions and post hoc comparisons followed the procedures described in Section 2.1. Results were expressed as germination percentages for interpretative clarity.

3 | Results

3.1 | Oospore Formation of *P. viticola* in Southern Brazil

The formation of oospores of *P. viticola* was confirmed in the leaf tissues of susceptible hosts in all the wine-growing regions under investigation in southern Brazil, including Serra Gaúcha (RS), Campanha Gaúcha (RS), Campos de Cima da Serra (RS), Serra Catarinense (SC), Vale do Rio do Peixe (SC) and the Curitiba Metropolitan Region (PR). Oospores were more abundant in the central areas of DM mosaic leaf symptoms. They were typically rounded, thick-walled and clustered near the

veins. Oospore density in leaf tissues ranged from 28 oospores mm⁻² in Farroupilha (FAR) to 212 oospores mm⁻² in Vacaria (VAC). A statistically significant difference in mean oospore density was found among the evaluated regions ($F=2.74$; $df=9$; $p=0.0138$; Figure 1a). The mean density ranged from 48 oospores mm⁻² in Campo Largo (CAM) to 123 oospores mm⁻² in Videira (VID). The other regions evaluated, including Monte Belo do Sul (MBS=107), Dom Pedrito (DPE=85), Bento Gonçalves (BG1=83 and BG2=72), VAC (=78), Água Doce (AGD=69), Nova Pádua (NPA=59) and Farroupilha (FAR=55), presented intermediate densities and did not differ significantly from each other. Significant regional variation was also observed for mean *P. viticola* oospore diameter ($\chi^2=2.56$; $df=9$; $p<0.0001$; Figure 1b). Mean diameters per site varied from 20.39 µm (AGD) to 24.00 µm (VID), with leaves from VID having larger oospores than those in other locations. Oospore diameters ranged from 15.95 µm (BG2) to 33.05 µm (VID).

3.2 | Influence of Resistance Genes and Environment on Oospore Formation

Oospores were detected within leaf tissues at all three study locations, and every host evaluated produced oospores in at least one of these environments (Figure 2). Table S4 presents the incidence, mean diameter and density of oospores observed in grapevine hosts cultivated across different environments in SC. The incidence of *P. viticola* oospores (IO) differed significantly among hosts ($\chi^2=83.42$; $df=8$; $p<0.0001$), across environments ($\chi^2=74.74$; $df=2$; $p<0.0001$) and in their interaction ($\chi^2=122.96$; $df=15$; $p<0.0001$). Overall, the VID and AGD environments favoured oospore formation, whereas URU provided less conducive conditions, particularly for partially resistant genotypes (Figure 3a). In the VID environment, all hosts exhibited IO values above 60% with no clear distinction between susceptible and resistant genotypes. In AGD, although most genotypes maintained IO values above 50%, oospores were completely absent in Isabella and Bronner. In contrast, oospore formation in URU was restricted to a subset of hosts. Genotypes carrying single *Rpv* alleles, such as Regent (*Rpv3.1*) and Souvignier Gris (*Rpv3.2*), did not produce oospores, whereas genotypes carrying pyramided *Rpv* alleles displayed contrasting responses. Cabernet Cortis and Calardis Blanc maintained IO values above 50%, while Bronner and GF-15 exhibited intermediate values (25%; Figure 3a).

There were also significant differences in oospore density between hosts ($F=13.68$; $df=8$; $p<0.0001$), environments ($F=71.54$; $df=2$; $p<0.0001$) and host × environment interaction ($F=8.33$; $df=15$; $p<0.0001$; Table S4). The VID environment showed the highest mean oospore densities for most genotypes (Figure 3b). In this environment, Regent, Souvignier Gris and Sauvignon Blanc exhibited high oospore densities (>75 oospores mm⁻²), whereas Isabella consistently showed low density (14 oospores mm⁻²).

In AGD, a general reduction in oospore density was observed, with values ranging from 9 to 39 oospores mm⁻² among genotypes that formed oospores, and complete absence in Isabella and Bronner. In URU, oospore densities were generally low

