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Recovery from Grapevine Flavescence Dorée in Areas of High Infection Pressure

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Abstract: Flavescence dorée (FD) is a quarantine grapevine disease caused by a phytoplasma transmitted by the leafhopper *Scaphoideus titanus* Ball. FD management relies on compulsory insecticide treatments, roguing of infected plants, and substitution with certified material. Some grapevine cultivars show a spontaneous remission of symptoms (recovery). To determine if recovery is a suitable strategy to co-exist with disease in areas of strong infestation, the qualitative aspects of grapes, musts, and wines obtained from recovered Barbera and Chardonnay grapevines were investigated in two productive vineyards. Following field observations, about 1500 plants in each vineyard were divided into healthy (asymptomatic and negative in phytoplasma molecular diagnosis) and recovered (asymptomatic the year of observation but infected the year before). Maturation curves and microvinification tests followed by oenological and sensory analyses showed that maturation trends of recovered grapes were in line with those from healthy plants and the final qualities of wines were comparable. The spread of FD has strongly increased in Piedmont (Italy) in recent decades. Management strategies to cope with the disease are necessary to preserve traditional wine production. Despite the yield from recovered grapevines is quantitatively lower than that from healthy ones, we showed here that the wine quality is, however, preserved.

Keywords: phytoplasma; *Vitis vinifera*; wine quality; Barbera; Chardonnay; integrated pests and disease control

1. Introduction

Flavescence dorée (FD) is a grapevine yellows (GY) disease caused by phytoplasmas transmitted by the leafhopper vector *Scaphoideus titanus* Ball (Hemiptera: Cicadellidae) [1]. This pathogen belongs to the 16SrV ribosomal group; its assignment to a '*Candidatus* Phytoplasma species' has been proposed but not yet accepted by the International Research Programme for Comparative Mycoplasmology (IRPCM) Phytoplasma/Spiroplasma Working Team—Phytoplasma taxonomy group [2]. Typical symptoms, such as yellowing, downward curling of leaves, inflorescence or bunch withering, and lack of lignification appear usually one year after inoculation on some or all shoots and progressively spread within the canopy during the vegetative season, causing a strong reduction in the yield and quality of grapes, eventually resulting in plant death [3]. Similar symptoms on grapevine can be also induced by Bois noir phytoplasma (BNp) ('*Candidatus* phytoplasma solani'), belonging to the

16SrXII ribosomal group [4]. Flavescence dorée phytoplasma (FDp) can colonize *Vitis vinifera* in its overall cultivation area without being significantly limited by eco-climatic conditions [5]. In the main grape-growing European countries (Austria, Croatia, France, Hungary, Italy, Portugal, Slovenia, and Spain), the simultaneous presence of the phytoplasma and its vector has been the cause of the severe epidemic outbreaks, which historically have occurred causing heavy economic damage to the wine industry [5]. FD epidemics are reported in Piedmont (north-western Italy) since 1999 [6], particularly in the southern part of the region, which, was, in 2014, included in the World Heritage Site list of UNESCO (vineyard landscape of Piedmont: Langhe-Roero and Monferrato). From 2003 to 2018, the 'FD-settlement areas' increased from a very restricted zone of one province (Alessandria) to almost 25% of the whole Piedmont Region of Italy, covering the whole territory of two provinces (Alessandria and Asti) [7]. Therefore, management strategies aimed at coexisting with rather than eradicating the disease are necessary to preserve vineyards and wine economy in the area. In this region, wine production is based on traditional cultivars (among which Barbera, Nebbiolo, Dolcetto, Bonarda, Cortese) showing distinct susceptibility to infection [3,8].

