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Pesticide Residues and Stuck Fermentation in Wine: New Evidences Indicate the Urgent Need of Tailored Regulations

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Abstract: For three consecutive years, an Italian winery in Apulia has dealt with sudden alcoholic stuck fermentation in the early stages of vinification process, i.e., typical defects addressable to bacterial spoilage. After a prescreening trial, we assessed, for the first time, the influence of the commercial fungicide preparation Ridomil Gold® (Combi Pepite), containing Metalaxyl-M (4.85%) and Folpet (40%) as active principles, on the growth of several yeasts (*Saccharomyces cerevisiae* and non-*Saccharomyces* spp.) and lactic acid bacteria of oenological interest. We also tested, separately and in combination, the effects of Metalaxyl-M and Folpet molecules on microbial growth both in culture media and in grape must. We recalled the attention on Folpet negative effect on yeasts, extending its inhibitory spectrum on non-*Saccharomyces* (e.g., *Candida* spp.). Moreover, we highlighted a synergic effect of Metalaxyl-M and Folpet used together and a possible inhibitory role of the fungicide excipients. Interestingly, we identified the autochthonous *S. cerevisiae* strain E4 as moderately resistant to the Folpet toxicity. Our findings clearly indicate the urgent need for integrating the screening procedures for admission of pesticides for use on wine grape with trials testing their effects on the physiology of protechnological microbes.

Keywords: pesticide; fungicide; wine; alcoholic fermentation; yeast; stuck fermentation

1. Introduction

Yeasts are responsible for the alcoholic fermentation process, i.e., the conversion of sugar into ethanol and CO_2, thus being the key player of the transformation of grape must into wine [1]. For this reason, among the different problems in wine production, the negative impacts on the fermentation process are of outstanding importance [2]. It is possible to distinguish two major types of fermentation problems: slow fermentative trends and stuck fermentation [3]. Due to the high risks of economic losses and of quality depreciation, the fermentation arrests represent one of the main challenges in winemaking [4,5]. Stuck fermentation is particularly hard to manage also because of the different possible physical, chemical and biological causes that make elaborate either the diagnosis and, the rectification too [4]. These considerations help explain the evidence suggesting that, despite considerable scientific advances and the existing panel of possible (bio)technological solutions, every year wine producers have to cope with consistent economic losses due to alcoholic stuck fermentation [6,7].

Grey mould (*Botrytis cinerea*), powdery mildew (*Erysiphe necator*) and downy mildew (*Plasmopara viticola*) are responsible for serious yield loss in the wine sector. They are the most damaging diseases of cultivated grapes (*Vitis vinifera*) worldwide, leading to severe injuries and resulting in significant commercial losses [8,9]. The use of several different fungicides to reduce the incidence of these viticulture pests can lead to the presence of organic residues, which must remain under the legal limit throughout the production process [10–14]. However, fungicides are often added without respecting the suppliers' prescription, thus causing the presence of organic residues in musts and wine over the legal limits. Moreover, chemical fungicides cannot be specific for the above pests and they can interfere with the biological function of other microbes, including protechnological organisms involved in food fermentations [15–17]. In fact, it has been demonstrated that, in some cases, fungicide residues can lead to modifications in the structure of the cellular membranes and in the metabolism of the yeast, affecting their activity during fermentation [18,19].

For three consecutive years, a winery in Apulia (southern Italy) has dealt with sudden fermentation arrests in the early stages of alcoholic fermentation. These sudden stucks in fermentation were difficult to justify and impossible to manage using classical (bio)technological approaches adopted by technical staff of the cellar (e.g., yeast nutrient integrations, addition of yeast starter cultures tailored for re-fermentation, variable concentration of free SO_2).

In order to identify possible physical, chemical or biological causes of observed stuck fermentations, we adopted a polyphasic approach suitable for considering some possible causes such as nutrients/oxygen starvation, high temperatures, low pH values and ethanol concentrations [4]. Among the other trials, we also preliminary tested the commercial pesticide formulations commonly used in the vineyard of this winery, for its capacity to inhibit the yeast starter cultures utilized in the cellar to promote the above stucked fermentations. As a result, a fungicide commercial preparation, Ridomil Gold® (Combi Pepite; Syngenta, USA), was found to provoke yeast growth inhibition. This commercial fungicide preparation contains Metalaxyl-M (4.85%) and Folpet (40%) as active principles, and it is commonly used in viticulture to fight the oomycete *Plasmopara viticola*, the causal agent of grapevine downy mildew.

In the present study, we examined the effect of this commercial fungicide preparation on the growth of *Saccharomyces* and non-*Saccharomyces* yeasts and lactic acid bacteria (LAB) of oenological interest, with the aim of determining its effects on the fermentation process. To the best of our knowledge, this is the first investigation aimed at assessing the impact of this commercial preparation on the growth/performance of the main protechnological microbes involved in winemaking.