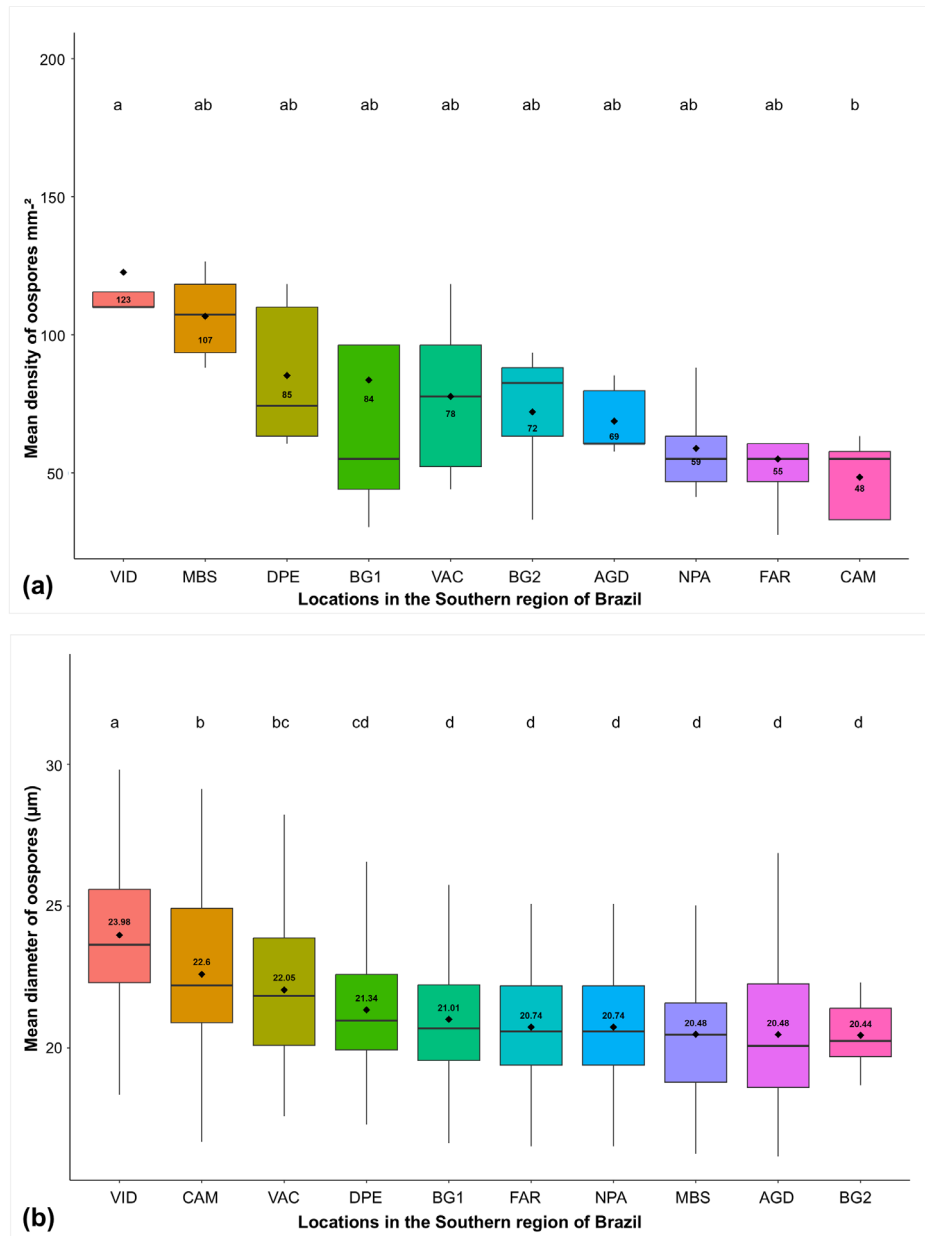


FIGURE 1 | Boxplot distribution of (a) mean density per mm² in the evaluated leaf discs ($n=5$) and (b) mean diameter of *Plasmopara viticola* oospores ($n=100$) measured in 10 locations in southern Brazil. Oospores were extracted from downy mildew symptomatic leaves sampled in the autumn from susceptible *Vitis vinifera* varieties in the following municipalities across the states of Paraná (PR), Santa Catarina (SC) and Rio Grande do Sul (RS). CAM—Campo Largo, PR; AGD—Água Doce, SC; VID—Videira, SC; VAC—Vacaria, RS; FAR—Farrroupilha, RS; NPA—Nova Pádua, RS; BG1 and BG2—Bento Gonçalves, RS; MBS—Monte Belo do Sul, RS and DPE—Dom Pedrito, RS. Different letters indicate significant differences in mean oospore density per mm² and mean oospore diameter between locations as detected by Tukey's test ($p < 0.05$).

(≤ 20 oospores mm⁻²), except for Cabernet Cortis, which showed high oospore density at this environment (> 50 oospores mm⁻²), contrasting with the pattern observed at the other locations for this same genotype (Figure 3b). In contrast, GF-15 exhibited low and relatively stable oospore densities across environments, suggesting reduced sensitivity for this variable. Statistically significant differences were also observed in the mean diameter of oospores between hosts ($\chi^2 = 1.45$; $df = 8$; $p < 0.0001$), environments ($\chi^2 = 5.33$; $df = 2$; $p < 0.0001$) and host $\times$ environment interaction ($\chi^2 = 0.96$; $df = 15$; $p < 0.0001$; Table S4). Consistently, larger mean oospore diameters were recorded in VID when compared to AGD and URU (Figure 3c).

In this environment, Bronner, Calardis Blanc and Sauvignon Blanc produced the largest oospores ($24 \mu\text{m}$), whereas Isabella exhibited the smallest diameters ($21 \mu\text{m}$). Of the hosts oospore mean diameters were measured in AGD and URU, GF-15 did not differ between environments; Felicia and Calardis Blanc presented larger oospores in AGD, and Cabernet Cortis exhibited larger oospores in URU. The susceptible host Sauvignon Blanc was not available in URU.

Taking all the results together, the presence of the *Rpv* resistance alleles did not prevent the formation of oospores. Even the genotypes containing *Rpv* alleles pyramided (e.g., Cabernet

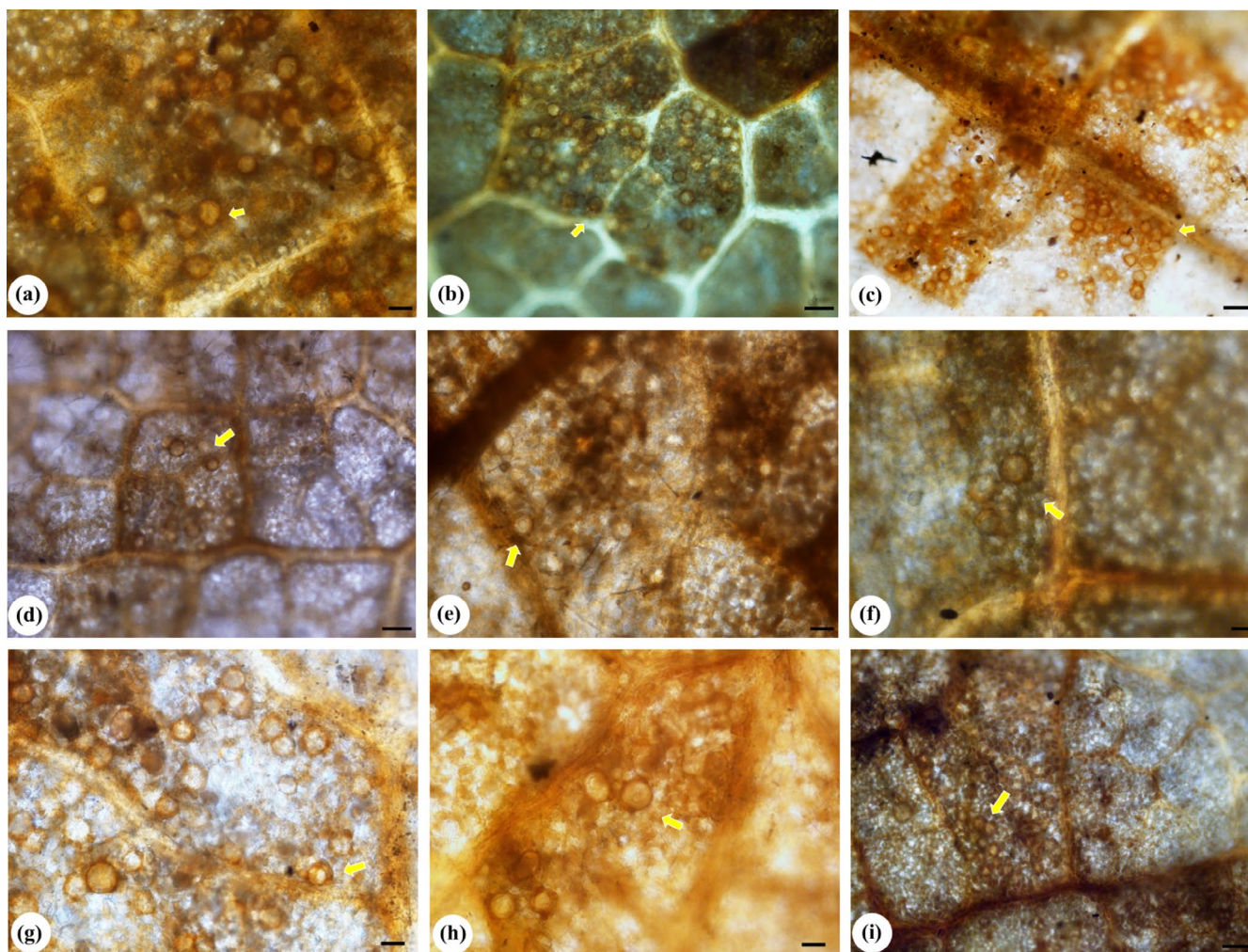


FIGURE 2 | Detection of *Plasmopara viticola* oospores in leaf discs excised from leaf areas manifesting downy mildew symptoms, collected in three locations of Santa Catarina state (AGD, Água Doce; URU, Urussanga; and VID, Videira) from eight resistant and one susceptible (Sauvignon Blanc) hosts. (a–i) Light microscopic images showing oospores in all hosts under investigation. (a) Sauvignon Blanc in VID, (b) Regent in VID, (c) Souvignier Gris in VID, (d) Calardis Blanc in VID, (e) Felicia in URU, (f) Gf. 2004–043–0015 in AGD, (g) Bronner in VID, (h) Cabernet Cortis in URU and (i) Isabella in VID. Scale bar of 50 μ m for images (b), (c), (d) and (i). Scale bar of 20 μ m for images (a), (e), (f), (g) and (h). Yellow arrows highlight the oospores.