No grapevine genetic resistance to FD is known and all grapevine cultivars are susceptible to infection, although at a different degree [9,10]. Moreover, cultivars with diverse susceptibility support different pathogen loads [8,11]. FD management traditionally relies on preventive control strategies, including compulsory insecticide treatments, roguing of infected plants, and their replacement with new grafted cuttings that will not be productive for 3–4 years [5]. The presence of highly sensitive cultivars may improve the efficiency of vector transmission [12] and, therefore, may influence FD epidemiology and the choice of the correct control strategy. FD-infected grapevines can spontaneously recover from the disease restoring their yield [3]. Recovery was first described for grapevine [13], then for apple [14], and for apricot [15,16]. Immunization or tolerance to new re-infections of apricots recovered from European stone fruit yellows' phytoplasma has been reported [16–18], although this is not the case for phytoplasma-recovered grapevines, which are susceptible to new infections [19]. Previous studies conducted on different plant species showed that recovery can be stimulated by abiotic stress, treatment with resistance inducers, antimicrobial molecules as well as application of mycorrhiza [20]. Furthermore, the involvement of endophytes in the recovery phenomenon is also reported [21]. Moreover, recovered grapevines do not contain detectable FDp and are not a source of phytoplasma for *S. titanus* [11]. Several works focus on molecular and physiological aspects of grapevine recovery from both Bois noir (BN) [22–25] and FD [26,27], addressing the comparison of infected and recovered plants. Healthy and FD-recovered grapevines show differences at both transcriptomic and proteomic levels [28–30]. Biochemical analyses also show a significantly higher level of hydrogen peroxide (H_2O_2) in the leaf phloem of recovered Glera grapevines compared to healthy and infected plants. Recovered plants can accumulate H_2O_2 because of the stable down-regulation of two genes that encode the main enzymatic H_2O_2 scavengers: catalase and ascorbate peroxidase [27]. Consistently, Barbera plants recovered from FD show up-regulation of several genes that encode enzymes involved in H_2O_2 metabolism, as well as high concentration of H_2O_2 [28]. Down-regulation of ascorbate peroxidase was confirmed in recovered Barbera vines [29], and lower catalase activity was measured in recovered vs. healthy Glera grapevines [27]. Flavescence dorée infection induces a wide metabolic alteration of most genes within the general metabolism category, with a long-lasting effect on recovered grapevines, which can be distinguished at the molecular level from healthy plants, even several years after infection [31]. Field observations suggest that recovery from FD infection is highly dependent on the grapevine cultivar: for instance, Barbera has a much higher attitude to recover than Glera [19]. Some sustainable strategies can be encountered as promising tools against FD: (i) the identification of molecular profiles linked to the grapevine cultivar-diverse susceptibility to develop less susceptible plants by breeding programs, (ii) the use of grapevine endophytic microorganisms with known biocontrol properties and endophytes living inside specialized insect cells, which can be potential candidates for FD vector control, (iii) the application of plant defense elicitors [32].

Nevertheless, more research is needed before these measures can be implemented in integrated pest management plans.

In the present work, the disease incidence, the recovery rate as well as the qualitative aspects of FD-recovered grapevines, musts, and wines were investigated for two cultivars, Barbera and Chardonnay, to support integration of recovery in the FD management strategy. Aim of this descriptive qualitative study was to ensure that qualities of recovered grapes and musts were within acceptable ranges for the respective wines. Indeed, if recovery is coupled to insecticide treatments for vector control, it might be a valuable tool for viticultural areas where the disease is endemic (FD-settlement areas).

