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Behavior of Vine Varieties Resistant to Fungal Diseases in the Somontano Region

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Abstract: The vine is highly susceptible to numerous fungal diseases, the incidence and severity of which are increasing because of climate change. To fight them, large amounts of phytosanitary products are generally used, although they entail important economic and environmental costs. The new fungus resistant vine varieties (PIWI) constitute one of the most active lines of research on plant material in viticulture and are regarded as a viable solution to respond to the requirements of European Directive 2009/128/EC. In the present work, the evolution and impact of the three main fungal diseases (powdery mildew, downy mildew and botrytis bunch rot) were monitored in plots in the Somontano region (Huesca, Spain), comparing three PIWI varieties (Sauvignon Kretos, Souvignier gris and Muscaris) with a susceptible control variety (Sauvignon blanc) in real field growing conditions over three successive years (2016–2018). The main ampelographic characters of each variety were studied and a weekly follow-up was carried out to track the development of each disease, relating it to climatic variables. Regardless of the climatic conditions (one of the growing seasons was rainy and the other two corresponded to dry years, with differences in the distribution of rainfall), the three PIWI varieties hardly presented any symptoms, demonstrating a high resistance to downy mildew and powdery mildew and even to botrytis. Principal component analysis results pointed to a differential behavior versus the control and treated Sauvignon blanc plants in terms of disease resistance. Based on production results, despite the youth of the plantation, the excellent potential of Sauvignon Kretos variety was observed. However, from the analysis of the quality of wine obtained in microvinifications from these fungus resistant varieties in the second and third year, the one obtained from Muscaris seemed to have the best organoleptic properties. Apart from environmental considerations, given that these fungus resistant varieties can significantly contribute to the sustainability of wineries, they may also respond to the demand of consumers who want zero-pesticide products or of the supporters of ecological products adjusted to European regulations.

Keywords: *Plasmopara viticola*; *Uncinula necator*; *Botrytis cinerea*; *Vitis* spp.; resistance; PIWI

1. Introduction

The vine (*Vitis vinifera* L.) is highly susceptible to numerous diseases of fungal origin, mainly powdery mildew (*Uncinula necator* (Schwein.) Burrill, syn. *Erysiphe necator* Schwein.), downy mildew (*Plasmopara viticola* (Berk. & M.A. Curtis) Berl. & De Toni) and botrytis bunch rot (*Botrytis cinerea*

Pers.) [1]. The pressure of these diseases is forcing viticulturists to use large amounts of phytosanitary products, which entail substantial economic and environmental costs and which, in many cases, quickly generate resistance. According to Eurostat data, the application of phytosanitary products per hectare and year in viticulture is the highest among all crops [2], since in some cases the number of applications per growing season is higher than 12 [3], reaching up to 16 applications in times of high disease pressure. For instance, in the case of the Burgundy wine region, on average 15 applications/year are needed to manage mildew [4].

European Directive 2009/128/EC establishes in its article 14 the bases for the use of pesticides in a sustainable manner, highlighting as a fundamental aspect the reduction of their use, particularly that of copper in viticulture [3]. In Spanish legislation, reference to the sustainable use of phytosanitary products is made in Royal Decree 1311/2012. One of the most promising strategies involves prevention based on the development of fungus resistant varieties [3] obtained through breeding programs, although this approach is constrained by the limits of natural resistance [5] and strategies aimed at limiting the risk of resistance breakdown and to enhance its durability need to be employed [6].

Marker assisted selection, combined with backcrossing with multiple varieties of *V. vinifera*, has allowed to develop fungus resistant grape varieties with two resistance genes (Rpv1 ... Rpv27 against downy mildew; Ren1 ... Ren10, Run1 ... Run2.1, Run2.2 against powdery mildew, and so forth [7]) and a significant percentage (over 85%) of *V. vinifera* in their pedigree. These varieties, known as "PIWI" (from the German *Pilzwiderstandsfähig*, "resistant to fungal diseases") or fungus-resistant grapes (FRG), are accepted as *V. vinifera* in European catalogs [8]. Regulation (EU) 1308/2013 includes the use of these PIWI varieties for the production of wines, provided they are previously registered in the register of varieties of the country, and their use will be likely permitted in the production of wines with a designation of origin after the conclusion of the EU agricultural policy reforms currently in progress [9]. At present, Germany is the country with the highest number of licensed mildew resistant grape varieties, listed in the database of the German Federal Office of Varietal Registration (Bundessortenamt).

The resistance of these varieties varies with cultivar genetics and location [10]. Therefore, most cultivars of resistant varieties show some susceptibility to different pathogens, including powdery mildew, downy mildew, botrytis, black rot and anthracnose. These diseases are generally controlled with sulfur-based fungicides [3,11] or with copper-based formulations, but with a much smaller number of applications than in traditional varieties [12]. In a study conducted in six European countries, which included 183 PIWI varieties, a reduction in the number of fungicidal treatments ranging from 73% to 82% was obtained for medium and low disease pressure levels, respectively [3]. A similar result was also obtained in a study involving 65 German vineyards under ecological management, whose producers reported that they sprayed the fungus-resistant varieties about 3.8 times on average per growing season [13].

It is expected that the use of PIWI varieties will result in very important cost savings in annual disease control—for instance, in France, it has been estimated that production with resistant varieties could reduce production costs by half [14]. In California, it has been foreseen that the use of varieties resistant to powdery mildew could save at least $48 million per year in the production of table grapes, raisins and wine [15]. In terms of pesticides annual costs, those associated with the control of downy mildew in conventional vineyards in Italy typically range from 8 to 16 M€/year, depending on disease pressure [16]. In California, Sambucci et al. [17], on the basis of pesticide use reports from California Department of Pesticide Regulation, estimated the statewide costs of managing powdery mildew in 2015 at about $240 million. Moreover, powdery mildew management would account for 89% of restricted material (pesticide) applications.