Cortis, Bronner and GF-15) showed high oospore density in at least one location. The outstanding host was Isabella, for which a low oospore density was observed in VID and no oospores were detected in AGD and URU.

3.3 | Climatic Data From the Sample Collection Location

The mean daily air temperature and the total amount of precipitation for the 30 days before sample collection in the main wine-growing regions of southern Brazil (Section 3.1) and in vineyards with resistant hosts (Section 3.2) are displayed in Figure 4. In PR State, the municipality of CAM recorded a mean temperature of 17.8°C and 113 mm of precipitation. The municipalities in SC registered a mean temperature of 18.4°C for AGD, 17.8°C for VID, and 23.7°C for URU and the total precipitation was 126, 157 and 200 mm, respectively. In RS, VAC registered 13.9°C and 293 mm of precipitation. The municipalities of FAR,

NPA, MBS and BGO integrate the “Serra Gaúcha” region, where the mean temperature ranged from 16.1°C to 18.3°C and 212 to 399 mm of precipitation. DPE had a mean temperature of 17.5°C and 412 mm of precipitation.

The mean daily air temperature range also varied among the evaluated municipalities, with values ranging from 7.9°C to 10.2°C (Figure S3). The lowest value was observed in NPA (7.9°C), while the highest occurred in AGD (10.2°C). Locations such as VID (9.8°C), VAC (9.9°C) and DPE (9.4°C) showed amplitudes similar to AGD. In contrast, municipalities located in the Serra Gaúcha region (such as BGO, FAR and MBS) and URU exhibited values close to those of NPA, ranging from 8.3°C to 8.5°C. The observed patterns indicate that, during the 30 days prior to sample collection, climatic conditions characterised by mild mean temperatures, moderate thermal amplitude and high precipitation (above 100 mm/month) favoured the formation of *P. viticola* oospores in the main grape-growing regions of southern Brazil.

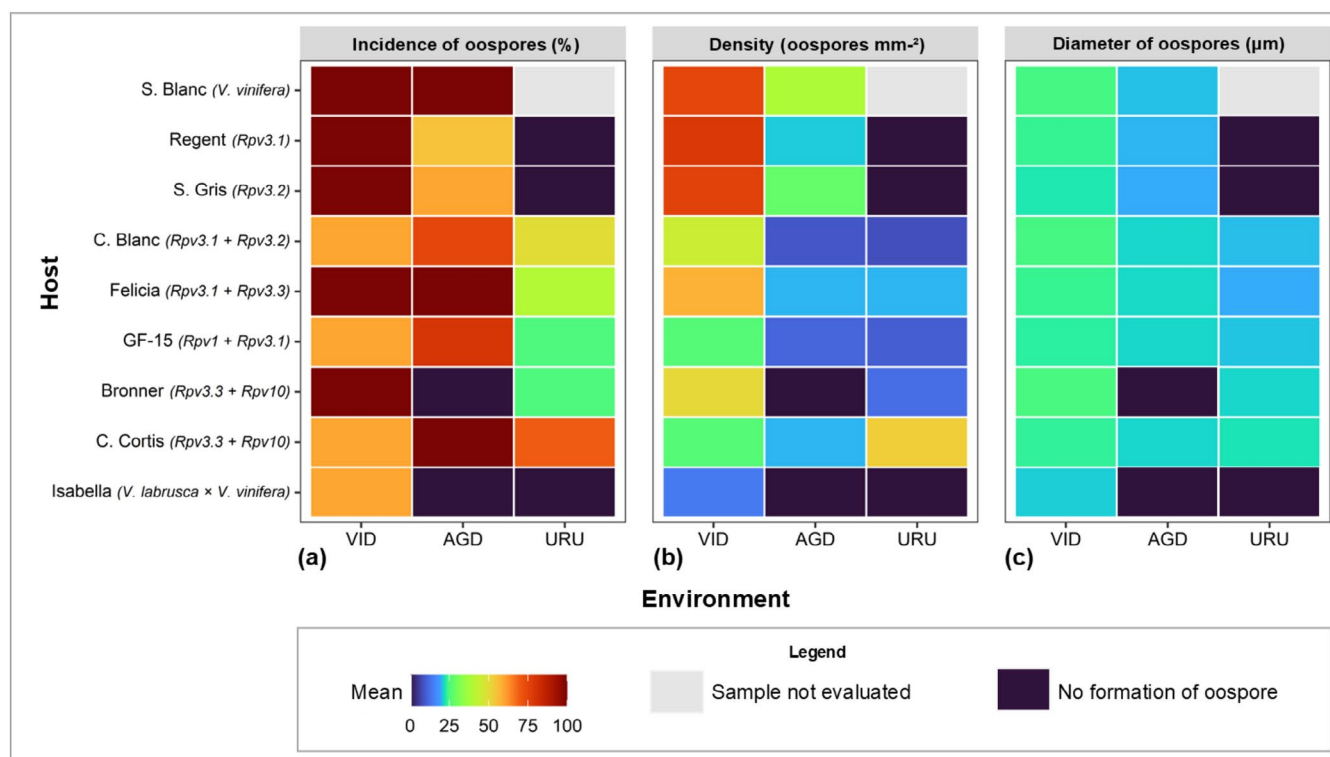


FIGURE 3 | Heatmaps illustrating the combined effects of host genotype and environment on (a) mean oospore incidence (IO, %), (b) mean oospore density (oospores mm⁻²) and (c) mean oospore diameter (μm) of *Plasmodiopsis viticola* in leaf discs from grapevine genotypes grown in Videira (VID), Água Doce (AGD) and Urussanga (URU), Santa Catarina, Brazil. Colours correspond to the mean values for each variable based on the indicated scale. Light grey cells denote samples that were not evaluated whereas dark purple cells indicate no formation of oospores.

3.4 | Climatic Suitability for *P. viticola* Oospore Formation in Southern Brazil

The ecological niche model exhibited high discriminatory accuracy in predicting environmentally suitable areas for *P. viticola* oospore formation. Model performance was supported by a high AUC value (0.878), along with a TSS of 0.691, sensitivity of 0.846, specificity of 0.845, and a Boyce Index of 0.884, indicating the predictive output was both reliable and robust (Figure S4).

The most influential environmental variables in the model are temperature (relative contribution of 73.8%), temperature range (16.8%) and ppt (9.4%). The response curve for mean temperature indicated a gradual decline in climatic suitability for oospore formation as monthly mean temperatures increased (monotonic negative relationship; Figure S5). Maximum suitability (0.93) was observed at 14.2°C, remaining above 0.6 up to 16.8°C and above 0.4 up to 18.0°C. Temperatures exceeding 19.7°C resulted in low suitability values (<0.20).

A similar monotonic negative pattern was observed for the diurnal temperature range, with maximum suitability (0.47) occurring at 7.6°C and dropping below 0.20 beyond 11.7°C. In contrast to the previous variables, the response curve for monthly ppt showed that climatic suitability increased progressively with rising precipitation levels (monotonic positive relationship). Suitability values increased from 0.11 (~89 mm) to 0.59

(~179 mm), with no evidence of saturation within the analysed range (Figure S5).