2. Materials and Methods

2.1. Microbial Strains and Growth Conditions

Four commercial strains of *Saccharomyces cerevisiae*, namely Maurivin™ Elegance (Mauri Yeast Australia), Fervens® SLC (Dal Cin Spa, Concorezzo, Italy), Enartis Ferm SB (Enartis, Trecate, Italy), Lalvin RBS133 (Lallemand, Castel D'Azzano, Italy), and two indigenous *S. cerevisiae* strains (I6 and E4) previously isolated from Nero di Troia must [20], were used in this study. Autochthonous non-*Saccharomyces* yeast strains *Hanseniaspora guilliermondii* M105A31, *Hanseniospora uvarum* B05B29, *Issatchenkia orientalis* B05B2, *Issatchenkia terricola* B05B8, *Candida zemplinina* B05B6, *Torulaspora delbrueckii* B05B12, *Kluyveromyces thermotolerans* B05B32, *Metschnikowia pulcherrima* B05A22 and B05A36, and *Pichia fermentans* B05A29 and M105A3 were tested. These strains were isolated from Uva di Troia grape cultivar [21]. Flavia® (Lallemand), a *M. pulcherrima* strain, was isolated from a commercial preparation. Lactic acid bacteria used in this study were strains *Lactobacillus brevis* IOEB9809, *Lactobacillus hilgardii* CECT4786, *Lactobacillus plantarum* UFG44, *Leuconostoc mesenteroides* OT54, and *Pediococcus parvulus* UFG126. Yeasts and LAB were routinely grown at 30 °C on YPD and MRS (Oxoid, Basingstoke, UK), respectively.

2.2. Growth Curves in Media Contaminated with the Pesticide

Saccharomyces cerevisiae, non-*Saccharomyces* spp., and LAB strains were inoculated from cryo-conserved stocks (1:1000 *v/v*) in 30 mL of YPD or MRS, for yeasts and bacteria, respectively. After 24 h of incubation at 30 °C without shaking, cultures were diluted in fresh medium (1:100 *v/v*) artificially contaminated or not with 20.61 mg/L of Ridomil Gold® in order to achieve a final concentration of 1 mg/L of metalaxyl-M, corresponding to the legal EU limit in grapevine. Growth was monitored spectrophotometrically by measuring the optical density (OD_{600}) during 30 h of incubation at 30 °C in static conditions. Three replicates were performed for each assay.

2.3. Laboratory-Scale Vinification Assay

The laboratory scale vinifications were carried out by inoculating 150 mL of must from "Uva di Troia" grapes (sugars 230 g/L, pH 3.4). Commercial and autochthonous *S. cerevisiae* strains were inoculated at an initial concentration of about 1×10^7 CFU/mL in must supplemented with 20.61 mg/L Ridomil Gold®. The initial microbial concentration was determined by plate counting onto YPD agar, after incubation at 30 °C for 48 h. Control microvinifications were carried out in must without the addition of the pesticide. Fermentation progress was daily monitored by weight loss (indicative of CO_2 generation) measurement at 20 °C. The trials were performed in triplicate.

2.4. Laboratory-Scale Vinification Assay with Analytical Grade Standards

The commercial strain Lalvin RBS133, *S. cerevisiae* E4, *S. cerevisiae* I6 and twelve non-*Saccharomyces* strains were inoculated at an initial concentration of about 1×10^7 CFU/mL in 150 mL of must (from "Uva di Troia" grapes; sugars 230 g/L, pH 3.4) supplemented or not with the commercial pesticide or the analytical standard Folpet (8.24 mg/L) and Metalaxyl-M (1 mg/L) (Sigma Aldrich, St. Louis, MO, USA). Standards were added independently or in combination (8.24 mg/L Folpet and 1 mg/L Metalaxyl-M). A further control was the untreated and spontaneously fermented must. Microvinifications were carried out at 20 °C and monitored daily by weight loss. Experiments were performed in triplicate.

2.5. Laboratory-Scale Vinification Assay in Complex Microbial Ecosystem

In order to reproduce a complex microbial ecosystem like must fermentation, microvinification assays (performed at the same conditions as reported above) were repeated by using a mixed-fermentation approach, as detailed in Table 1. Non-*Saccharomyces* strains were divided into two panels, each including six strains, based on their resistance (panel R) or susceptibility (panel S) to the fungicide compounds. Both panels contained 1×10^7 CFU/mL of each strain. Three more resistant *S. cerevisiae* strains were independently co-inoculated with each panel at an initial concentration of 1×10^7 CFU/mL, as determined by plate counting. Samples were also contaminated with a mix of five oenological LAB (10^4 CFU/mL of each species). Microvinifications, including untreated must as control, were carried out at 20 °C and daily monitored by weight loss. Experiments were performed in triplicate.