Apart from aforementioned advantages in terms of environmental safety, reduced production costs and reduced carbon footprint, it has also been shown that conditions for natural pest control are improved in fungus-resistant grapevines because of reduced fungicide applications [18,19]. Regarding their yield, fungus resistant varieties are generally more vigorous and show greater productivity [3,20–22]. A comprehensive and up-to-date panorama of the benefits, limits and challenges associated with the use of these varieties for organic wine production may be found in the review papers by Pedneault et al. [23] and Yobregat [24].

Nonetheless, despite the advocated advantages, fungus resistant varieties are practically absent from the wine market of most producing countries due to an unjustified distrust of both their use and of the quality of wine [13]. This suspicion arose because PIWI carry non-*V. vinifera* genes (even at low levels), which led to the presumption that they could result in lower quality wines [15]. Currently, there are studies that show that the quality of wines from fungus resistant varieties is generally equivalent to those produced from *V. vinifera* [3,12]. However, as noted in a recent review paper by Pertot et al. [25], growers and winemakers still need to acquire sufficient experience to optimize both agronomic practices and oenological processing in order to exploit the full potential of these new resistant genotypes.

In Spain, the only experimental test fields with PIWI, in addition to that of Viñas del Vero—in which the present work has been carried out—are those located in Olite and in Baztán valley in Navarra [26]. All of them were planted in 2015 and no details on production and/or on use of phytosanitary products have been disclosed.

In this study, we report the results for three PIWI varieties (Sauvignon Kretos, Souvignier gris and Muscaris) over the 2016–2018 period (three growing seasons), in a pioneering study in Spain.

2. Material and Methods

2.1. Location

The experimental vineyard is located on a plot owned by Viñas del Vero S.A., in the area referred to as 'Las Almunietas,' in the municipality of Barbastro, heading. The Universal Transverse Mercator (UTM) coordinates of the plot are: X = 261706, Y = 4652417, zone 31.

Regarding the soil, in Badía et al. [27] a detailed study of the area of 'Las Almunietas' is carried out, specifically the section referring to 'Torre Fierro.' This area is formed by gypsisols, that is, soils developed on gypsum marls that present a secondary accumulation of gypsum in powder and/or lenticular forms at a certain depth. They are loamy soils, with low stoniness, a moderate available water retention capacity and low cation exchange capacity.

2.2. Plant Material

The plant material used in the study consisted of three white PIWI varieties (chosen by the winery) and a control variety (Sauvignon blanc). The first PIWI variety, Sauvignon Kretos (UD 76-026), was obtained by the Udine Institute of Applied Genomics (Italy), from the cross of Sauvignon blanc with 20/3 and was registered in 2015 in the "Registro Nazionale delle Varietà di Vite" [28]. The second PIWI variety was Souvignier gris (FR 392-83), obtained by the Stainliches Weinbauinstitut Freiburg (Germany) in 1983, by crossing Cabernet Sauvignon (Sauvignon blanc × Cabernet Franc) with Bronner (Merzling × Gm 6494 (Zarya Severa × St. Laurent)). It was registered in the Bundessortenamt in 2008 [29] and in France in the "Catalogue des variétés de vigne" [30]. The third PIWI variety, Muscaris (FR 493-87) was also obtained by the Stainliches Weinbauinstitut Freiburg in 1987 from the cross of Solaris × Gelber Muskateller [31]. The resistance loci were Rpv12 for Sauvignon Kretos; Rpv3.3, Rpv10, Ren 3 and Ren 9 for Souvignier gris; and Rpv10, Ren 3 and Ren 9 for Muscaris [32].

The plants were certified by the Vivai Cooperativi Rauscedo nursery (Rauscedo, Italy) in the case of Sauvignon Kretos and by Pepinières Viticoles Mercier nursery (Vix, France) for the other two varieties.

Two hundred and twenty four individual PIWI varieties were studied—69 of Sauvignon Kretos, 76 of Souvignier gris and 79 of Muscaris, all planted in April 2015 as grafted plants on an SO4 rootstock. For comparison purposes, Sauvignon blanc (clone 376) was used as the susceptible control and treated variety (183 plants, with the same rootstock and planted at the same date).

2.3. Experimental Design and Cultural Techniques

The varieties to be studied were distributed in two adjacent plots, with rows of control plants located on both sides of the fungus resistant ones and then rows of treated Sauvignon blanc (Figure S1). To distinguish the specific plot in which the rows were located, subgroups of the latter two were labelled as '#1' and '#2.' Each variety was planted in two rows of about 40 m, with a plantation frame of 2.8 × 0.9 m (i.e., a planting density of 3968 plants/ha), using a single curtain bi-lateral cordon (High Trellis) training system with a training height of 1.4 m.

The plants were subjected to demanding conditions in terms of yield, forcing some management aspects (irrigation, grass, canopy ...) in order to assess the resistance of the experimental cultivars.

Watering was conducted with integrated drip hoses every 75 cm, with a flow rate of 4 L/h. The hoses were raised 50 cm above the ground. Fertilization-related information is summarized in Table S1.

In connection with canopy management, in 2016 only winter pruning was carried out, while in 2017 and 2018 growing seasons both winter and summer pruning were conducted.