Climatic suitability for *P. viticola* oospore formation in southern Brazil is presented in Figure 5 and Table S5. Ecological niche modelling indicates that, under mean climatic conditions during March, April and May, the mean suitability was 0.41, with values ranging from 0.01 to 1.00 (Figure 5a). Approximately 35.4% of the study area was classified as having moderate suitability (>0.2–0.4), 24.4% as high (>0.4–0.6), 20.9% as very high (>0.6–1.0) and only 19.2% as low suitability (0–0.2). The map highlights that the highest-risk areas (suitability >0.80) are concentrated in highland vineyards, particularly in intermediate latitudes (27.6°S from 29.8°S), localised in the states of SC and RS.

The climatic suitability of the places representing the main wine-growing regions in southern Brazil, where the formation of oospores was confirmed, ranged from 0.22 (URU) to 0.92 (SJ), both located in SC State. High suitability values were observed in wine-growing regions of RS State, particularly in VAC (0.86 ± 0.05), NPA (0.85 ± 0.06), BG1 and BG2 (0.83 ± 0.12), and MBS (0.71 ± 0.09), and SC State, in SJ (0.92), AGD (0.81 ± 0.07), CUR (0.79 ± 0.04), and VID (0.64 ± 0.06). In contrast, CAM (0.38 ± 0.09) in PR State and DPE (0.33 ± 0.05) in RS State, and URU (0.22 ± 0.06) in SC State showed moderate suitability reflecting less favourable climatic conditions during the March–May period.

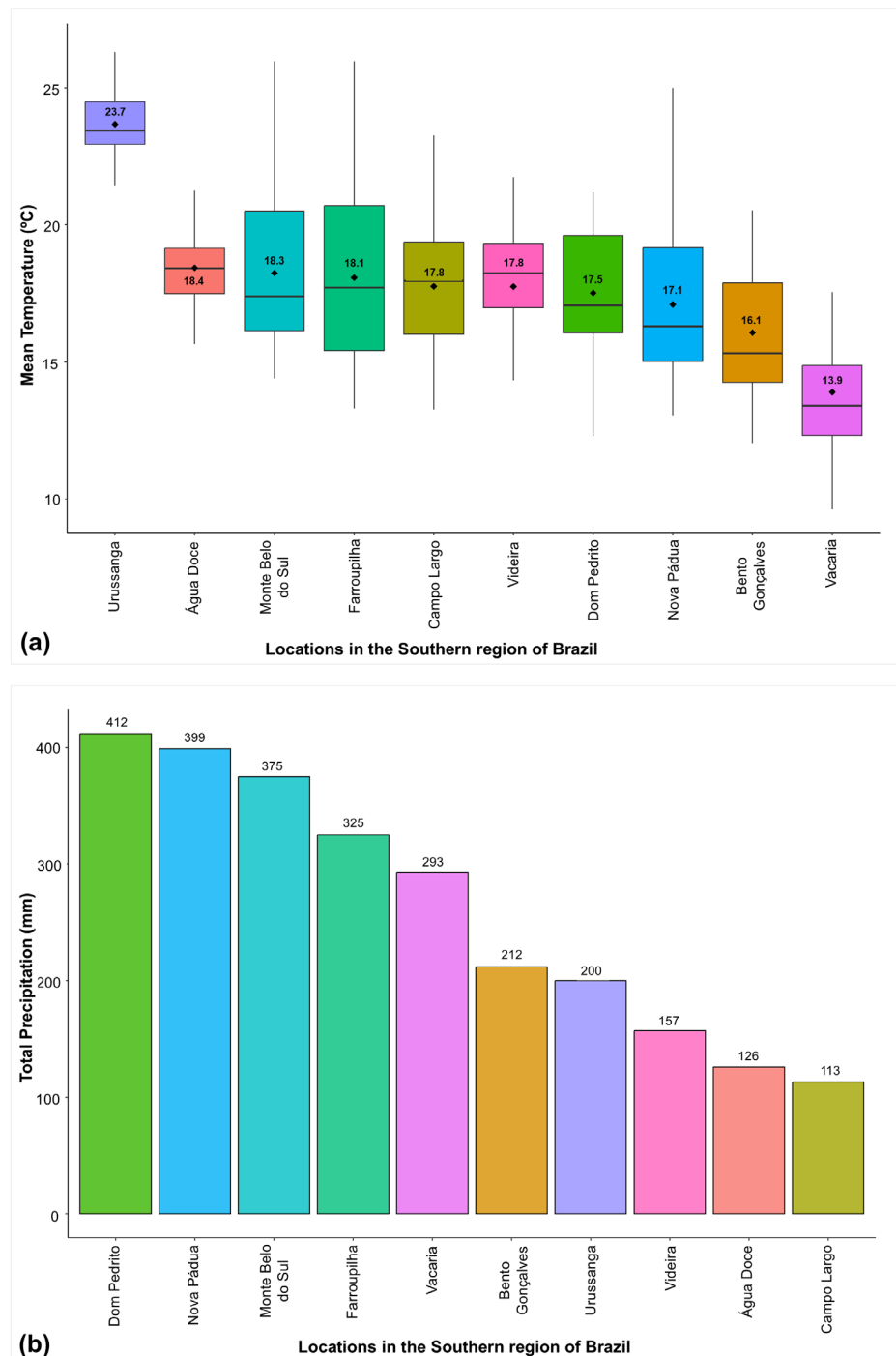


FIGURE 4 | Climatic data from the study sites during the 30 days prior to sample collection. (a) Boxplot distribution of daily mean air temperature (°C) for each sampling location. (b) Total accumulated precipitation (ppt) during the same period at each sampled location (bars).

In this context the regions of Serra Catarinense (SC) (mean = 0.92 ± 0.05), Campos de Cima da Serra (RS) (0.89 ± 0.04) and Serra Gaúcha (RS) (0.82 ± 0.07) emerged as key risk areas, with all municipalities within these regions classified as having very high suitability (>0.60), according to the thresholds proposed by Cohen (2023) (Table S5). Other regions with high suitability included Planalto Catarinense (SC) (0.76 ± 0.04) and Alto Vale do Rio do Peixe (SC) (0.63 ± 0.06), where most municipalities were classified as high to very high. In contrast, Campanha Gaúcha (RS) (0.33 ± 0.04) and Vale da Uva Goethe (SC) (0.23 ± 0.09) showed lower mean suitability, with most

municipalities falling into the low to moderate categories, indicating reduced climatic favourability for sexual reproduction during the study period. Pronounced intraregional heterogeneity was also observed, particularly in southeastern PR, where suitability for oospore formation ranged from a low of 0.17 in Cerro Azul to very high values of 0.62 and 0.64 in Quatro Barras and Tijucas do Sul, respectively.