Table 1. Strains of non-*Saccharomyces* and oenological lactic acid bacteria (LAB) co-inoculated with *Saccharomyces cerevisiae* strains in the laboratory-scale vinification assay in complex microbial ecosystem.

Non-*Saccharomyces*		**Lactic Acid Bacteria**
Panel S	Panel R	
I. orientalis B05B2	*P. fermentans* B05A29	*L. brevis* IOEB9809
M. pulcherrima B05A22	*P. fermentans* M105A3	*L. hilgardii* CECT4786
H. guillermondi M105A31	*C. zemplinina* B05B6	*L. plantarum* UFG44
H. uvarum B05B29	*T. delbrueckii* B05B12	*L. mesenteroides* OT54
M. pulcherrima B05A36	*I. terricola* B05B8	*P. parvulus* UFG126
K. thermotolerans B05B32	*Flavia*®	

2.6. Fungicide Residue Analysis

The analytical determinations of the fungicidal active principles were performed according to the European Standard Method EN 15662:2008 "Foods of plant origin—Determination of pesticide residues using GC-MS following acetonitrile extraction/partitioning and cleanup by dispersive SPE—QuEChERS-method" [22]. In particular, the analysis was performed using a GC/MS Agilent device (Little Falls, DE, USA) gas chromatograph 7890A, coupled with triple quadrupole spectrometry (7000C, Agilent). Pesticides were separated on an HP-5 ms UI capillary column from Agilent (0.25 mm i.d. × 30 m, 0.25 µm film thickness). The column was set at a constant flow rate of 1 mL min^{-1} using helium as carrier gas. The oven temperature was programmed as follows: the initial temperature was 60 °C, held for 1 min, increased to 120 °C at 40 °C/min, then ramped to 310 °C at 5 °C/min; the total run time was 40.5 min. The injector temperature was 280 °C and injection volume was 1 µL in splitless mode. The ion source and transfer line temperature was set at 280 °C. The data were processed with MassHunter software (B.07.00 Agilent). High purity pesticide analytical standards and internal standards were purchased from Sigma–Aldrich (Sigma Aldrich, St. Louis, MO, USA).

2.7. Statistical Analysis

Data were subjected to one-way analysis of variance (ANOVA). Pairwise comparison of treatment means was achieved by Tukey's procedure with a significance level of p values < 0.05, using the statistical software Past 3.0.

3. Results and Discussion

The aim of the present study was to investigate the causes of stuck fermentation detected for three consecutive years in the same winery. Initially, we adopted a polyphasic approach suitable to exclude some possible causes of stuck fermentation already described by other authors [4,23]. For these purposes, we studied the effects of nutrients/oxygen starvation, high temperatures, low pH values and ethanol concentrations on the same starter cultures utilized in the cellar to promote the alcoholic fermentation process. The obtained results indicated that none of the above parameters influenced, under the real fermentation conditions, the yeast starter growth [24].

Based on the above evidence, we decided to enlarge the scale of our investigation to assess the effects on fermentations of the pesticide sprayed on the grapes utilized for vinifications. The commercial fungicide routinely employed in the vineyard was the Ridomil Gold®, Combi Pepite (Syngenta). This pesticide, which contains Metalaxyl-M (4.85%) and Folpet (40%) as active principles, is commonly used in viticulture to fight the oomycete pathogen *Plasmopara viticola*, agent of the downy mildew in grapevine [25].

In a preliminary assay, we monitored the growth of *Saccharomyces* spp., non-*Saccharomyces* spp. and LAB in synthetic culture media (YPD and MRS for yeasts and LAB, respectively) supplemented with Ridomil Gold® (Combi Pepite). Currently, the EU limit in wine grapes for Metalaxyl-M and Folpet are 1 and 20 mg/L, respectively [14]. Therefore, based on the pesticide composition, containing Metalaxyl-M (4.85%) and Folpet (40%), the assays were performed by adding 20.61 mg/L of Ridomil Gold, an amount corresponding to 1 and 8.24 mg/L of Metalaxyl-M and Folpet, respectively.