All plants (both the fungus resistant varieties and the control and treated Sauvignon blanc) had to be treated against vine leaf blister mite in the 2016 growing season (5 treatments with abamectin 1.8% and one treatment with azadirachtin 3.2%), because they were severely affected (the effect of the attack is more pronounced in young plants). Apart from that, no other treatments against pests or diseases were applied to the PIWI and control rows. Regarding the fungicides applied to the treated rows, a preventive fungicide (cupric hydroxide) and a systemic one (metalaxyl + mancozeb) were used against downy mildew; and for powdery mildew, myclobutanil was applied as a preventive fungicide, together with several systemic fungicides (bupirimate, tebuconazole and proquinazid). Further details on these treatments are provided in Table S2. To exclude that the fungicides applied on Sauvignon blanc could have reached the control or resistant varieties, phytosanitary products drift was analyzed with hydro-sensitive paper cards.

2.4. Data Collection

Climate monitoring was carried out using moisture meter dataloggers supplied by Hobby Boards [33], with leaf wetness and temperature sensors, located in the plot under study. The data from these measurements were complemented with climatic data from the Ebro Hydrographic Confederation (CHE) and the Spanish Agroclimatic Information System for Irrigation (SIAR).

The weekly field monitoring of all varieties was conducted from March to September for the three years. The phenological states were tracked according to the following scales: Eichhorn & Lorenz code [34], *Les stades repères de la vigne* [35] and the Biologische Bundesanstalt, Bundessortenamt und CHemische Industrie (BBCH) scale [36]. The main ampelographic characters were also monitored by taking 10 reference elements and using the list of descriptors of the International Organization of Vine and Wine (OIV) for grape varieties and *Vitis* species [37]. The descriptors used were those for young leaf, adult leaf, flower, cluster, berry, production and disease. The ampelographic characterization for the Sauvignon blanc variety was only performed on the control rows.

For disease monitoring, 55 randomly chosen leaves and, in turn, 25 clusters were observed for each variety each week. For the evaluation of the parameters related to the presence and severity of the attack of these diseases, the European and Mediterranean Plant Protection Organization (EPPO) proposal was followed. This scale establishes a scale based on the percentage of area of the organ affected by the disease [38]. To calculate the degree of attack, both in leaves and clusters, the Townsend-Heuberger formula was used, and for the evaluation of the efficacy of the treatment (i.e., the ratio between the degree of attack in control vines versus in treated vines), Abbott's formula was chosen [39].

The harvest was done manually. In 2017, it was carried out on a single date for the three varieties (on August 22), while in 2018 it was split into two dates—August 22 for Muscaris and September 3 for Sauvignon Kretos and Souvignier gris. In 2016, when the vines were 2-years-old, there was no harvest.

For the evaluation of the balance between production and plant development, the Ravaz index [40] was calculated, using harvest weight and pruned wood weight data.

2.5. Disease Forecast Models

The accuracy of the forecast models used by winegrowers in this area was tested: in the case of downy mildew, the potential moment of infection was estimated by calculating the latent period, based on Goidanich's model [41]; for powdery mildew, the Thomas and Gubler model was chosen [42].

2.6. Winemaking

Two hundred kilograms of grapes per variety were processed. A pressing without stripping was performed, with the addition of pectolytic enzyme (Pectazine, 3 g/100 kg) and potassium metabisulfite (30 ppm of SO_2), with a subsequent 24 h cold stripping. After racking, yeasts (*Saccharomyces bayanus* at 20 g/hL) were added and alcoholic fermentation was monitored and controlled, ending after 13 days (Muscaris), 20 days (Sauvignon Kretos) and 16 days (Souvignier gris). After a final racking and sulphite addition (metabisulfite, 4.5 g/hL), the finished wines were analyzed.

2.7. Statistical Analyses

Statistical analyses were carried out with IBM (Armonk, NY, USA) SPSS Statistics v.22 software. Prior to the analyses, the assumptions of independence, normality and homoscedasticity were checked for all groups. Since the disease monitoring data did not meet the normality requirement—checked with a Shapiro-Wilks test and the homoscedasticity requirement and checked with a Bartlett's test—the Kruskal-Wallis non-parametric test was used, with Conover-Iman test for post hoc multiple pairwise comparisons. Disease monitoring data were also subjected to principal component analysis (PCA) with the Varimax rotation method with Kaiser normalization. In the PCA, the components that accounted for most of the variance were selected and those that contained only a small percentage of the variance of the original data were removed. For the initial values of musts data, analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) post hoc test was used instead.

3. Results

3.1. Climatic Data

The average monthly temperatures and rainfall for the study period (March–September) in the three years under study are shown in Table 1. The years of 2016 and 2017 were dry years (243.6 and 294.3 mm, respectively), although 2016 presented a more irregular distribution of rainfall than 2017, and 2018 had an above-average rainfall (499 mm). Leaf wetness data for the three growing seasons is shown in Figure S2.

Table 1. *Top*: average monthly temperatures (°C); *bottom*: average monthly rainfall (mm) for the three growing seasons.

Year	March	April	May	June	July	August	September
2016	9.3	12.5	16.1	21.6	24.9	24.0	21.4
2017	11.8	13.5	18.2	23.7	24.8	24.3	18.7
2018	8.9	13.4	17.1	22.0	25.6	25.2	22.2
Year	**March**	**April**	**May**	**June**	**July**	**August**	**September**
2016	69.2	93.0	33.0	13.6	11.8	1.0	22.0
2017	109.4	34.0	45.4	47.8	23.3	7.0	27.4
2018	60.7	117.0	142.6	28.4	13.0	101.9	35.4

3.2. Phenology and Ampelography

Phenological and ampelographical data are summarized in Tables S3 and S4. The results obtained were in good agreement with the varietal files for the majority of the descriptors in the three years and, in the case of Muscaris and Souvignier gris, with the findings of Reference [43]. The small differences detected, especially in the 2016 growing season, may be attributed to youthfulness expression. In general, these fungus resistant varieties showed no adaptation problems.