Seasonal maps of climatic suitability for each month (March, April and May) revealed notable spatial and temporal variability across the study area (Figure 5b–d). In March (Figure 5b), high

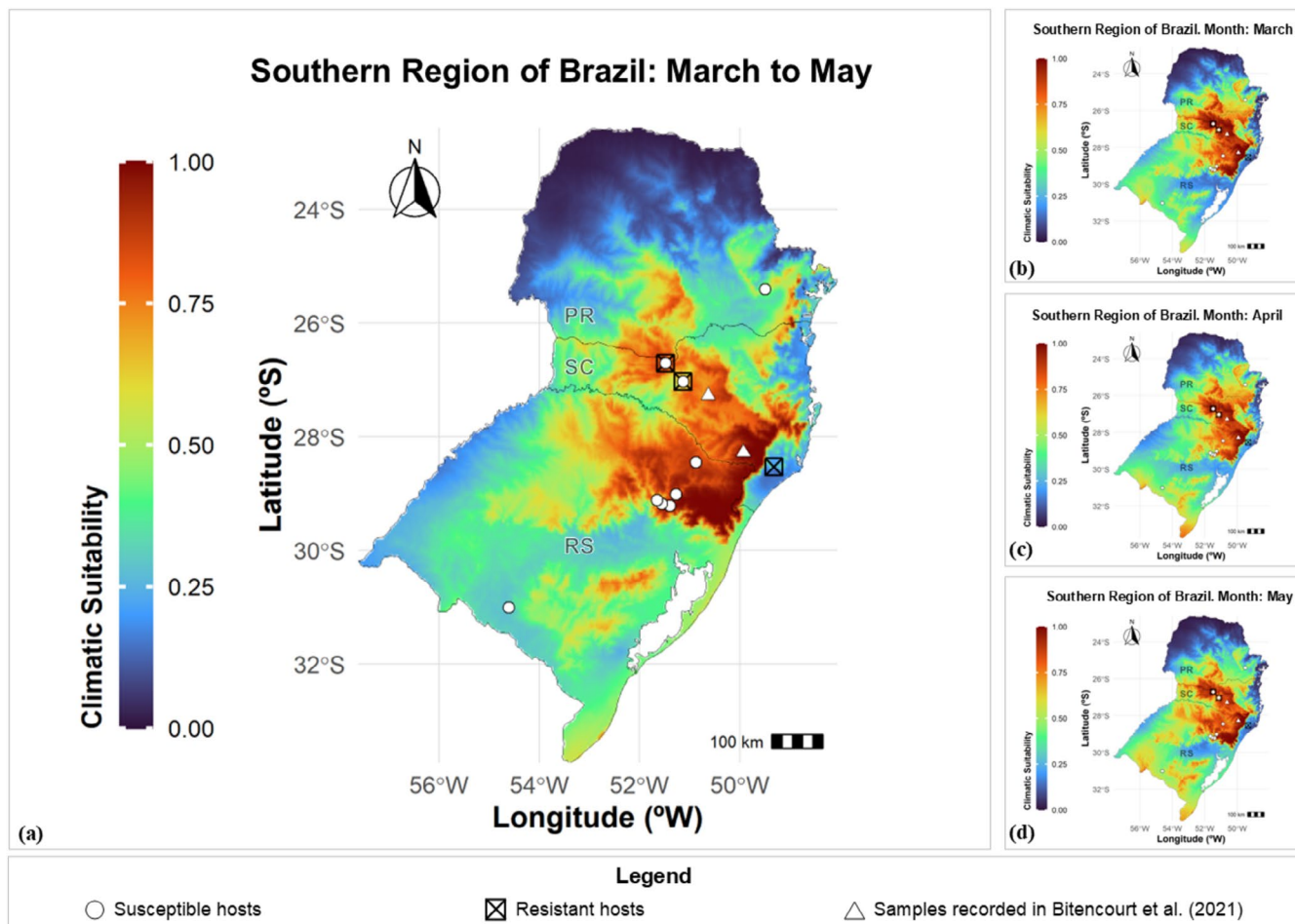


FIGURE 5 | Climatic suitability for *Plasmopara viticola* oospore formation in southern Brazil as predicted by the MaxEnt model for (a) the mean period from March–May, (b) March, (c) April and (d) May. White circles indicate sites of oospore occurrence in susceptible hosts; white triangles represent locations previously reported by Bitencourt et al. (2021) and the square with an internal “x” marks occurrence sites in resistant hosts. In the colour scale shown on the left side of the figure, the orange-to-red gradient denotes a very high probability of suitable conditions (> 0.6–1.0), light green indicates high suitability (> 0.4–0.6), light blue reflects moderate suitability (> 0.2–0.4) and dark blue shades represent low predicted suitability (0–0.2).

suitability was observed in regions such as Campos de Cima da Serra (RS), Serra Gaúcha (RS), Serra Catarinense (SC), Planalto Catarinense (SC) and Alto Vale do Rio do Peixe (SC). However, in April and May (Figure 5c,d), a substantial expansion of areas classified as very highly suitable was detected, including a marked increase in suitability indices in the Campanha Gaúcha and southeastern PR regions. This temporal pattern coincides with the sample collection, when DM mosaic symptoms were abundant. In contrast, the Vale da Uva Goethe exhibited minimal temporal variation in suitability throughout the trimester, consistently presenting low to moderate values.

3.5 | Germination of *P. viticola* Oospore in the Highland Region of Santa Catarina

The temperature and precipitation data for Curitiba (SC) during the oospore overwintering period that was tested (9 May to 16 September 2024) are presented in Figure 6. The mean temperature and ppt during the period were 14.4°C and 591.7 mm, respectively. Ten events of temperatures under 0°C and frequent rainfall were recorded.

Following *P. viticola* oospore overwintering in the field, germination assays confirmed oospore germination for a susceptible and a panel of resistant hosts. During germination, the thick oospore walls were disrupted, allowing the emergence of single germ tubes that terminated in macrosporangia containing zoospores (Figure 7). Statistically significant differences in oospore germination were observed among the different hosts ($\chi^2 = 45.59$; $df = 5$; $p < 0.0001$), as shown in Figure 7f. Higher rates of oospore germination were observed for the susceptible host Cabernet Sauvignon (59%) and the resistant hosts Calardis Blanc (51%), Cabernet Cortis (50%) and GF-15 (44%). The resistant hosts Felicia (20%) and Souvignier Gris (30%) showed significantly lower oospore germination rates, although Souvignier Gris did not differ significantly from GF-15.

4 | Discussion

The biological function of *P. viticola* oospores, which determines their role in the disease cycle, depends on the successful completion of their formation, maturation and germination stages

Curitibanos, SC (2024)