As reported in Figure 1, the growth of *S. cerevisiae* was always lower in pesticide-enriched media when compared with the untreated control. In particular, the commercial strains Fermens and Elegance were mainly affected by the presence of Ridomil Gold (Figure 1). Interestingly, non-*Saccharomyces* strains showed a variable reduction in their growth rate in media with the pesticide formulation. For example, the growth of *H. uvarum*, *H. guillermondii*, *I. terricola*, *I. orientalis*, and some strains of *M. pulcherrima*, was completely inhibited in media containing Ridomil Gold [24], whereas *T. delbrueckii*, *P. fermentans*, *C. zemplinina*, and the commercial *M. pulcherrima* strains were more resistant at the same conditions. Interestingly, *T. delbrueckii* B05B12 seems to be more resistant than *S. cerevisiae* strains. Indeed, after a pre-adaptation time of about 24 h, the kinetics of growth of B05B12 did not seem to be

influenced by the presence of the pesticide (Figure 1B). In contrast, contamination of Ridomil Gold in MRS broth did not affect the growth of any of the tested LAB (Figure 1C).

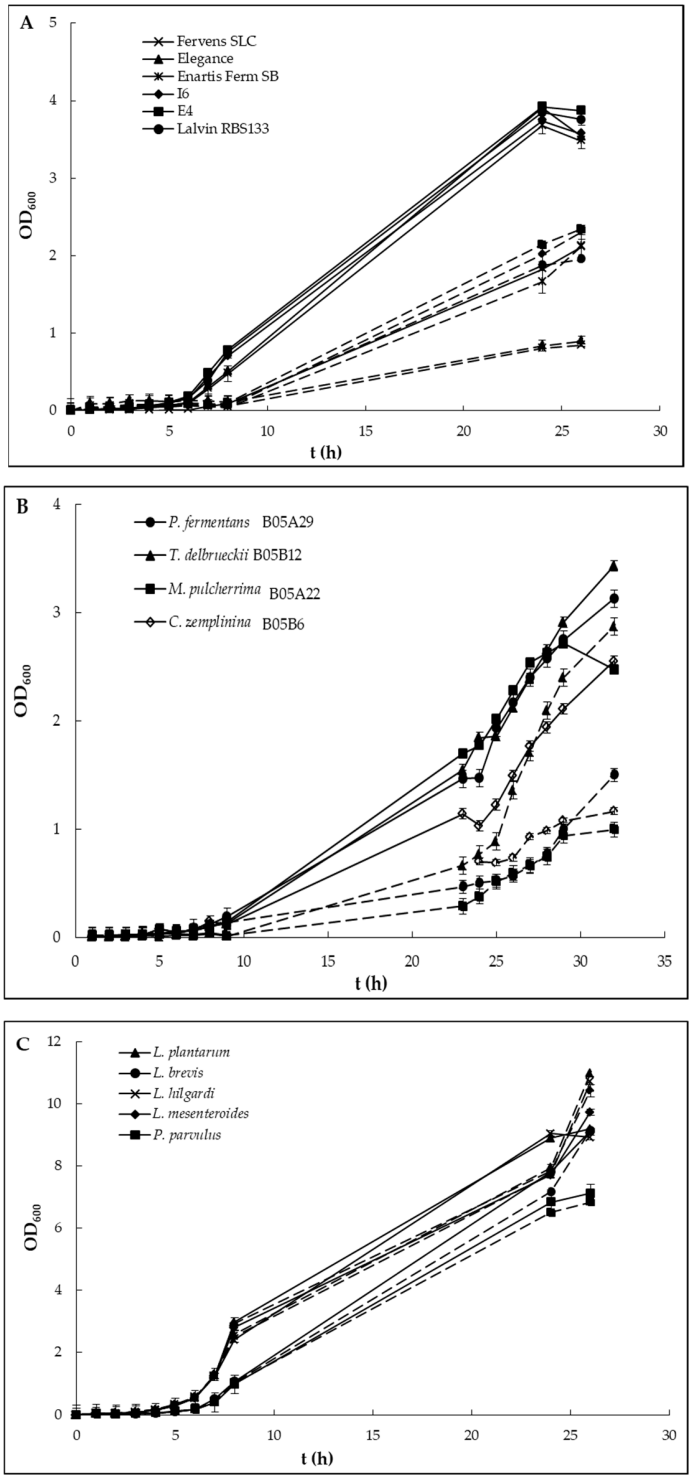

Figure 1. Growth of strains of *S. cerevisiae* (**A**), non-*Saccharomyces* (**B**), and LAB (**C**) inoculated in YPD (yeasts) and MRS (bacteria) supplemented (dashed lines) or not (continuous lines) with 20.6 mg/L of Ridomil Gold.