2. Materials and Methods

2.1. Vineyards, Plot Selection, and Assessment of Flavescence Dorée-Infected Vines

The agronomical and epidemiological effects of recovery were studied in two grapevine cultivars 'Barbera' (red grape) and 'Chardonnay' (white grape) located in two commercial vineyards at Cocconato d'Asti, Piedmont (north-western Italy) (45°04'58.4'' N 8°03'21.1'' E, N-S orientation). Both vineyards were conducted with integrated pest management and regularly treated with mandatory insecticides against *S. titanus* according to the annual directives of the Regional Phytosanitary Service. In particular, the vineyards have been sprayed with thiamethoxam insecticide (200 g ha^{-1}) in the second half of June to control *S. titanus* nymphs, and with Chlorpyriphos-ethyl (2.4 L ha^{-1}, 600 g ha^{-1}) one month later to control adults. The two insecticides were allowed for use in viticulture at the time of the experiments. During the years of the study (2011–2015), infected (symptomatic) plants were not uprooted. We selected two highly infected vineyards subjected to routine agronomic practices, and for the purpose of the experiment, control of FD relied only on insecticide applications against the vector, without roguing of infected plants and removal of symptomatic branches, therefore resulting in continuous increase in the number of infected vines in the two vineyards. In Chardonnay plots, dead plants were replaced with grafted cuttings. Dead and newly replaced plants were not included in calculation of incidence and recovery rates for Chardonnay plots. Barbera and Chardonnay vineyards included about 9000 and 4500 plants, respectively. The average plant density in the vineyards was about 5000 ha^{-1} (0.5 m^{-2}). In both vineyards, 3 plots, each of about 500 plants, were used for the experiments: plots 1 and 3 (B1 and B3 for Barbera, C1 and C3 for Chardonnay) included the external plant rows, whereas plots 2 (B2 and C2 for Barbera and Chardonnay, respectively) contained few internal rows (Figure 1). The selected plots were designed to include a similar average number of total plants per block. The whole vineyards were regularly managed by wine-grower, irrespective of the selected experimental plots. All Barbera and Chardonnay plants in the selected plots were monitored for FD-specific symptoms from 2011 to 2015 and 2013 to 2015, respectively. Yellowing, downward curling of leaves, fruit abortion, stunting, and presence of black spots on the new canes are among the most frequently observed symptoms. Each year, in August, plants were visually inspected for symptoms of FD, and 10 to 20 plants for each cultivar were randomly sampled across the vineyard plots and singly analyzed to confirm FDp presence by PCR and exclude Bois noir phytoplasma (BNp) infection. Plants showing at least three of FD-specific symptoms were sampled. All symptomatic plants were labelled and year of infection recorded. According to field observation and molecular analysis, the selected plants in the plots were classified as: (1) recovered grapevines (infected in the previous year of observation, but symptomless and negative in FD and BN-specific PCR assays in the following year); (2) healthy control grapevines (which never showed GYs symptoms in the period of observation and tested negative for the presence of FD and BN phytoplasmas in molecular analyses). Recovery rate for each year was calculated as the percentage of recovered plants (among those infected in the previous year) over total infected plants of the previous year. Recovered (r) plants were identified in external rows (plots B1, B3 and C1, C3), whereas healthy (h) plants in the internal parts (plots B2 and C2), also considering the strong border effect demonstrated in FD epidemics [33,34].

Indeed, four different categories were identified according to the sanitary status for both Barbera (B) and Chardonnay (C): Br, Cr, Bh, and Ch.

Figure 1. Experimental plots. Selected areas of Barbera (**B1**–**B3**) and Chardonnay (**C1**–**C3**) vineyards are indicated (Cocconato municipality, Asti province, Piedmont region, Italy). Red, yellows, and green squares indicate symptomatic, recovered, and healthy plants, respectively, according to field observation performed in 2014, depicted as example. Satellite image from Google.

2.2. Nucleic Acid Extraction and Phytoplasma Molecular Detection

During the first year of observation (2011 for Barbera and 2013 for Chardonnay), the reliability of symptom observation was validated by PCR analysis, and phytoplasma genotypes present in the vineyards were characterized. Leaf samples were collected from 20 symptomatic grapevines (10 samples from 10 Barbera plants and 10 samples from 10 Chardonnay plants) randomly distributed within the vineyard plots and singly analyzed to confirm FDp presence by PCR and exclude BNp infection. Each sample generally consisted of basal, median, and apical leaves from shoots with symptoms, for a total of 10 leaves per plant.

During summer 2015 (year of harvest for microvinification), the absence of phytoplasmas was confirmed by molecular analyses for all the plants (Bh and Ch) selected for further measurements. To this purpose, on July 2015 leaf samples were collected from 100 symptomless plants randomly distributed in internal plots (B2 and C2) of each cultivar and pooled in mixed samples (each representative of 5 plants).

