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Indigenous *Aureobasidium pullulans* Strains as Biocontrol Agents of *Botrytis cinerea* on Grape Berries

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Abstract: *Aureobasidium pullulans* is a yeast-like fungus found on the surface of the grape berries that has been proven to act as a biocontrol agent for the management of grey mould disease caused by *Botrytis cinerea*. In this work, an indigenous strain of *A. pullulans* isolated from grape berries and selected according to the in vitro activity against *B. cinerea*, was used in vineyards of the winery where it originated, in comparison with a commercial product containing two *A. pullulans* strains with the aim of assessing its effectiveness as a biocontrol agent. The experimental design included daily meteorological data registration and the early defoliation of grapevines as treatments. The monitoring of *A. pullulans* strains on grape berries by plate counts and molecular methods as well as of *B. cinerea* symptoms on grape bunches was performed in the different trials from the end of flowering to the harvest time. Results highlighted that although no significant differences ($p < 0.05$) in the occurrence of *B. cinerea* were detected according to different treatments, the mean incidence of symptomatic berries ranged from 7 to 16%, with the lowest values recorded in bunches treated with the indigenous *A. pullulans* strain. The efficacy of the biocontrol agent was affected more by meteorological conditions than the defoliation practice.

Keywords: *Aureobasidium pullulans*; biocontrol; grapevine; *Botrytis cinerea*; viticulture; grape berries; grey mould; yeasts; sustainability; *Botrytis* bunch rot

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1. Introduction

Aureobasidium pullulans (also known as black yeast) is an oligotrophic, saprophytic and polymorphic yeast-like fungus naturally present in association with the endophyte populations in the phyllosphere and carposphere of various species of plants including both diseased and healthy grapevines [1,2]. Indeed, it is one of the predominant yeast species found on the surface of the grape berries at all stages of maturity according to several studies [2–7] or mostly at veraison time as stated by Renouf at al., 2005 [8]. The widespread occurrence of *A. pullulans* is attributed to its elevated tolerance to different ecological stresses and its high antagonistic activity against bacteria and fungi [1,9,10]. Because of these characteristics, *A. pullulans* has been exploited as a biocontrol agent for the management of *Botrytis* bunch rot (BBR) caused by the fungus *Botrytis cinerea*, and thus also called grey mould, which is responsible for significant economic damage in vineyards and the postharvest decay of table grape [1,11,12]. In fact, the use of anti-*Botrytis* synthetic fungicides is not considered sustainable due to their negative effects on the environment and human health, and the high costs for the synthesis of new chemicals required due to the increase in fungicide-resistant strains of *B. cinerea* within vineyard populations [12–15]. Therefore, biological control represents a sustainable approach to produce high-quality grapes and wines with high standards of food safety without residues

of synthetic fungicides. Furthermore, the successful application of biocontrol agents to avoid BBR can be an alternative to the addition of sulfur dioxide (SO_2), thus contributing to a reduction in its content in grapes and a decrease in potential allergic reactions to sulfites in wine consumers [11–13]. For these reasons biocontrol practices can be regarded as a key enabler for achieving the objectives of the European Green Deal that include, by 2030, a reduction of 50% of chemical and hazardous pesticides, in order to minimise negative impacts on human health and the environment and to increase sustainability.

Many different modes of action have been reported for the *A. pullulans* biocontrol agent including the competition for nutrients and space, production of cell wall-degrading enzymes, synthesis of antifungal compounds, and mycoparasitism [12,16–19]. Moreover, *A. pullulans* is known to exhibit a high phenotypic and genotypic diversity [1,20], so the choice of which strains to use as biocontrol agents is a critical step.

Despite the availability of the *A. pullulans*-based product currently marketed [10,13,21] which, according to European Union Pesticides Database [22], contains the DSM14940 and DSM14941 strains (Botector®, BIO-FERM, Austria) [23], new candidates to control BBR in vineyards are being sought. The first step in developing biocontrol agents is the isolation and the screening process and the best sources of antagonistic microbial strains are the natural environments in which they compete with plant pathogens; therefore, they are better adapted to specific ecosystems [11,16]. Therefore, in this work, with the aim of selecting *A. pullulans* strains as potential biocontrol agents against BBR, several fungal isolates from vineyards of the same winery during three vintage processes were characterised by both molecular methods and in vitro assays to assess their anti-botrytis activity. Subsequently, the selected *A. pullulans* strain which possessed the highest activity was applied in vineyards of the winery where it came from, in comparison with the commercial product containing the DSM14940 and DSM14941 strains with the aim to assess its effectiveness as biocontrol agent. Moreover, an early defoliation (pre-flowering) treatment was considered in the experimental design because it was a non-microbial practice suggested to improve the microclimate and prevent the development of *B. cinerea* thus reducing the incidence of BBR. Finally, during the experimental trials in vineyards, the main meteorological variables (air temperature, relative humidity, and rainfall) were monitored to evaluate their trend in correlation with potential *B. cinerea* symptoms in grape bunches.