3.3. Disease Monitoring

3.3.1. Powdery Mildew

In 2016 and specifically on July 5, very slight attacks on leaves were observed in the control and treated varieties, while in the resistant ones there was no attack. No statistically significant differences were found for the attack over the entire growing season (Table S5). The attack on clusters appeared in the week of July 19 (Figure 1a) and it was particularly noticeable in the control 1 rows, in which approximately a 20% degree of attack was reached; the attack in control 2 and treated rows was moderate and in the resistant varieties no affected clusters were observed. Significant differences were detected (Table S6).

In 2017, there was no powdery mildew attack in the PIWI but attack was detected in the control and treated varieties, both in leaves (Figure 1b) and in clusters (Figure 1c), with statistically significant differences between the PIWIs and the control in both cases (but not with the treated rows, see Tables S7 and S8).

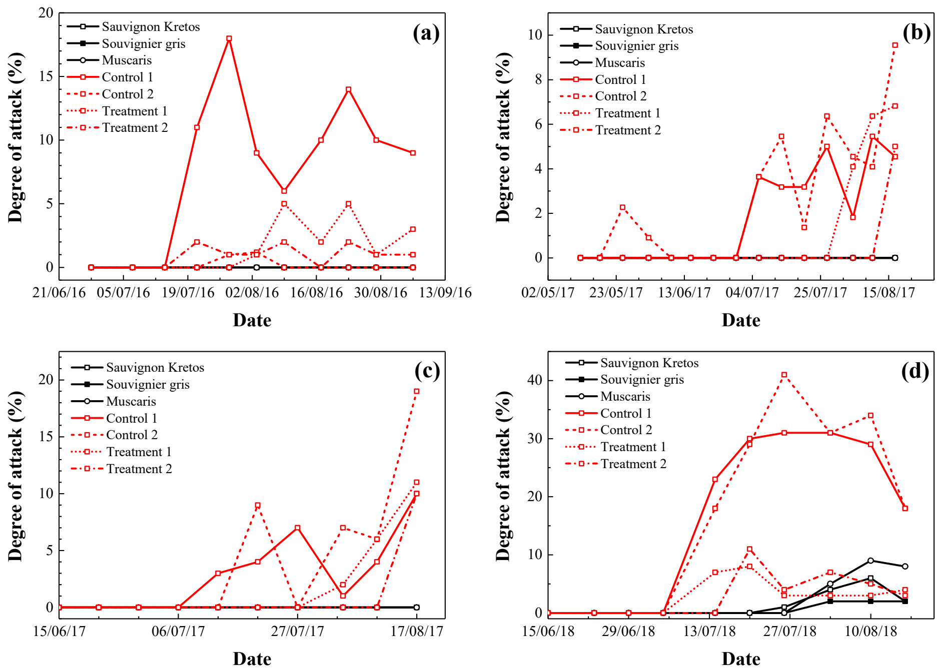

Figure 1. Temporal evolution of the powdery mildew degree of attack in: (**a**) clusters in 2016; (**b**) leaves in 2017; (**c**) clusters in 2017; and (**d**) clusters in 2018. Dates in which no attack was detected have been omitted for clarity. n = 25 for clusters and n = 55 for leaves.

In the 2018 growing season, no attack was detected in leaves (neither for the control, nor for the treated, nor for the PIWI vines) but there was powdery mildew attack in clusters (Figure 1d). In Sauvignon Kretos, a low degree of attack was recorded (in a total of 13 samples, all with a disease severity <25%) from July 26 to August 16. In Souvignier gris, the degree of attack was even lower (6 annotations, all with a severity <25%) from August 3 to August 16. In Muscaris, there were 20 annotations with a severity <25% (from August 3 to August 16) and only one in the 25–50% range of severity in the last sampling date (August 16). Comparatively, the degree of attack in the susceptible control plants was markedly higher but with no statistically significant differences (Table S9).

3.3.2. Downy Mildew

In 2016, leaf damage was detected in all vines (Figure 2a). The resistant varieties presented a low degree of attack, while the control and treated vines were the most affected but with no statistically significant differences (Table S10). With regard to cluster attack (Figure 2b), it was only detected for the control and treated vines: from June 28 to July 12, totally destroyed clusters were observed before the pea berry size was reached, followed by another attack that affected larger clusters, in which, over the weeks, the drying of grains was observed. This resulted in significant differences between the three PIWIs and the control varieties and also between the PIWIs and treatment 1 rows (Table S11).

In the 2017 growing season, no damage was detected in any of the PIWI varieties, in the treated vines or in group #2 susceptible control vines. Anecdotally, attacks of mild severity (<25%) were detected in leaves of group #1 control vines on July 13 (3 out of 55 samples, 5.77%). No damage was detected in clusters.