For each sample, about 1.5 g of leaf veins were excised and total DNA was extracted and dissolved in 100 µL of sterile double distilled water (SDW), as reported previously [6]. Nucleic acid concentration and purity were analyzed by a NanoDrop spectrophotometer. To verify the absence of FDp, total DNA was used as template for direct PCR with universal primer pair P1/P7, followed by nested reactions driven by primers R16(V)F1/R1, following the protocol described in the literature [6]. Amplicons were separated by electrophoresis on 1% agarose gels buffered in 0.5 X TBE (90 mM Tris-borate, 2 mM EDTA) and visualized under UV light after staining with ethidium bromide.

Since BN phytoplasma may also infect grapevines in the area, and infected plants cannot be easily distinguished from those infected by FD upon symptom observations, absence of BNp was confirmed by nested-PCR of P1/P7 amplicons with R16(I)F1/R1 primers [35].

Characterization of FDp isolates present in the two vineyards was done by restriction analysis of the partial 16S–23S rDNA operon and *secY* gene, as described in the original papers [36,37]. In the

first case, fragments amplified with the P1/P7 primer pair [38,39] were diluted 1:40 with SDW and re-amplified with primers M1/B6 [36], as previously described. For the partial amplification of the *secY* gene, total grapevine DNAs were amplified in direct PCR with primers FD9f2/FD9r and then, following 1:40 dilution with SDW, amplified with primers FD9f3/FD9r2 [40].

2.3. Maturation Curves

Maturation of grapes from healthy and recovered plants of the two cultivars was analyzed. Berry composition was measured weekly from veraison through harvest, on a sample of about 1000 berries collected randomly from each experimental block. To ensure homogeneous coverage of the plots, a suitable number of plants randomly dislocated within the block rows was selected. For each plant, five berries per bunch were collected: two at the top, two in the middle, and one at the base of the bunch. Berries were then pressed, and the must, after a gentle centrifugation, was analyzed to measure pH, total acidity (mg L^{-1} of tartaric acid), sugar content (°Brix), and sugar concentration (mg L^{-1}), by using Wine Scan Foss. To confirm the obtained results with officially accepted methods, sugar content was also calculated by densimeter Anton Paar (accredited in-house method), pH was verified by pHmeter Mettler Toledo according to OIV (International Organisation of Vine and Wine, OIV-MA-AS313-15), and total acidity was verified by titration with 0.1 M NaOH (OIV-MA-AS313-01). For each grape sample, also the potential alcohol was determined by Wine Scan Foss and expressed in % vol. Maturation curves were performed with two technical replicates of each measurement on representative samples (one for each treatment).

2.4. Microvinification, Enological, and Sensory Analyses

Microvinification tests followed by enological and sensory analyzes were carried out on the grapes of healthy and recovered plants of the two cultivars to characterize wine quality. Microvinifications were carried out by Cantina sperimentale Istituto Bonafous (Chieri, TO, Italy) and were performed on one representative sample for each treatment, consisting of grapes harvested from about 50 plants for each category. The harvest date was determined for each cultivar when the increase in sugar accumulation slowed and the organic acid content began to decrease. For both cultivars, selected plants in experimental plots were harvested at the same time as the whole vineyard, according to oenological evaluations customized on the final desired wine, to reflect the production reality. Microvinifications were carried out using 100 kg of grapes from each experimental category. For red wine production, destemmed and crushed grapes were vinified in stainless steel tanks of 100 L, adding 10 mg L^{-1} of potassium metabisulfite and 20 g hL^{-1} of active dry yeast. Maceration was conducted by pumping the juice over, twice a day. Fermentation and maceration lasted 14 days for all vinifications. In all cases, malolactic fermentation was fully completed. Wines received no wood treatment and were bottled after a storage period of two months. For white wine production, grapes were destemmed and crushed, and then, they were macerated for 12 h at 8 °C, after the addition of 10 mg L^{-1} of potassium metabisulfite and 1 g hL^{-1} of pectolytic enzymes. Crushed grapes were then pressed and, after static settling, fermented at 16 °C. At the end of the alcoholic fermentation, 50 mg L^{-1} of potassium metabisulfite was added to the wine, which was stored three months in stainless steel tanks, and then bottled. Wine analysis was performed three months after harvest. Alcoholic degree and titrable acidity of the wines were determined according to OIV methods (OIV-AS312-01A and OIV-AS313-01). The alcohol content was indicated as alcoholic strength percentage (% vol). The content of malic and lactic acid was determined by Wine Scan Foss and by enzymatic titration with automatic titrator Steroglass. The value of pH was measured by Wine Scan Foss and by pHmeter Mettler Toledo.