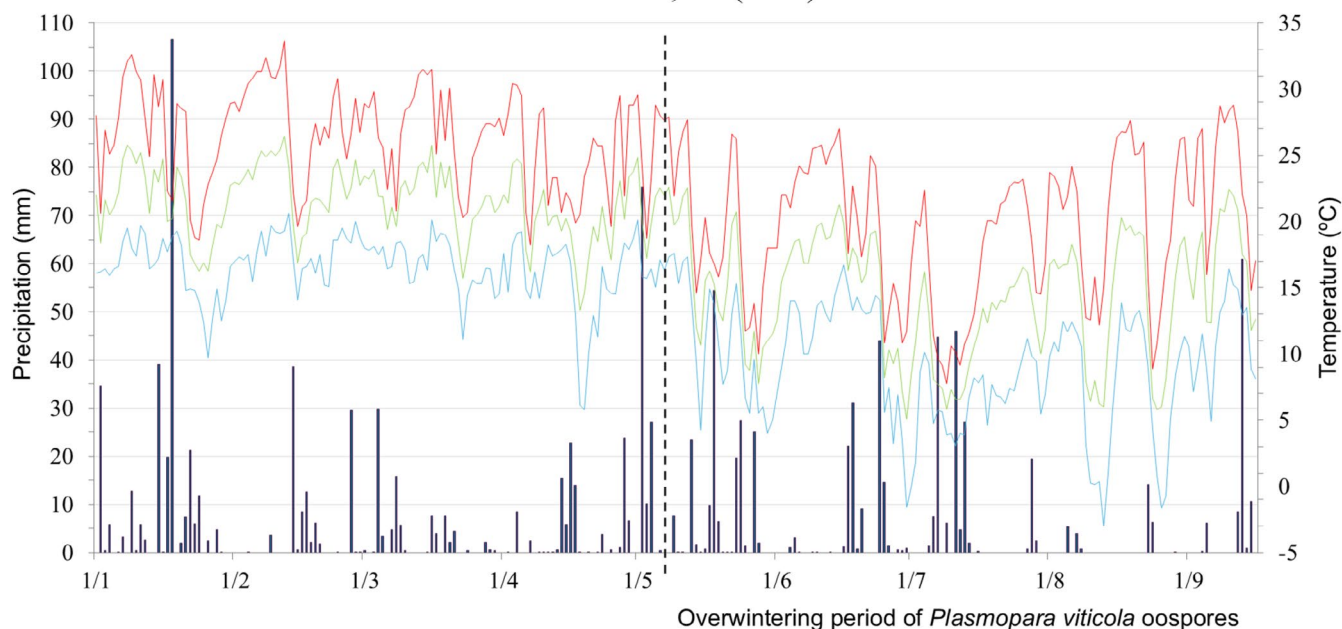


FIGURE 6 | Daily precipitation (blue histogram) and minimum (blue line), mean (green line) and maximum mean temperature (red line) recorded from January to September 2024 in Curitibanos, Santa Catarina, Brazil. The grey dashed line indicates the date of collection of leaf samples and the start of sample overwintering in the environment to evaluate germination of *Plasmopara viticola* oospores.

(Ronzon-Tran Manh Sung and Clerjeau 1988). The results obtained in the present study confirm the sexual reproduction of *P. viticola* in leaves of grapevine in southern Brazil, demonstrating not only the formation of oospores in susceptible and resistant hosts but also their subsequent maturation and germination. As the sexual reproduction of *P. viticola* is heterothallic (Dussert et al. 2020; Wong et al. 2001), our findings also strongly suggest that both the P1 and P2 mating types are widely distributed in the southern region of Brazil. These results are in accordance with the high genetic diversity reported for *P. viticola* in RS state (Santos et al. 2020).

Our study detected the presence of *P. viticola* oospores in the main wine-growing regions of southern Brazil. The morphology and diameter of the observed oospores were consistent with previous descriptions in the literature (Lehoczký 1956; Vercesi et al. 1999). High numbers of oospores were detected in the analysed samples, reaching up to 212 oospores mm^{-2} in a leaf disc excised from a mosaic lesion from a susceptible host. These results are consistent with Gessler et al. (2011), who reported up to 250 oospores mm^{-2} within a single polygonal leaf lesion. In the soil, concentrations of up to 1858 oospores per gram have been reported, originating from infected leaf tissue that falls to the ground (Poeydebat et al. 2024). These results demonstrate that in southern Brazil, leaf tissue of grapevine can accumulate significant quantities of oospores, reinforcing the need for integrated disease management strategies that address both the asexual and sexual stages of *P. viticola* to disrupt oospore formation.

Additionally, the locations where leaf samples were collected recorded mean temperatures ranging from 13.9°C to 23.7°C during the 30 days preceding sampling, corroborating the temperature range for sexual reproduction reported by Gehmann

et al. (1987). Although oospore formation had previously been confirmed in highland regions of SC (above 980 m.a.s.l.) (Bitencourt et al. 2021), our results demonstrate that sexual reproduction occurs across a broader range of latitudes (from 25°S in CAM to 31°S in DPE) and altitudes (from 49 m a.s.l. in URU to 1250 m a.s.l. in AGD). These findings expand the known geographic distribution of the sexual cycle of *P. viticola* in the southern hemisphere and indicate that, under field conditions in southern Brazil, oospore formation is not restricted to highland regions.

Evaluation of the same hosts in contrasting SC environments demonstrated that *P. viticola* oospore development is not determined solely by host genotype and/or environmental conditions but rather by a complex interplay between these variables. Higher oospore incidence, density and diameter were observed in VID, most likely reflecting a combination of agronomic, environmental and epidemiological factors. Sample collection in VID occurred later than in AGD and URU, and the mean temperature in VID was also lower. Consequently, *P. viticola* is expected to have had a more favourable condition for oospore formation in that location. Interestingly, for susceptible hosts, oospore diameter in VID was larger than in the Serra Gaúcha regions, where samples were collected later in the same year and with lower mean temperatures (e.g., NPA and BGO). The differences observed between VID, URU and AGD for resistant hosts can be explained by the fact that VID is the most traditional grape-producing region in SC, with the largest vineyard area. This may promote a more uniform and widespread distribution of the two mating types required for sexual reproduction. Additionally, a high disease incidence and severity on resistant hosts were reported at the end of the season in VID (Souza et al. 2025). High severity eases the interactions between compatible hyphae, thereby enhancing oospore formation. The direct correlation