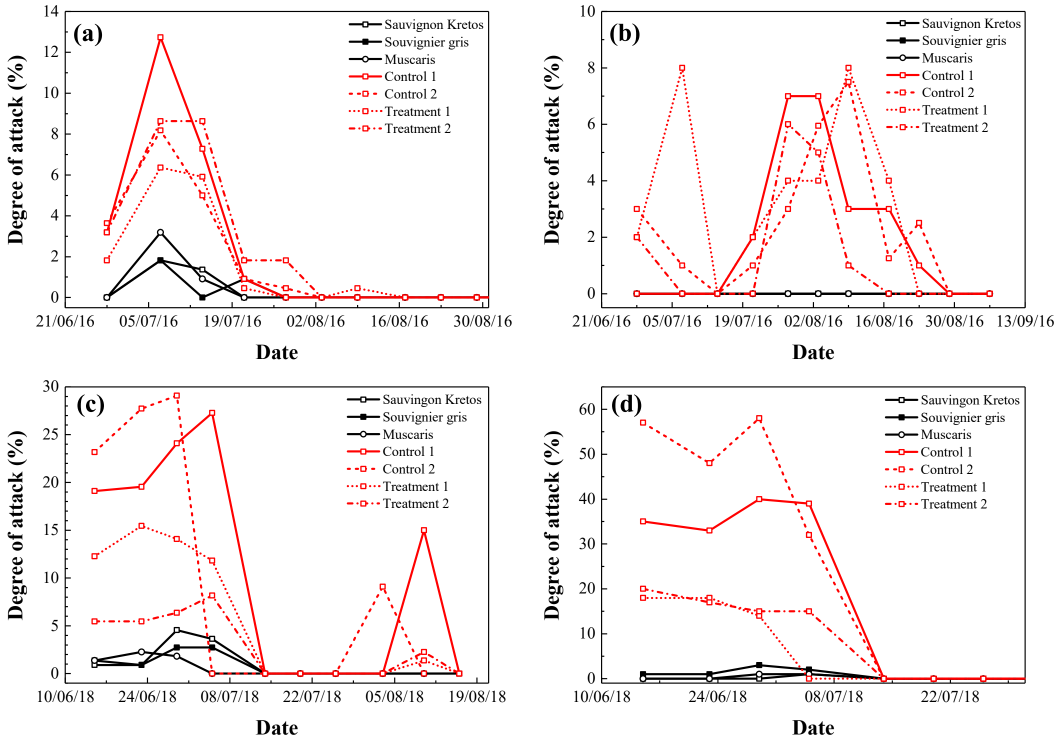

Figure 2. Temporal evolution of downy mildew degree of attack in: (**a**) leaves in 2016; (**b**) clusters in 2016; (**c**) leaves in 2018; and (**d**) clusters in 2018. Dates in which no attack was detected have been omitted for clarity. n = 25 for clusters and n = 55 for leaves.

In 2018, downy mildew attack was detected both in leaves (Figure 2c) and in clusters (Figure 2d). Regarding the attack in leaves, 22 cases were detected in Sauvignon Kretos vines, with a severity <25%, from June 15 to July 5; in Souvignier gris, a total of 17 cases were detected, all with a severity <25%, in the same period and, in Muscaris, a total of 15 cases were detected, with a severity <25%, also from June 15 to July 5. No statistically significant differences were detected between PIWIs, control and treated rows (Table S12). In relation to cluster damage, in Sauvignon Kretos a single case was detected, with a severity <25%, on July 5; in Souvignier gris a total of 7 cases were detected, all with a severity <25%, from June 15 to July 5; and in Muscaris two cases were detected, with a severity <25%, from June 29 to July 5. Damage to Sauvignon blanc control and treated plants was found to be much higher, reaching a degree of attack of up to 58% for clusters but with no significant differences (Table S13).

The decrease in downy mildew incidence along the season may be tentatively attributed to increases in temperature [32].

3.3.3. Botrytis Bunch Rot

In 2016 (Figure 3a), Botrytis attack was detected at the end of August in the control and treated vines, whereas no symptoms were observed in the PIWI varieties but no significant differences were found (Table S14). The damaged clusters mostly showed an attack severity in the 25%–50% range. In a similar fashion, in 2017 (Figure 3b) there was no attack on the PIWI varieties. In the control and treated vines, some cluster damage was detected on August 10 and on August 17 (with a severity of up to 50%), which affected less than 4% of the sampled clusters and which did not result in significant differences (Table S15). In the 2018 growing season (not shown), no botrytis bunch rot damage was detected in any vines (neither in control, nor in treated, nor in PIWI plants).

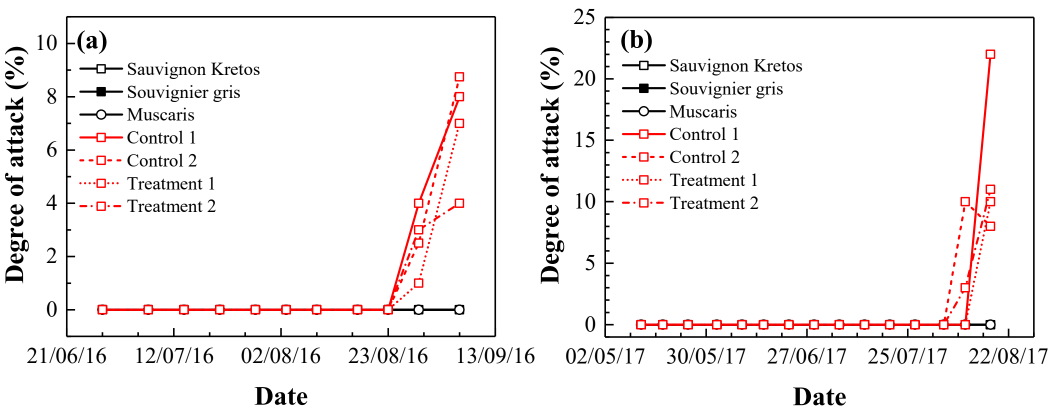

Figure 3. Temporal evolution of botrytis bunch rot in: (**a**) 2016 and (**b**) 2017. $n = 25$ clusters.