Sensory analysis of the experimental wines was conducted by a panel of 11 experienced tasters drawn from Enocontrol S.c.a.r.l. (Alba, Italy). Differences in the sensory perceptions between treatments of Barbera and Chardonnay wines were evaluated with a triangular test (according to ISO 4120). The samples were then subjected to a bilateral pair comparison test with the mandatory response to the preference. Each of the wines was descriptively assessed for its appearance, nose, and palate.

The scores of all judges for each wine were summed. Wine scores (n = 11 for Barbera and n = 11 for Chardonnay) were statistically analyzed with t-test with Microsoft Excel software.

2.5. Virus Detection

Diagnosis of common grapevine viruses was performed to monitor other vine diseases with symptoms similar to those caused by FD. Virus population in the plants of the four categories (Br, Cr, Bh, and Ch) yielded for the microvinification was analyzed by serological assays. To this purpose, branches from 200 plants (50 for each category) were collected during fall 2015, and bark scrapings were pooled in mixed samples (each representative of 5 plants). The presence of the grapevine viruses commonly reported in Piedmont, as Grapevine leafroll-associated virus-1 and 3 (GLRaV-1, -3), Grapevine fan leaf (GFLV), Grapevine virus A (GVA), was assessed by direct double antibody sandwich (DAS)-ELISA, while GFkV infections were assessed by indirect double antibody sandwich (DASI)-ELISA using commercial kits (Agritest), according to the manufacturer's instructions.

3. Results

3.1. Disease Incidence and Recovery Rate

During each experimental season, a total of 1652 Barbera and 1115 Chardonnay grapevines were visually inspected to identify plants showing FD symptoms. The observed plants were distributed in different field blocks on the borders (B1/C1 and B3/C3) and in the center (B2/C2) of each vineyard. The incidence of symptomatic plants in Barbera plots varied from 1 to 8% in 2011, from 1 to 16% in 2012, from 0 to 34% in 2013, from 11 to 56% in 2014, and from 13 to 52% in 2015, in the three blocks. The highest disease incidence was always recorded in block 1 (B1), while the lowest in the mid-vineyard block (B2) (Table 1). The incidence of symptomatic plants in the Chardonnay plots varied from 15 to 41% in 2013, from 14 to 55% in 2014, and from 14 to 49% in 2015, in the three blocks. In addition, for Chardonnay, the highest disease incidence was always recorded in block 1 (C1), while the lowest in the mid-vineyard block (C2) (Table 1). Field observation performed in 2014 is depicted in Figure 1, as an example of distribution of the symptomatic/recovered/healthy plants among the experimental plots.

Table 1. Number of plants, disease incidence, and recovery rates calculated over years in selected plots of Barbera and Chardonnay vineyards. Percentage (% flavescence dorée (FD)) of FD-infected plants and recovery rate (% Rec) in each plot are reported for both cultivars. Recovery rate was calculated as percentage of recovered plants (infected the previous year and symptomless the year of the analyses) out of the infected plants during the previous season in the same plot. Dead and newly replaced plants were not included in calculation of incidence and recovery rates for Chardonnay plots.