In a subsequent step, we monitored the fermentation process of grape must of the variety "Uva di Troia", artificially contaminated with the same concentration of Ridomil and inoculated with

commercial and indigenous strains of *S. cerevisiae*. Using fungicide residue analysis, we verified that the concentration of active principles in the artificially contaminated sample corresponded to the dilution of quantities claimed in the product specification [24]. As shown in Figure 2, we found that, in the must without Ridomil the fermentation happened regularly, resulting in approximately 10% of weight reduction within two weeks. In contrast, when must was supplemented with the commercial formulation of pesticide, a slower and incomplete fermentation occurred, in all the assays, because the weight loss after 2 weeks was about 3% for all the investigated strains (Figure 2). These evidences strongly suggested that the presence of Ridomil was responsible for the stuck fermentation observed for three consecutive years in the winery.

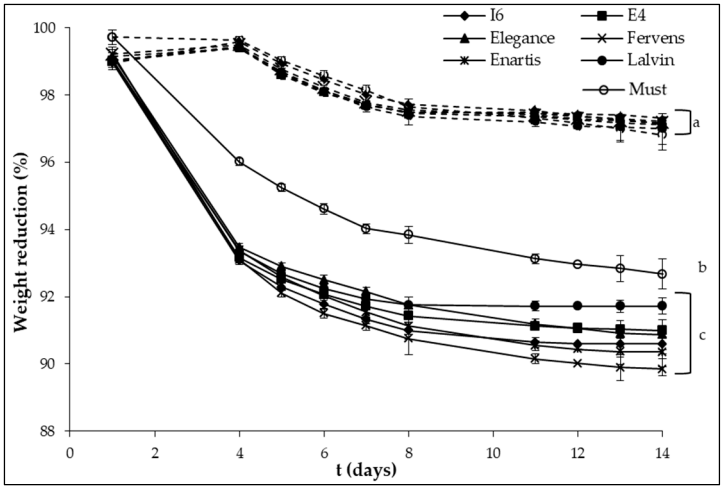

Figure 2. Microvinifications monitored for 14 days by weight reduction (%) of must not contaminated (dashed lines) or contaminated (continuous lines) with a Ridomil Gold® preparation containing 1 mg/L of Metalaxyl-M and inoculated with commercial and autochthonous *S. cerevisiae* strains. Sample "Must" was submitted to spontaneous fermentation. Different superscript letters indicate statistically significant differences ($p < 0.05$) in the weight reduction as determined by one-way analysis of variance (ANOVA).

In order to investigate if the stuck fermentation resulted from the specific action of the Ridomil active principles (i.e., Metalaxyl-M and Folpet) alone or in synergistic action, the analytical grade standards of these chemical compounds were also used as additive in the laboratory-scale vinification assays in comparison with the addition of the Ridomil itself. In order to challenge the interspecific and intraspecific variability in the tolerance to the excipients included in the commercial preparation twelve non-*Saccharomyces* and three *Saccharomyces* yeasts (namely, Lalvin RBS133, E4, and I6) were further investigated.

Samples were individually inoculated in must supplemented with: (i) nothing as control, (ii) Folpet (8.24 mg/L), (iii) Metalaxyl-M (1 mg/L), (iv) Folpet (8.24 mg/L) and Metalaxyl-M (1 mg/L), and (v) Ridomil Gold® (20.6 mg/L).

The results reported in Figure 3 and Supplementary Material S1 showed a great variability, indicating a different microbial susceptibility that could be either species- and/or strain-specific. In particular, when Ridomil was added to the must, the weight loss was always minimal compared with the other experimental conditions, and after 8 days the must was more than 98% of the initial weight (Figure 3 and Figure S1). A further reduction, detected during the second week corresponding to about 97% of the initial weight), was presumably associated with metabolic activities other than alcoholic fermentation. In general, no differences were observed in must untreated or supplemented with Metalaxyl-M. In contrast, Folpet strongly inhibited the fermentation, indicating that residuals of this molecule could be a serious concern during wine-making. Overall, three main different scenarios were observed. As model strains, we chose *T. delbrueckii* B05B12, *C. zemplinina* B05B6 and *S. cerevisiae* E4, and the corresponding profiles in the microvinification assays are summarized in

Figure 3. In general, no significant differences were found between samples supplemented with only Folpet or when Folpet was combined with Metalaxyl-M. This most abundant pattern was typical of twelve of the examined fermentations, including the spontaneous fermented control (Figure S1A–M). However, among the strains of this group, a markedly higher effect of the commercial Ridomil rather than Folpet or its combination with Metalaxyl-M was detected only for *Torulaspora delbrueckii* B05B12, and the commercial strains Flavia and Lalvin RBS133 (Figure 3A and Figure S1A–M). Only three samples, inoculated with *C. zemplinina* B05B6, *P. fermentans* B05A29, and *P. fermentans* M105A3, were characterized by a lower weight loss in must supplemented with Folpet and Metalaxyl-M than with only Folpet, suggesting a synergistic effect of both molecules (Figure 3B and Figure S1N–O). Intriguingly, the autochthonous *S. cerevisiae* E4 was the only strain unsusceptible to Folpet and Metalaxyl-M when added either independently or simultaneously to the must. Nonetheless, the same strain was unable to perform the alcoholic fermentation in samples contaminated with Ridomil Gold (Figure 3C), suggesting that different molecules, other than Folpet and Metalaxyl-M, could increase the effect of the commercial pesticide.