2. Materials and Methods

2.1. Aureobasidium pullulans Isolation and Culture Conditions

During three different vintages from 2015 to 2018, in a winery located in Montalcino (Siena, Italy), healthy grape berries were randomly sampled from Sangiovese vineyards from the flowering to harvest time.

Ten grams of grape samples, transferred into 90 mL of sterile physiological saline solution (9 g/L NaCl) were homogenised for 2 min in a Stomacher Lab Blender 400 (Seward Ltd., Worthing, West Sussex, UK). Then, 100 µL of these suspensions were plated directly (1 mL) or after decimal dilutions on MYPG agar (5 g/L malt extract, 3 g/L yeast extract, 5 g/L meat extract, 10 g/L glucose, and 20 g/L agar) by using the pour plate method. Plates were performed in duplicate and incubated for 120 h at 30 °C under aerobic conditions. At least 10 colonies, showing characteristic *Aureobasidium* cell morphology at light microscopy, for each point of sampling, were randomly selected from the plates containing the two highest sample dilutions, purified and stored at −80 °C for further analyses by molecular methods.

2.2. Identification and Typing of Aureobasidium pullulans Isolates

Aureobasidium spp. isolates were identified by PCR-RFLP analysis of the rDNA-ITS (Internal Transcribed Spacer) region according to Granchi et al., 1999 [24], using *Hae*III, *Hinf*I, and *Cfo*I (Fermentas Inc, Burlington, ON, Canada) as restriction enzymes. The restriction fragments were separated (at 100 volts for 2.5 h) on 2 % (w/v) agarose gel

(Lonza Group Ltd., Basel, Switzerland), containing ethidium bromide (Sigma-Aldrich, St Louis, MO, USA) and TEB buffer (1 M Tris, 10 mM EDTA, 0.9 M boric acid, pH 8.3). The obtained restriction patterns were captured and observed by UV transillumination and compared with those reported in the literature [24–26].

Intraspecific biodiversity of *A. pullulans* was performed by Randomly Amplified Polymorphic DNA-Polymerase Chain Reaction (RAPD-PCR) analysis as described by Venturi et al., 2012 [27], using the primer P4 (50 CCGCAGCGTT 30), first described by De Angelis et al., 2001 [28]. Profiles were analysed on 2 % (w/v) agarose gel (Lonza Group Ltd., Basel, Switzerland) stained with ethidium bromide (Sigma-Aldrich, St Louis, MO, USA) in TEB buffer for 2.5 h at 100 V and observed after UV transillumination.

2.3. Biocontrol Activity against Botrytis cinerea In Vitro

A screening to evaluate the inhibition activity of *A. pullulans* strains on *Botrytis cinerea* was carried out on potato dextrose agar (PDA; 4 g/L potato extract, 20 g/L glucose, 20 g/L agar) as a medium. The radial growth inhibition test was performed following the method reported by Raspor et al., 2010 [16] with few modifications. In particular, on 90 mm Petri dishes containing PDA, *A. pullulans* strains were streaked 20 mm from the edge and, in the middle, 32 mm from the *A. pullulans* line and 32 mm from the opposite edge, a single drop of 50 µL of *B. cinerea* conidial suspension in the concentration of 10^4 conidia per mL was observed. The Petri dishes were incubated at 25 °C for 14 days and the mould growth was monitored daily. When the mycelium reached the Petri dish edge, the inhibition distance, defined as the gap between *A. pullulans* line and the mycelium of *B. cinerea*, was measured and then expressed as a percentage with respect to the distance of 32 mm.

In addition to the indigenous strains, the two *A. pullulans* strains (coded BOT1 and BOT2) used in the commercial product Botector® (BIO-FERM, Austria) were included in the tests for comparison. The best performing strain was selected to be used for the further trials in the vineyard.

2.4. Experimental Design and Botrytis Control Strategies Tested

Experimental trials were conducted from May to September 2019 in a pruned-spur cordon Sangiovese vineyard planted in 2000, located in Montalcino (Siena, Italy), facing N-S, row distance 3 m and plant distance 0.9 m, in a randomised block design including 12 blocks (Figure 1).

Figure 1. Photo and scheme of the grapevine rows considered in the randomised block experimental design. C: not treated grapevines; DEF: defoliated grapevines; BOT: grapevines treated with the commercial product Botector®; AP: grapevines treated with the indigenous *A. pullulans* strain AP159-18. Not under investigation: grapevines rows managed according to common company practices.