3.4. Treatment Efficacies and Associated Costs

In 2016, treatment efficacies against downy mildew were very low (18%) in leaves and somewhat higher in clusters (47%); in the case of powdery mildew, average treatment efficiencies above 50% were observed, both in leaves and in clusters. In 2017, treatment efficiencies against powdery mildew were higher than 60% in leaves (reaching 93% in group #2 treated vines). In 2018, the efficacy of the treatments against downy mildew was 66% in leaves and 60% in clusters and the efficacy against powdery mildew was as high as 82%.

Concerning treatment costs against downy and powdery mildew (for Sauvignon blanc), they accounted for 260 €/ha in 2016, 169 €/ha in 2017 and 322 €/ha in 2018.

3.5. Disease Forecast Models

In the case of downy mildew, Goidanich's model did not accurately predict the latent period under Somontano conditions: the expect dates in the 2016 and 2018 were off by several weeks. This would be in agreement with the findings of Kennelly et al. [44], who suggested that the latent periods can be much longer than expected. The use of other models (see [45]) should thus be encouraged in this region. On the other hand, Thomas and Gubler's model was found to be very accurate for prediction of powdery mildew in the three years of the study, with offsets smaller than a week.

3.6. Production and Ravaz indices

The yields and calculated Ravaz indices for the PIWIs and for the treated Sauvignon blanc variety in 2017 and 2018, based on harvest and pruned wood weight data, are shown in Table 2. No production data were available for the control rows, in which the attack was so severe that clusters were not even harvested (production may be roughly estimated at ca. 20% of that of the treated rows).

Table 2. Harvest and pruned wood weight data and calculated Ravaz indices.

Variety	2017				2018			
	100-Berry Weight (g)	Harvest Weight (kg/vine)	Pruned Wood Weight (kg/vine)	Ravaz Index	100-Berry Weight (g)	Harvest Weight (kg/vine)	Pruned Wood Weight (kg/vine)	Ravaz Index
Sauvignon Kretos	95.67	3.50	0.86	4.07	99.25	3.27	0.80	4.09
Souvignier gris	132.50	2.03	0.15	13.53	136.30	2.27	0.49	4.63
Muscaris	132.95	1.48	0.17	8.71	128.30	2.01	0.12	16.75
Sauvignon blanc	130.02	2.44	0.62	3.94	151.05	3.11	0.78	3.99

3.7. Initial Values of Musts

The results of the analyses performed on musts upon their grape reception at the winery are summarized in Table 3. The pH and probable alcohol contents were in average values obtained for other varieties grown in the same territory, although Muscaris variety reached a very high alcoholic degree and a too low total acidity. Souvignier gris was the only variety that manifested deficiencies in easily assimilable nitrogen, which would pose problems in fermentation, if not corrected.

Table 3. Initial values of musts from the three fungus resistant (PIWI) varieties and the control variety in 2017 and 2018.

Variety	Year	pH	Total Acidity (g/L)	Probable Alcohol Content (°)	YAN
Sauvignon Kretos	2017	3.14 ± 0.12 a	6.61 ± 1.96	12.90 ± 1.62	223
	2018	3.16 ± 0.11 ab	6.10 ± 1.99	13.66 ± 1.80	100
Souvignier gris	2017	3.35 ± 0.09 ab	6.08 ± 1.00	14.30 ± 1.01	190
	2018	3.38 ± 0.12 ab	5.50 ± 0.97	14.96 ± 1.62	84
Muscaris	2017	3.15 ± 0.11 a	3.79 ± 1.01	16.20 ± 1.12	165
	2018	3.71 ± 0.10 c	3.90 ± 1.50	15.60 ± 1.33	224
Sauvignon blanc	2017	3.47 ± 0.09 bc	3.95 ± 1.22	13.45 ± 1.10	168
	2018	3.31 ± 0.07 ab	4.28 ± 1.35	14.01 ± 1.24	180

YAN: yeast assimilable nitrogen. All values are expressed as the average across 3 replicates ± standard deviation. pH values labelled with the same lowercase letters are not significantly different at $p < 0.05$ by Tukey's test.

Statistically significant differences for variety*year interaction were only found for pH. In the case of the probable alcohol content, significant differences were observed for the variety factor (between Sauvignon Kretos and Muscaris).

3.8. Winemaking Results

The analytical parameters of the vines prepared from the PIWI varieties are presented in Table 4. The obtained wines were subjected to a sensory analysis in the Aroma Analysis and Oenology Laboratory (LAAE) of Zaragoza. Tasting results for the wine from Muscaris—with descriptors of bitter almond and dried fruit and a high intensity terpenic profile—were consistent—it had the best reception in both years, with higher ratings than the control. The one from Sauvignon Kretos featured white, tropical and stone fruit notes, with ratings slightly lower than those of the Sauvignon blanc wine. It may have an acceptance similar to it if only the acidity and some unpleasant notes are modulated by carrying out vinification tests with different ripening stages. On the other hand, the wine from Souvignier gris variety had a very low acceptance rate in 2017 but a good acceptance in 2018.

Table 4. Analytical parameters of wines prepared in 2017 and 2018 from the three PIWI varieties.