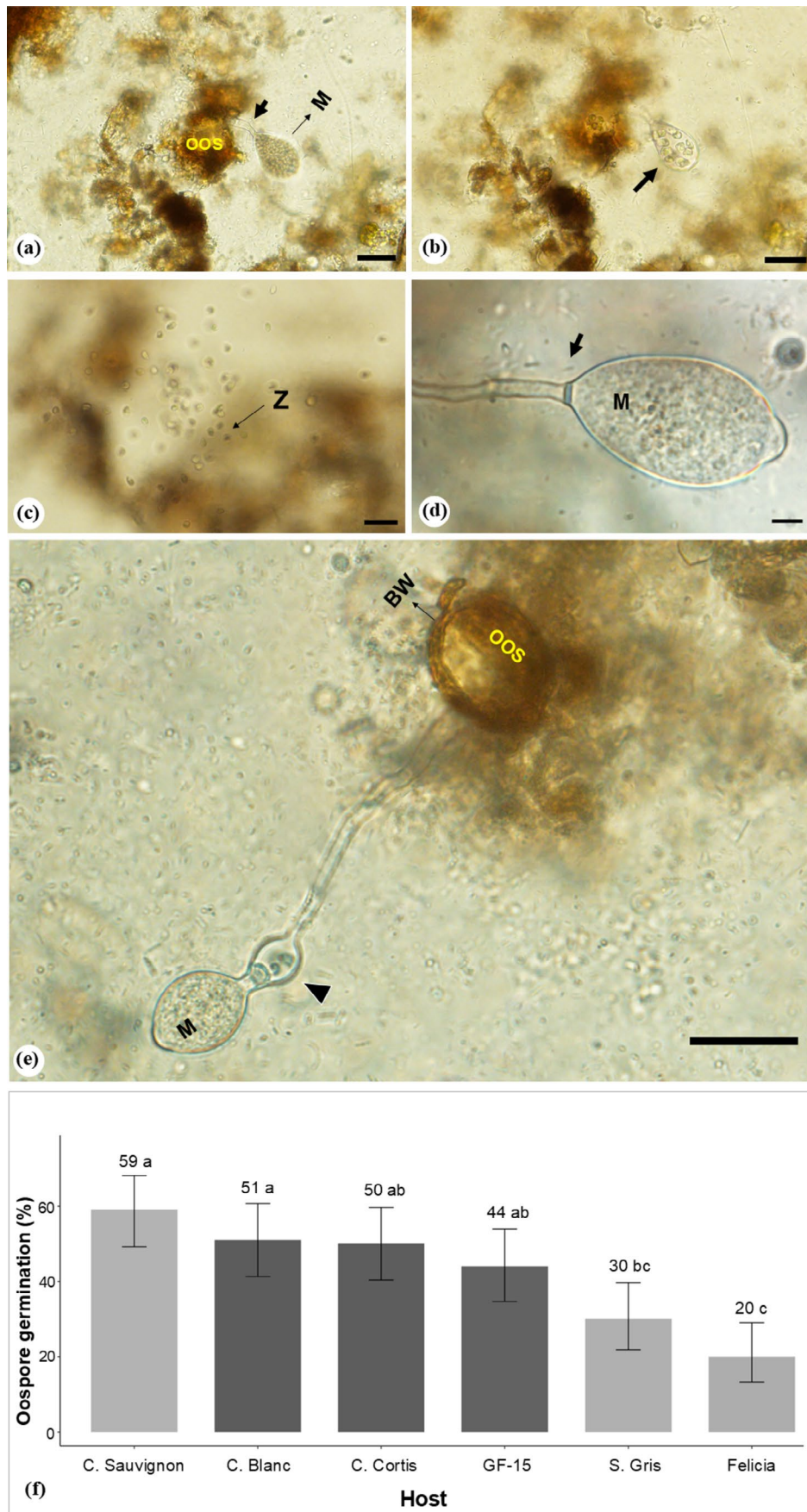


FIGURE 7 | Legend on next page.

FIGURE 7 | Germination of *Plasmopara viticola* oospores overwintered in Curitibanos, Santa Catarina, southern Brazil. (a–e) Light microscopy images showing oospores germinating. (a) Oospore of *P. viticola* (OOS) emitting a single germ tube (black arrow) that ends with a sporangium (macrosporangium [M]). (b) The same germinating oospore with the macrosporangium recently ruptured (black arrow). (c) Zoospores (Z) (approximately 64) released from the recently ruptured macrosporangium. (d) Mature macrosporangium (M) connected to the germ tube (black arrow). (e) Germinating oospore showing the broken thick wall (black arrow pointing to BW) and material being transferred (black arrowhead) from the oospore through the germ tube to the macrosporangium (M). Scale bar of 20 µm for images (a), (b), (c) and (e). Scale bar of 5 µm for image (d). (f) Percentage of *P. viticola* oospores germinated from susceptible and resistant hosts (Susceptible host: Cabernet Sauvignon; Resistant hosts: Calardis Blanc, Cabernet Cortis, GF-15, Sauvignier Gris and Felicia). Different letters indicate a significant difference in the germination percentage between hosts detected by the Tukey's test ($p < 0.05$).

between the severity of DM and oospore concentration in leaf litter was already reported (Carisse 2016; Fedele et al. 2025). We can also not disregard the possibility of the presence of isolates of *P. viticola* that overcome the resistance alleles in grapevine.

In URU and AGD, the samples were collected on the same day, and the oospore density for most of the hosts did not differ significantly. The two locations are the most contrasting in altitude and, consequently, temperature. AGD and URU showed a difference of 5.2°C in mean temperature in the month samples were collected, which corresponded to 18.4°C and 23.7°C, respectively. This suggests that oospore formation was more likely associated with a physiological inhibition of asexual reproduction (Grünzel 1961) rather than the effect of low temperature because the temperature observed in URU is within the optimal range for asexual reproduction (Mouafo-Tchinda et al. 2020; Williams et al. 2007). Furthermore, oospores can form at temperatures ranging from 10°C to 25°C, with an optimum between 14°C and 18°C (Gehmann et al. 1987), which encompasses the conditions recorded at both locations. This evidence supports our historical temperature data, which is the most influential variable in the ecological niche model.

The *Rpv* alleles present in the hosts under investigation did not prevent oospore formation, even when pyramided. Oospores were detected in all the hosts carrying *Rpv* alleles in at least one location. Some resistant hosts exhibited similar oospore densities to those observed on susceptible hosts in both VID and AGD, including hosts carrying the pyramidal resistance alleles *Rpv3.3* + *Rpv10*. For instance, we also monitored oospore formation in the field on hosts carrying combinations of resistance alleles not included in the main methodology of this study. In the autumn of 2025, oospores of *P. viticola* were detected on the hosts Pinot Regina (*Rpv1* + *Rpv12*) (Figure S6) and Sauvignon Kretos (*Rpv12*), as well as on two advanced selections from the UFSC/Epagri grapevine breeding programme, each carrying pyramided resistance alleles (*Rpv10* + *Rpv3* + *Rpv1*). A previous study conducted in SC State reported that Felicia, Calardis Blanc, Bronner and GF-15 showed significantly lower maximum disease severity and AUDPC (Area Under the Disease Progress Curve) when compared to the susceptible host, particularly for the hosts containing two resistance genes pyramided (Zanghelini et al. 2019). However, an increase in DM severity was observed from berry ripening onwards, reaching maximum severity after grape harvest, even in Bronner (*Rpv3* + *Rpv10*) and GF-15 (*Rpv1* + *Rpv3.1*), for which a slight *P. viticola* sporulation could be visualised. These results demonstrate that in the field, although *Rpv* alleles are significantly efficient in the asexual phase during most of the growing season, they are not sufficient to prevent late infections that lead to sexual reproduction.

On the other hand, it is important to emphasise that the present study analysed only regions with mosaic DM symptoms, and DM severity was not assessed on the sampled leaves. As reported by Zanghelini et al. (2019), DM severity at the end of the growing season was lower on hosts carrying *Rpv* alleles, meaning that a smaller leaf area was colonised and a lower number of oospores per leaf would be expected (Fedele et al. 2025). Therefore, future investigations need to consider disease severity and adjust the methodology for estimating the number of oospores formed per leaf. The formation of oospores in resistant hosts has already been reported by Delbac et al. (2019) and Bitencourt et al. (2021). Delbac et al. (2019) observed a lower number of oospores per leaf in hosts containing the *Rpv* allele when compared to the susceptible host. In this study, the host Isabella, an interspecific hybrid between *V. vinifera* and *V. labrusca*, exhibited the lowest oospore formation across all evaluated locations. This is due, at least in part, to the presence of trichomes on the abaxial surface of its leaves (a trait inherited from *V. labrusca*), which serves as a physical barrier to pathogen penetration (Nascimento-Gavioli et al. 2019) and may limit oospore development.

All occurrence locations of *P. viticola* oospores in southern Brazil were used to develop the ecological niche model. Despite the limited number of sampling points, the model exhibited high predictive performance. The AUC value of 0.878 was comparable to those reported in previous studies based on larger occurrence datasets, such as Ikeda and Osawa (2020) (AUC = 0.861) and Cohen (2023) (AUC = 0.935), all considered satisfactory according to the classification proposed by Li et al. (2020). Model robustness was further supported by the Boyce index (0.884), sensitivity (0.846), specificity (0.845) and TSS statistic (0.691), indicating a balanced and reliable predictive capacity (Hirzel et al. 2006; Ikeda and Osawa 2020). These findings pave the way for future studies, such as broader regional assessments and the prediction of climate change impacts on *P. viticola* sexual reproduction.