Interestingly, after about 2 weeks of the fermentation process, a thick gelatinous coating appeared on the surface of treated must, whereas, in untreated must, this coating was never detected (Figure 4). This finding clearly indicated that the arrest of fermentation turned the winemaking condition towards alterations of the wine, probably due to bacterial development.

On the basis of the biological information recently reported and related to the non-*Saccharomyces* biodiversity during the early steps of spontaneous alcoholic fermentation of "Nero di Troia" wines [26], we assessed the effect of the residual pesticide on the fermentation process in a more complex ecosystem resembling the microbial conditions of the must. "Uva di Troia" must was co-inoculated with each *S. cerevisiae* strain previously tested and two mixtures containing commercial and autochthonous non-*Saccharomyces* and LAB strains, as detailed in Table 1. According to the results obtained in the lab-scale vinification assay, the non-*Saccharomyces* yeasts were grouped into two panels, each containing six strains, based on their tolerance to Folpet: panel S including the most susceptible, and panel R including strains showing some resistance (Figure 5). A mix of LAB strains was also inoculated at a concentration of about 10^4 CFU/mL, according to their typical concentration in must [27]. The results showed that, in uncontaminated must, fermentation took place regularly, and a weight loss corresponding to 14 and 8% was detected (Figure 5A). As expected, alcoholic fermentation was faster in samples inoculated with the commercial *S. cerevisae* strains than with autochthonous yeasts. Interestingly, the weight reduction in presence of the panel R was higher, suggesting that non-*Saccharomyces* strains could differently affect the fermentation kinetics. In contrast, a stuck fermentation was always detected when must was supplemented with a Ridomil preparation containing 1 mg/L metalaxyl-M (Figure 5B). In general, no significant differences were detected, indicating that in the case of a complex microbial consortium the pesticide did not have an impact on the fermentation capabilities under these experimental conditions. However, samples inoculated with the autochthonous *S. cerevisiae* E4, which in our previous assay was resistant to Folpet, achieve the highest weight loss of about 5% after two weeks.

To the best of our knowledge, it is the first time that a commercial fungicide formulation recommended for control of pathogen on wine grapes and used within the legal limit was found responsible for grape stuck fermentation inoculated with *Saccharomyces* and non-*Saccharomyces* starter cultures. The potential of some pesticide formulations to induce fermentation arrests or to interact with the *S. cerevisiae* during fermentation has been already suggested [17,28]. In addition, the reported evidences testify that preliminary in vitro screenings are not always adequate to describe the possible negative effects of a commercial pesticide formulation on the fermentative performances of protechnological microbes. It was the case of *T. delbrueckii*, *P. fermentans*, *C. zemplinina*, and the commercial *M. pulcherrima* strain that displayed inhibitory behaviors under in vivo (in grape must) but not in vitro (culture media) trial conditions. Moreover, our results confirm a general scarce effect of fungicide preparation on malolactic bacteria [17]. Further studies will consider the

effect of the commercial preparation on spoilage microbes of relevant interest in the wine industry (e.g., *Dekkera*/*Brettanomyces* [29–31]) and the application of specific timing management of microbial resources in vinification (e.g., coinoculation of yeasts and bacteria [32]).