Variety	Year	ABV (%vol)	pH	Total Acidity (g/L)	Volatile Acidity (g/L)	Malic Acid (g/L)	Glucose + Fructose (g/L)
Sauvignon Kretos	2017	12.80	3.02	5.07	0.34	1.74	3.53
	2018	14.35	3.09	6.80	0.48	1.08	1.92
Sauvignier gris	2017	13.80	3.35	4.22	0.23	1.87	<1
	2018	15.55	3.41	6.01	0.80	1.95	1.41
Muscaris	2017	18.02	3.90	2.96	0.62	1.51	2.29
	2018	16.22	3.54	5.72	0.58	1.55	1.39
Sauvignon blanc	2017	13.05	3.39	3.52	0.27	1.65	2.45
	2018	13.2	3.3	3.29	0.33	1.21	0.71

ABV: Alcohol by volume.

4. Discussion

With regard to the disease monitoring data and the validation of the resistance of the PIWI varieties against fungal diseases in this study, statistically significant differences were not found in all cases when the comparisons were made for each disease and growing season separately. In an attempt to gain insight on their global performance, a PCA was conducted with the data of the degree of attack of the three fungal diseases over the entire period of study (3 years). As shown in Table S16, the strongest underlying trend in the feature set (component 1) was associated with Sauvignon blanc (both control and treated rows), the second strongest underlying trend (component 2) was only associated with the PIWI varieties and the third strongest underlying trend (component 3) was related to the date. Hence, a differential behavior for these varieties (in this location) versus the control would be evidenced, independently of the year and disease.

Field studies on the performance of fungus resistant varieties are scarce in the literature [46–50] and, as noted above, the resistance of PIWI cultivars varies not only with genetics but also with location [10], so comparisons should be taken with caution. For instance, one of the most comprehensive studies was the one by Zamboni et al. [51], who evaluated the adaptability of 26 resistant varieties in terms of yield, quality and resistance against fungal diseases to Emilia-Romagna hills (Ravenna, Italy) over a 5-year period (1994–1998). However, the three PIWI varieties studied herein were not assessed.

Muscaris was evaluated by Vezzulli et al. [52] in a study in which downy mildew resistance in 28 grapevine hybrids (from various European institutions or nurseries located in France, Germany, Austria, Hungary and Czech Republic) was assessed in an untreated field trial conducted in Trentino region (Italy) over three successive years. In 2011, 16 hybrids showed a high level of resistance on leaves (OIV 452 scores 7–9), while during 2012 and 2013 only 6 and 8 hybrids, respectively, showed such scores. Over the evaluated triennium, a consistent level of downy mildew resistance on leaves was observed in 4 hybrids—Bronner, Solaris, Prior and Muscaris. Regarding disease resistance on clusters, 21 hybrids showed a high level (OIV 453 scores 7–9) in 2011, while during 2012 and 2013 the number of hybrids showing such scores decreased to 14 and 8, respectively. Over the 3-year period, a consistent level of downy mildew resistance on clusters was observed for four hybrids: Bronner, Solaris, Prior and Bianca. Considering the level of downy mildew resistance both on leaves and clusters over the 3 years, the authors concluded that the best results were achieved by Bronner, Solaris and Prior. In the particular case of Muscaris, it obtained a score of 9 in both OIV descriptors (452 and 453, for leaf and cluster based on symptom visual observation, respectively) in 2011 and 2012 but it scored 7 and 5 in 2013 in OIV 452 and OIV 453, respectively. Interestingly, such behavior differs from that observed in our study, in which it showed more downy mildew resistance in clusters than in leaf. This discrepancy can be explained with the variability of the plant-pathogen interaction phenomenon. Besides the environmental factors, plants can be affected by rootstock, while for the pathogen the existence of various and specific *P. viticola* genotypes may not be excluded [53]. Moreover, it should also be taken into consideration that the appearance of *P. viticola* genotypes able to breakdown the resistance cannot be left out [54], which points to the importance of designing breeding programs that optimize the durability of the resistances.

In another recent study by Nicolini et al. [43], both Muscaris and Souvignier gris were also tested in the Trentino region, as part of VEViR project. They reported that Souvignier gris showed a high tolerance to powdery mildew and medium-high tolerance to downy mildew. However, no disease resistance data for Muscaris were provided. According to AREDVI [55] data in two locations in France, both PIWI varieties showed high resistance to powdery mildew attack in leaves and bunches, with results similar to those found in this study, in Domaine de Cazes (Alaigne, Aude department). Nonetheless, the tolerance to the attack in clusters in the plot located Marsillargues (Hérault department), by the sea, was much lower for Muscaris. As regards Sauvignon Kretos, to the best of the authors' knowledge, no field performance data have been reported in the literature.

Regarding the between-years variability in the disease rating, it should be attributed to differences in rainfall [56]. As in the case of the study by Vezzulli et al. [52], the mean temperatures registered during the three considered growing seasons were very similar, while the total rainfalls differed. This different annual amount of rainfall might explain the significant difference in the disease symptoms found on leaves and clusters in 2017 (less severe) than in 2018 (more severe).