Cultivar	Plot	N° of Plants	2015		2014		2013		2012		2011
			% FD	% Rec	% FD	% Rec	% FD	% Rec	% FD	% Rec	% FD
Barbera	B1	537	52%	14%	56%	19%	34%	46%	16%	61%	8%
	B2	569	13%	18%	11%	0%	0%	100%	1%	100%	1%
	B3	546	39%	13%	39%	15%	20%	27%	5%	58%	4%
Chardonnay	C1	442	49%	11%	55%	10%	41%	/	/	/	/
	C2	334	14%	12%	14%	16%	15%	/	/	/	/
	C3	339	40%	3%	41%	6%	39%	/	/	/	/

Amplicons of expected size were obtained following nested FDp-PCR assays using as template the DNA extracted from all tested symptomatic grapevines of both cultivars, whereas BNp was never detected. The results of molecular characterization showed that FD-C was the prevalent phytoplasma strain (16Sr-C; *sec*Y-C). Indeed, this latter was detected in all Chardonnay samples and six out of ten Barbera grapevines. Mixed profiles were found in Barbera (2 out of ten 16Sr-C/SecY-D, and 2 out of ten 16Sr-D/SecY-C samples). Molecular tests carried out on Bh and Ch samples confirmed the absence of phytoplasmas in all the selected grapevines.

Recovery rates for Barbera varied according to the year, while they were similar across the three blocks (Table 1). In block 2 (B2), the few infected plants of 2011 and 2012 all recovered the next year. For Chardonnay, mean recovery rates of 11 and 9% were recorded in 2014 and 2015, respectively.

Monitoring of viruses present in the vineyard was carried out on woody material collected in autumn 2015. Results of serological assays showed the presence of GFkV in one Cr and three Bh pooled samples, while GLRaV-3 was detected in three Br and one Bh pooled samples.

3.2. Maturation Curves

The different parameters (pH, °Brix, sugar, total acidity, and potential alcohol) measured for healthy and recovered samples of Barbera and Chardonnay along with different sampling points from August to September are reported in Tables 2 and 3, respectively. In all categories, the sugar content and potential alcohol gradually increased and in parallel a constant decrease in total acidity was observed.

Table 2. Composition of berries from healthy and recovered Barbera measured from veraison through harvest.

Sample	Sampling Date	pH	°Brix	Sugar (g L^{-1})	Total Acidity (Tartaric Acid g L^{-1})	Alcoholic Potential Degree (% vol)
Bh	17 August 2015	2.77	21.3	194.9	15.53	11.46
	24 August 2015	2.96	24.3	226.6	11.62	13.32
	28 August 2015	3.05	26.2	249.2	10.18	14.65
	07 September 2015	3.13	29.4	285.1	8.91	17.11
	15 September 2015	3.24	28.9	277.4	8.86	16.65
Br	17 August 2015	2.76	22.2	204.4	14.23	12.02
	24 August 2015	3.03	22.4	207.7	11.63	12.21
	28 August 2015	3.05	25.8	241.3	12.38	14.19
	07 September 2015	3.06	27.5	267.8	9.52	16.07
	15 September 2015	3.29	28.3	273.5	8.43	16.41

Table 3. Composition of berries from healthy and recovered Chardonnay measured from veraison through harvest.

Sample	Sampling Date	pH	°Brix	Sugar (g L^{-1})	Total Acidity (Tartaric Acid g L^{-1})	Alcoholic Potential Degree (% vol)
Ch	03 August 2015	2.87	17.3	-	14.30	9.50
	07 August 2015	3.05	19.6	-	11.10	11.05
	17 August 2015	3.11	22.6	217.38	8.15	12.78
	24 August 2015	3.21	22.7	216.19	7.06	12.71
Cr	03 August 2015	2.90	15.8	-	12.92	8.50
	07 August 2015	2.93	16.2	-	12.81	8.80
	17 August 2015	3.08	19.2	177.11	9.92	10.41
	24 August 2015	3.22	22.5	214.17	6.91	12.59

3.3. Microvinifications and Enological and Sensory Analyses

The different parameters measured for musts and wines from healthy and recovered grapes of the both cultivars are reported in Table 4. The musts obtained from Barbera grapes were characterized by high sugar concentrations, and both Bh and Br showed a fermentation trend with a regular decrease in sugars, although the malolactic fermentation took place more rapidly in Br than in Bh (not shown). In addition, in the case of Chardonnay, fermentation trend with a regular decrease in sugars was observed (Table 4).