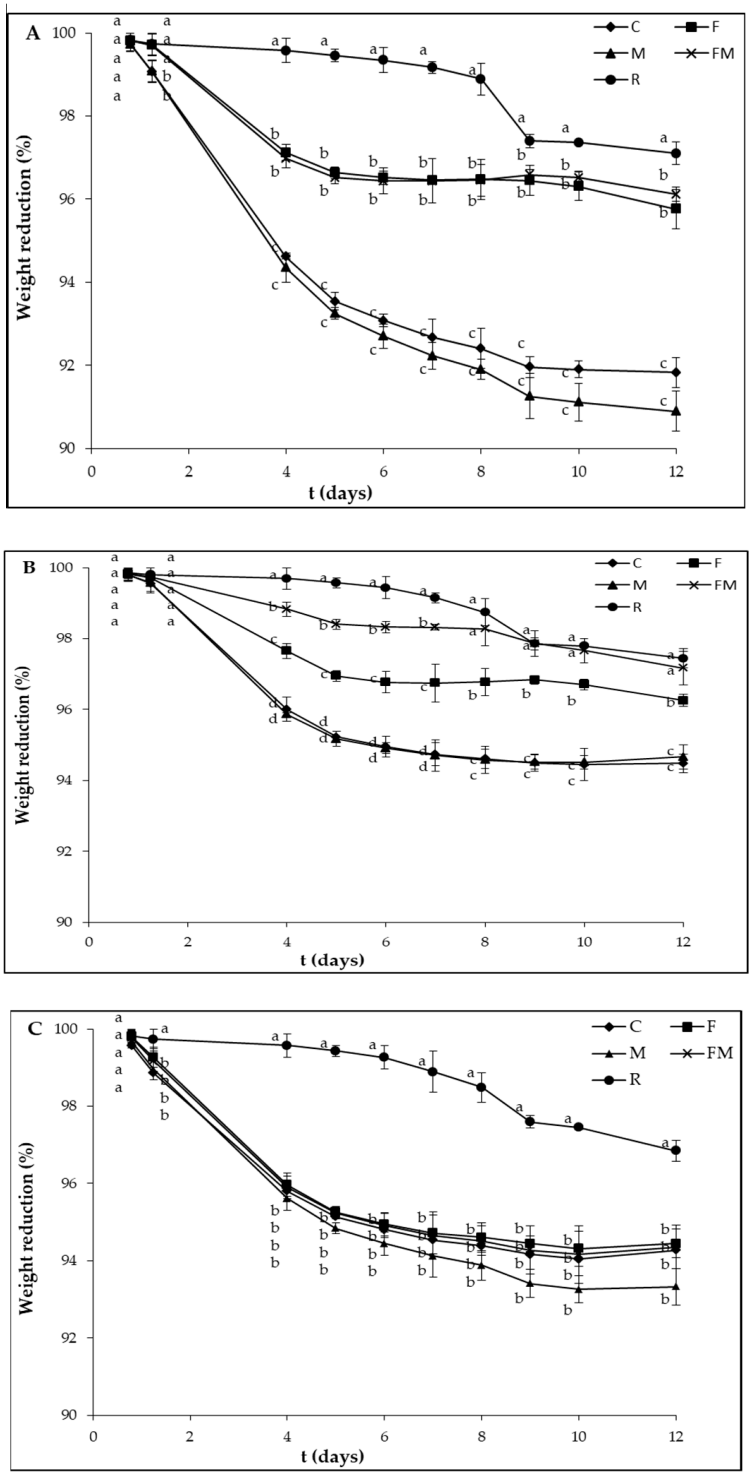

Figure 3. Microvinifications monitored for 12 days by weight reduction (%) performed by *T. delbrueckii* B05B12 (**A**), *C. zemplinina* B05B6 (**B**), and *S. cerevisiae* E4 (**C**) in untreated must (C), or supplemented with Folpet (8.24 g/L) (F), Metalaxyl-M (1 mg/L) (M), Folpet and Metalaxyl-M (FM), and Ridomil Gold® (R). Different superscript letters indicate statistically significant differences ($p < 0.05$) in the weight reduction as determined by one-way ANOVA.

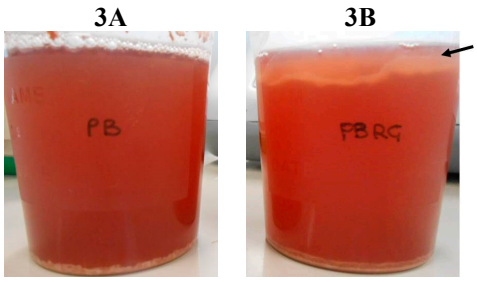

Figure 4. Appearance of the microvinification assay in must without (**A**) or supplemented (**B**) with 20.61 mg/L of Ridomil Gold® and inoculated with *S. cerevisiae* E4 after two weeks of fermentation. The arrow indicates the gelatinous coating on the surface of fermented must added with the pesticide.