With regard to the productivity of the PIWI varieties, Pedò et al. [57] reported a yield of 1.967 kg/vine for Muscaris and of 2.291 kg/vine for Souvignier gris cultivated in Trentino (Italy), with Ravaz indices of 5.8 and 9, respectively. Nicolini et al. [43] reported a yield of 1.63 kg/vine for Muscaris and 2.08 kg/vine for Souvignier gris and Ravaz indices of 3.46 and 5.3, respectively. While for Souvignier gris the yields in our study were very similar, for Muscaris in 2017 a lower yield was attained. This may be ascribed to the youth of the plantation (3rd year), given that in 2018 the yield was comparable to those reported above. Concerning Ravaz indices, values in the 5–7 range are advised to avoid imbalances in the vine, although other authors have suggested a wider range (5-12), depending on the cultivar, load, irrigation dose and driving systems [58,59]. Very high values—above 10—indicate that the vine has produced a lot of grape for its vigor, that is, that it presents a mismatch due to excess of production or defect in vigor. On the contrary, values below 3 would indicate an excessive vigor of the vine, that is, a low productivity for its fertility conditions. Therefore, it follows that for the two years for which production data could be registered (2017 and 2018, Table 2), there were significant imbalances for Souvignier gris in 2017 and for Muscaris in 2018, although they can be justified by the youth of the plantation.

In relation to the analytical data of musts, Pedò et al. [57] reported a pH of 3.38, total acidity (TA) = 6.53 g/L and yeast assimilable nitrogen (YAN) = 81 mg/L for Muscaris; and a pH of 3.27, TA = 6.97 g/L and YAN = 57 mg/L for Souvignier gris. Nicolini et al. [43] and Moser et al. [60] reported a pH of 3.36, TA = 6.3–7.3 g/L and YAN = 23–94 mg/L for Muscaris; and a pH of 3.17–3.39, TA = 7.4–7.5 g/L and YAN = 26–58 mg/L for Souvignier gris. In comparison with the results obtained in this study, shown in Table 3, pH values were in good agreement. TA values for Souvignier gris were slightly lower but in a normal range but those of Muscaris were much lower. In connection with YAN, the musts in this study would not require nitrogen supplementation, whereas those reported above would not meet the requirements for low risk fermentation or for clean/fruity flavor.

With reference to the wines, Moser et al. [60,61] reported an ABV = 13.15%vol, pH = 4.16, TA = 6.8 g/L, VA = 0.67 g/L, glucose+fructose<1 g/L for Muscaris; and an ABV = 13.09%vol, pH = 3.62, TA = 5.9 g/L, VA = 0.38 g/L, glucose+fructose = 2.5 g/L. Upon comparison with the results summarized in Table 4, it is worth noting that, in our study, the ABV values were noticeably higher for Muscaris and that TA values were lower for both PIWI varieties. About the organoleptic characteristics, the tasting results agreed with those reported by Moser et al. [60], who indicated that Muscaris is a variety characterized by the prevalence of the fruity and aromatic intensity descriptors. A non-negligible fruity component is also present in Souvignier gris, which is associated with medium floral notes and a limited presence of vegetal notes.

It should be clarified that a large variability according to the winemaking process has been reported for PIWI wines [62]. Salmon et al. [63], who studied the sensory perception of wines prepared from a disease resistant grapevine variety obtained by the French Institut National de la Recherche Agronomique−INRA (ref A. Bouquet, var. 3160-12-3), found that the descriptors used by the panelists reflected different and homogenous sensory perceptions, depending on the type of winemaking. Hence, the qualitative valorization of these new grape varieties must be screened through different winemaking methods so as not reject or support one variety over another.

Espinoza et al. [64] analyzed consumers' evaluations of a white wine from a resistant variety (Bouquet 3159), produced in the Languedoc (France). They found that, on a purely sensory level, consumers had difficulty in accepting wine from a resistant variety. However, communication focusing on environmental and health performances remarkably improved the position of the resistant vine variety, putting it at the top of the average qualitative evaluations. According to the authors of the study, such promotion results in high market share, gained from conventional wines. In the same line, in a recent review paper by Schäufele et al. [65], it was concluded that the producing and marketing wine with sustainability features is a promising strategy for quality differentiation, particularly for wine that is both local and organic. A more in detail analysis on how these new varieties will change the competitive scenario of the wine market may be found in Schäufele et al. [65] and Montaigne et al. [66].

5. Conclusions

From the comparison of the results obtained for each of the diseases and varieties with their varietal files, it was concluded that, in general terms, the three PIWI varieties under study showed a high resistance to downy mildew, to powdery mildew and even to botrytis bunch rot (for which a medium level of resistance had been established). Statistically significant differences versus the Sauvignon blanc control were detected for downy and powdery mildew in two of the growing seasons and PCA results evidenced clear differences in the underlying trend in terms of resistance against the fungal diseases versus both the control and treated Sauvignon blanc plants. The Sauvignon Kretos variety exhibited a higher yield than that of the control variety and its wine may have an acceptance similar if only the acidity and some unpleasant notes are modulated. Souvignier gris and Muscaris showed lower yields than the control. However, the Muscaris variety may be the most interesting for pilot scale production, due to its good organoleptic profile. These resistant varieties pose a production alternative in environments subjected to high fungal pressure and may occupy a niche market aimed at consumers who value the reduction of phytosanitary products in viticulture. Moreover, remarkable savings in phytosanitary products may be entailed, estimated in 250 € per ha and year for this particular location.

Supplementary Materials: The following are available online at http://www.mdpi.com/2073-4395/9/11/738/s1, Figure S1. Distribution of the different grapevine varieties under study in the two adjacent plots; Figure S2. Electrical resistance measured by the leaf wetness sensors and rainfall data from the weather station; Table S1. Fertilization data; Table S2. Treatments against fungal diseases; Table S3. Phenological data for the three growing seasons (2016–2018); Table S4. Ampelographical data for the three growing seasons (2016–2018); Tables S5–15: Kruskal-Wallis test and multiple pairwise comparisons using the Conover-Iman procedure for the different diseases in leaves and clusters in the three growing seasons; Table S16. PCA results.

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