Table 4. Oenological and chemical analyses of musts and wines obtained from grapevines Barbera and Chardonnay in different sanitary status; Bh: healthy Barbera; Br: recovered Barbera; Ch: healthy Chardonnay; Cr: recovered Chardonnay.

Sample	Must	End of Fermentation			Refinement Phase
	°Brix	Alcoholic Strength (% vol)	pH	Total Acidity (Tartaric Acid g L^{-1})	Total Acidity (Tartaric Acid g L^{-1})
Bh	29.1	15.34	3.22	9.17	6.50
Br	28.2	14.56	3.44	8.00	5.41
Ch	22.9	13.60	3.33	6.98	5.53
Cr	20.4	12.39	3.31	6.80	5.56

As far as concerns the triangular test of the Chardonnay wine, there were no statistically significant differences, with a 5% probability of error, between the Ch and the Cr samples. However, with an error probability of 10%, the Ch wine sample was the preferred one. For Barbera wines, the triangular test showed that, also in this case, there were no statistically significant differences between wines from healthy and recovered plants (with a 5% probability of error). Conversely from Chardonnay, even with an error risk greater than 5%, a favorite Barbera wine was not identified.

4. Discussion

Flavescence dorée of grapevine severely impacted wine-growing industry in Piedmont since the late 1990s [41] up to present days [7]. The exploitation of recovery, when feasible, would ensure significant economic savings due to the lack of removal of infected plants and their replacement with young plants, which remain unproductive for some years. Moreover, removal and replanting leads to the presence of different aged vines within the vineyard, with severe impact on its management. As suggested in previous studies, the substitution of FD-infected grapevines is not profitable for cultivars with high recovery rate such as Barbera. For cultivars with intermediate recovery attitude (Chardonnay), the decision of maintenance or replacement varies in relation to agronomic/economic factors and to the risk of new infections [42]. In particular, a lower yield per hectare made maintenance of infected plants more profitable, whereas the longest productive lifetimes as well as a lower density of plants per hectare and an increase in the grape price made replacement of infected grapevines with new plantlets relatively more profitable [42].

Although in BN-infected grapevines a time lag occurs between remission of symptoms and permanent recovery [43], in our condition recovered vines were assayed as early as possible. Indeed, recovered vines are productive since their first recovery year according to the data reported by Morone et al. [3], and they do not represent a source of FDp for the insect vector [11]. The quality of berries and wines from FD-recovered plants has not been addressed yet and few data are available for BN-recovered plants [44]. Previous studies have shown that FD-recovered plants are productive from the first year of symptom remission, although less than healthy ones [3]. In the present work, we analyzed maturation and wine parameters of FD-recovered and healthy grapevines of Barbera and Chardonnay cultivars, used for production of high-quality wines and well-known for their susceptibility to the disease [8,10]. Both cultivars have a good aptitude to recover from phytoplasma infection [3,20,45].

Detection and characterization analyses on symptomatic grapevines distributed at various points in both vineyards confirmed the complete correspondence between symptom observation and FDp presence. The presence of both FD-C and FD-D strains in Barbera and Chardonnay vineyards is in line with the genetic characterization of FDp populations in several agroecosystems of Piedmont [46]. At the same time, no BNp infections were detected, confirming the absence of this phytoplasma in both vineyards. Similarly, the absence of phytoplasmas was confirmed by molecular analyses in all symptomless plants (Bh and Ch) used for maturation curves and microvinification trials.

Viral infections are potentially associated with severe reduction in grapevine yield and wine quality, although the precise loss estimate depends on several factors involved in the plant-virus interaction (plant/virus genotypes, rootstock, vineyard management, environment, etc.). In most cases, the real damages induced by viruses are not even perceived at both quantitative and qualitative levels [47]. Consistently, among the five viruses surveyed in this work, only GFkV and GLRaV-3 were found in few symptomless plants, without any direct effect on the plant phenotype and quality of the products.