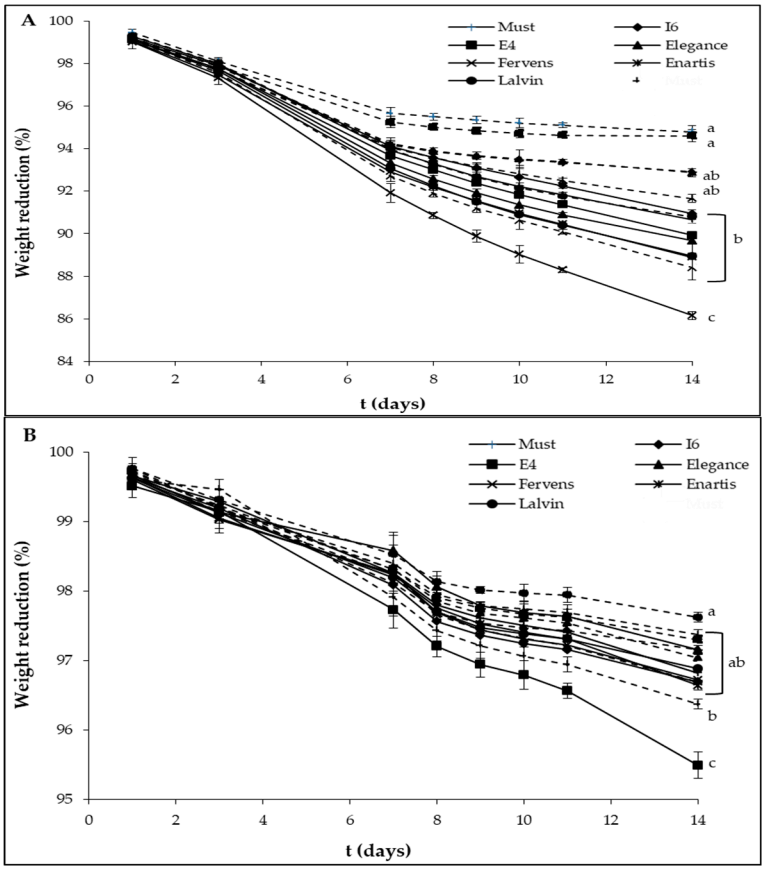

Figure 5. Microvinifications monitored for 14 days by weight reduction (%) of must not contaminated (**A**) or contaminated (**B**) with a Ridomil Gold® preparation containing 1 mg/L of Metalaxyl-M and inoculated with *S. cerevisiae* strains and panel S (susceptible non-*Saccharomyces* spp.—dashed lines), or panel R (resistant non-*Saccharomyces* spp.—continuous lines), and LAB. Different superscript letters indicate statistically significant differences ($p < 0.05$) in the weight reduction as determined by one-way ANOVA.

In the past, several molecules were investigated for their actions on wine microorganisms [17,33–35]. Considering the active principles present in Ridomil, different previous studies investigated the effects of Folpet on wine yeasts [17,36]. However, no data are available on the possible effects of Metalaxyl-M, whereas the effects of different pesticides used in the vineyard on oenological microbes have been described [37]. Our results generally confirmed the Folpet-induced reduction of fermentation activity of *S. cerevisiae* strains [17,35] but were unable to increase the fermentation rate of *Candida* spp. [17]. Furthermore, our findings underline the importance of testing the commercial formulation of

fungicides rather than the single active principles and evaluating the effect in vivo rather than in vitro by direct assessment of pesticide formulation in the specific matrix.

4. Conclusions

In summary, for the first time, we have described the effect of two active agents present in the same commercial formulation on *Saccharomyces* and non-*Saccharomyces* species, underlining the possible increasing risks of fermentation arrests in case of pesticide formulation containing more active molecules. Furthermore, the fermentative behavior observed for the autochthonous strain *S. cerevisiae* E4 points out the possible inhibitory role of excipients included in the fungicide preparation and representing more than 50 % of the product. Our results shed new light on the need for an urgent tailored regulatory intervention on the mandatory characterization of pesticide formulations allowed for use on crops destined for the production of fermented food/beverages. All our findings were consistent with the recent literature on the influence of pesticides on other food-stuffs fermentations [17,19,38], and they clearly indicate the urgent need for integrating the screening procedures for admission of pesticides in agriculture with trials on the possible negative effects on protechnological microbes.

Supplementary Materials: Supplementary materials can be found at http://www.mdpi.com/2311-5637/5/1/23/s1. Figure S1: Weight reduction (%) of must in microvinification assays of twelve *Saccharomyces* and non-*Saccharomyces* strains inin untreated must (C), or supplemented with Folpet (8.24 g/L) (F), Metalaxyl-M (1 mg/L) (M), Folpet and Metalaxyl-M (FM), and Ridomil Gold® (R).

Author Contributions: conceptualization, P.R., F.G., G.S. and V.C.; methodology, P.R., C.B., C.D.C., F.G., G.S. and V.C.; investigation, P.R., C.B. and C.D.C.; resources, P.R., F.G., G.S. and V.C.; data curation, P.R., C.B., C.D.C., F.G., G.S. and V.C.; writing—original draft preparation, P.R.; writing—review and editing, P.R., F.G., G.S. and V.C.; supervision, F.G. and G.S.; project administration, F.G., G.S. and V.C.; funding acquisition, F.G., G.S. and V.C.

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Conflicts of Interest: The authors declare no conflict of interest.

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