Ecological niche modelling indicates that 80.8% of the southern region of Brazil exhibits moderate to very high suitability for *P. viticola* oospore formation. Notably, oospores were observed even in regions such as URU and DPE, which presented relatively low suitability values of 0.22 and 0.33, respectively. The concern is even greater in areas of highest risk (suitability > 0.80), which, according to our results, are concentrated in the highland regions of SC and RS, between latitudes 27.6°S and 29.8°S. These regions contain the largest area of planted vineyards and are the main regions for producing fine wines in Brazil (OIV 2024). Therefore, the climate risk is greater precisely in places where viticulture has cultural and economic

importance. During the grape harvest, fungicide applications are restricted, opening a window for *P. viticola* infection and, consequently, oospore formation. In most wine regions of Brazil, the environmental conditions after harvest are still favourable for the asexual reproduction of *P. viticola*. After harvest, many wine growers reduce disease management measures, allowing the pathogen to infect leaf tissues. Our results emphasise the importance of late-season disease management, even in resistant and partially resistant cultivars. For effective management of DM, it is essential to prevent late-season *P. viticola* infections in senescent plant material (Poeydebat et al. 2022).

To our knowledge, this is the first study confirming *P. viticola* oospore germination in Brazil and in the southern hemisphere. During the overwintering period, the mean temperature was 14.4°C, with recorded drops below 0°C, and a total ppt of 591.7 mm. After this period, germination of oospores was observed in all assays conducted on both susceptible and resistant hosts. Once dormancy is overcome, oospore germination is influenced by temperature ($\geq 11^\circ\text{C}$), precipitation, and humidity (Rouzet and Jacquin 2003). Our results indicate that most oospores in the field are already mature at the beginning of the grape-growing season (budbreak), corroborating the northern hemisphere findings by Maddalena et al. (2021). These authors also noted that oospores are highly adaptable to host phenology, adjusting their behaviour according to the plant's developmental stage. Thus, continuous monitoring of oospore germination throughout the grapevine cycle is essential to better understand the dynamics of the pathogen under southern hemisphere conditions.

All morphological structures previously described by Vercesi et al. (1999) during oospore germination were observed (Figure 7). The oospores produced a single germ tube, at the tip of which a macrosporangium containing zoospores was formed (Figure 7d). The transport of cytoplasmic material from the oospore to the macrosporangium through the germ tube is visible in Figure 7e. According to Vercesi et al. (1999), this material includes numerous nuclei, vacuoles and ribosomes. We successfully recorded the exact moment of macrosporangium cleavage and the release of at least 64 zoospores in Cabernet Sauvignon (Figure 7a,b) and 53 zoospores in the Cabernet Cortis host (images not shown). The zoospores exhibited rapid movement in water (video in Figure S7), consistent with the descriptions provided by Burruano (2000).

Only the hosts Souvignier Gris and Felicia showed lower oospore germination rates than Cabernet Sauvignon. Studies evaluating oospore germination after overwintering under natural field conditions in resistant hosts are still scarce. However, Delbac et al. (2019) reported that *P. viticola* oospores formed in resistant hosts are also fertile, supporting our findings. The reduced germination rate observed for Souvignier Gris and Felicia may be resulting from the formation of non-viable oospores (type III) (Vercesi et al. 1999) or the need for a longer maturation period (Maddalena et al. 2021). Further investigation is needed to confirm these hypotheses.

Our results have important implications for grapevine breeding and DM management in southern Brazil. Sexual reproduction enhances the genetic diversity and evolutionary potential of pathogens (McDonald and Linde 2002). Indeed, isolates that

overcome resistance alleles (Delmas et al. 2016; Heyman et al. 2021; Peressotti et al. 2010) and show fungicide resistance (Gullino et al. 2004; Santos et al. 2020) have already been reported for *P. viticola*. Given the perennial nature of grapevines and the prolonged period for developing new resistant cultivars, durable resistance is essential. Achieving this requires integrating multiple and complementary DM management approaches, such as pyramiding resistance genes alongside cultural practices and chemical and biological controls. To delay the development of fungicide resistance, rational and preventive use is key, rotating products with different modes of action and adhering to dosage and timing recommendations. Disease forecasting systems should also be used to support decision-making and optimise fungicide application timing (Chen et al. 2020). Finally, further research is needed to develop strategies that disrupt oospore formation and germination.

Author Contributions

Aparecido Lima da Silva: funding acquisition, writing – review and editing, resources, supervision. **Camila Bitencourt:** conceptualization, investigation, writing – original draft, methodology, validation, visualization, writing – review and editing, software, formal analysis, data curation, resources. **Valdecir Perazzoli:** conceptualization, writing – original draft, methodology, validation, visualization, writing – review and editing, formal analysis. **Fabio Ribeiro de Freitas:** writing – review and editing, resources. **Cristian Soldi:** resources, writing – review and editing, funding acquisition. **Leocir José Welter:** conceptualization, funding acquisition, writing – review and editing, project administration, supervision, resources.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Figure S1:** Location of main wine-growing regions in southern Brazil and the sampling sites of leaves exhibiting typical mosaic symptoms of downy mildew. **Figure S2:** Symptoms of grapevine downy mildew (*Plasmopara viticola*) on leaves: (a) initial “oil spot” lesions on the adaxial leaf surface; (b) advanced stage of the disease with sporulation on the abaxial leaf surface; (c) grapevine leaf showing mosaic symptoms of grapevine downy mildew. **Figure S3:** Boxplot of daily temperature range (°C) during the 30 days before sample collection at the study locations. **Figure S4:** Comparison between the AUC of empirical and null models. Red lines are the 0.05 and 0.95 quantiles, respectively, whereas the blue line represents the AUC obtained by the empirical model. The lower-left panel presents the performance metric values: Boyce Index, True Skill Statistic (TSS), sensitivity and specificity. **Figure S5:** The response curves generated from the MaxEnt model were used to illustrate the relationship between each predictor variable and the predicted probability of climatic suitability during the three-month period (March, April and May) for the formation of *Plasmopara viticola* oospores. **Figure S6:** *Plasmopara viticola* oospores on leaf disc of host Pinot Regina (*Rpv1* + *Rpv12*) (scale bar = 20 µm). **Figure S7:** Video showing the exact moment of rupture of a mature macrosporangium and the release of zoospores, resulting from the germination of a *Plasmopara viticola* oospore (scale bar = 50 µm). **Table S1:** Information about the locations, hosts and sampling dates from the grape leaves naturally infected with *Plasmopara viticola* that were screened for oospore in southern Brazil, and methodological stations from which meteorological data were recorded. **Table S2:** Hosts, resistance alleles, and sample collection locations of grape leaves naturally infected with *Plasmopara viticola* screened for oospore formation in Santa Catarina State. **Table S3:** Data from susceptible and resistant hosts collected in two vineyards in Curitiba, Santa Catarina, Brazil, for the evaluation of *Plasmopara viticola* oospore germination. **Table S4:** Incidence of oospores, mean oospore diameter and oospore density of *Plasmopara viticola* observed in resistant and susceptible hosts grown in three contrasting environments in Santa Catarina State. **Table S5:** Mean and standard deviation of climate suitability for oospore formation based on historical quarterly climate data (March, April, May) from 2017 to 2022 in the municipalities of the main grape-growing regions in southern Brazil.