During the 2011–2015 surveyed period, mean disease incidence in Barbera dramatically increased from 4 to about 35%. During the same period, mean recovery rate substantially decreased from over 60 to about 15%. Interestingly, at low disease incidence in a given year, recovery rate of the following year was high, while it decreased along with increasing disease incidence, suggesting frequent re-infections of recovering grapevines. Indeed, infected vines show FD symptoms about one year after inoculation [5], and recovered vines are fully susceptible to new FD infections [19]. Disease incidence in Chardonnay was above 30% from 2013 to 2015, and as for Barbera, low recovery rates followed high disease incidences the year before. For both cultivars, disease incidence measured in the central plots was lower than those measured within edge plots. This observation confirms the importance of primary infections due to infected vectors coming from outside the vineyards [48,49]. The application of insecticides inside the vineyard targets resident vector population but has limited effects on incoming leafhoppers that developed on gone-wild *Vitis* outside the vineyards. The observed border effect in spatial distribution of disease was already reported in Barbera and Merlot plants infected with FD [33,34] as well as in Chardonnay vineyards affected by BN [50]. Although measured only under high disease incidence, recovery rate of Chardonnay was lower than that of Barbera under the same conditions. This finding is consistent with previously reported data [42], and this can be explained by the frequent death of infected Chardonnay.

Maturation curves observed for Barbera and Chardonnay were in line with those measured in Piedmont in previous years [51] and compliant with the values expected for the two cultivars. Despite the similar maturation trends observed for Bh and Br, the high alcoholic strength and total acidity of Bh wines did not affect the final quality perceived by the expert panel. The maturation of Chardonnay grapes was characterized by an initial low sugar level measured in Cr samples, which nevertheless reached a value in line to that obtained from Ch grapes at the harvest. During the maturation of Chardonnay, the alcoholic potential degree followed the same trend for Ch and Cr, and the differences of one–two alcoholic degrees measured in the wines and musts of the two categories did not affect the quality of the two wines, which were perceived as similar by the expert panel. A recent study on grape berry quality revealed similar levels of fruit sugars (total soluble solids) and titratable acidity between FD positive and negative plants, despite FD infection delaying the grapevine development and leading to drastic production losses [52]. Our work is the first qualitative description of must and wine characteristics from recovered vines, although the influence of seasonal variations among different years could not be evaluated, as measurements were performed only in 2015. Indeed, the Funding Project financed to the winegrower the eventual loss of vineyard production for only one year. The quality of grapes and wines obtained from recovered plants were satisfactory and recovered vines were immediately productive in line with very similar miRNA profiles compared to healthy ones [53], despite a differential activation of several genes [31]. The few differences suggest a reduced photosynthetic efficiency or less efficient gas exchange performances of recovered plants [26] and may account for the lower alcoholic potential of berries from recovered Chardonnay plants. It cannot be excluded that an altered sugar metabolism may compensate the reduced photosynthetic activity, as demonstrated for other plant pathogens [54].

5. Conclusions

Recovery can be exploited in an integrated management of FD, together with control of vector population with insecticides, pruning of the infected shoots at first symptom appearance, removal

of gone-wild *Vitis* surrounding the vineyard, with a cultivar-specific approach. Since Barbera has a good recovery attitude, yield, and quality of berries from recovered plants are in line with those from healthy grapevines, recovery can be considered as an extra tool to cope with the disease in settlement areas, if FD incidence does not exceed 10%. Actually, with higher disease incidence, uprooting of the whole vineyard is compulsory by law. Indeed, FDp is a quarantine pathogen, and removal of diseased grapevines is implemented by all the European Member States, following official inspection by the national phytosanitary services. In vineyards where the infection rate exceeds 20–30% of the grapevines, the whole vineyard is removed [5]. Thus, recovery from FD, combined with conventional control methods, might represent a potentially useful strategy to coexist with the disease, especially in well-established vineyards several years after planting, when replanting is no more economically sustainable [42] and in areas, such as the Piedmont, where epidemics are still ongoing.

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