The experimental trials included three treatments against *Botrytis cinerea* in which the grapevines were managed in three different ways: pre-flowering defoliation (DEF), application of the commercial product Botector® (BOT), and application of an indigenous selected *A. pullulans* strain (AP159-18). Moreover, some grapevine rows without any treatment were included as controls for comparison (C). Other grapevine rows of the vineyards were managed following the usual company practices. As reported in Table 1, defoliation was conducted once, while Botector® and indigenous *A. pullulans* solutions were sprayed four times with cell concentration and procedure suggesting by the Botector® producer. The sprayer equipment was different for commercial and indigenous strains in order to avoid potential contamination. Samplings were carried out on 8 different ocassions, and microbiological and molecular analyses were performed for the enumerating and typing of *A. pullulans* populations.

Table 1. Experimental treatments during the vintage of 2019. Botector® and AP159-18 indicate the *A. pullulans*-based commercial product and the indigenous *A. pullulans* strain, respectively.

Treatment	Treatments Dates								
	28 May	13 Jun.	27 Jun.	8 Jul.	22 Jul.	31 Jul.	29 Aug.	12 Sep.	26 Sep.
Defoliation	X	-	-	-	-	-	-	-	-
Botector®/AP159-18	-	X	X	-	-	X	X	-	-
Sampling	-	X	X	X	X	X	X	X	X

2.5. Microbiological and Molecular Analyses

Grape berries, sampled from grapevines and subjected to different experimental treatments, were analysed as reported above (Section 2.1) in order to quantify and characterise, at strain level, *A. pullulans* populations.

2.6. Measurement of Meteorological Variables

Meteorological measurements (air temperature, relative humidity, and rainfall) were carried out during phenological growth stages of grapevines (budbreak, BBCH9; flowering, BBCH68; veraison, BBCH83 and, at harvest, BBCH85-89). Data were collected by an agrometeorological station, A753 GPRS RTU SEN-R (Davis Instruments Corp., Hayward, CA, USA), located close to the investigated vineyards, equipped with sensors for measuring temperature and relative humidity (Combisensor Temp/RH TR1; Adcon Telemetry, Klosterneuburg, Austria), and pluviometer (RG1 Rain Gauge, 200 cm^2, 0.2 mm, unheated; Adcon Telemetry, Klosterneuburg, Austria).

2.7. Assessment of Biocontrol Activity against Botrytis cinerea on Grapes

For each experimental trial, at the harvest, grey mould caused by *B. cinerea* on grape clusters was visually assessed. The disease incidence was calculated by the formula [29]: Disease incidence (%) = (Number of decayed bunches/Total number of bunches) $\times$ 100. Grape clusters were considered "decayed" when at least the 5% of berries were mouldy ($\geq$3 in the European and Mediterranean Plant Protection Organization (EPPO) scale [30]).

2.8. Statistical Analysis

Microbiological determinations and the biocontrol of grapes were elaborated according to *t*-test procedures or nonparametric one-way ANOVA, followed by Tukey's test (Statistica 7.0 software package). Data differences were reported at a significance level of $p \leq 0.05$.

3. Results and Discussion

3.1. Isolation of Indigenous A. pullulans

With the aim of selecting indigenous *A. pullulans* strains as biocontrol agents for BBR management, 180 *Aureobasidium* spp. were isolated from grape berries sampled during three vintages from different Sangiovese vineyards of a winery located in Montalcino

(Siena, Italy). Based on the results of the RFLP analysis of rITS region, all the isolates belonged to the *A. pullulans* species. In agreement with Nisiotou et al., 2010 [25], they showed a PCR product of ca. 600 bp in the ITS region and restriction profiles of 190, 180, 110 and 100 bp fragments with *Cfo*I, of 440 and 150 bp with *Hae*III and of 290, 180 and 140 with *Hinf*I. The molecular biotyping of the *A. pullulans* isolates performed by RAPD-PCR with the primer P4, revealed the presence of 58 strains (Table 2) confirming the high polymorphism reported for this species by other authors [10,20,31].

Table 2. *Aureobasidium pullulans* strains isolated from Sangiovese vineyards during three vintages.

N. Isolates	*A. pullulans* Strains	Vintage
64	AP95-15, AP96-15, AP97-15, AP116-15, AP117-15, AP118-15 AP175-15, AP176-15, AP180-15, AP185-15, AP188-15, AP196-15 AP202-15, AP209-15, AP218-15, AP230-15, AP239-15, AP246-15 AP248-15, AP249-15, AP253-15.	2015
56	AP18-17, AP38-17, AP106-17, AP107-17, AP110-17, AP115-17 AP120-17, AP125-17, AP127-17, AP130-17, AP132-17, AP140-17 AP164-17, AP165-17, AP173-17, AP180-17, AP186-17, AP199-17, AP206-17, AP232-17.	2017
60	AP45-18, AP85-18, AP88-18, AP111-18, AP112-18, AP159-18 AP160-18, AP170-18, AP177-18, AP183-18, AP187-18, AP206-18 AP214-18, AP216-18, AP232-18, AP-254-18, AP267-18	2018

3.2. Selection of *A. pullulans* with Anti-Botrytis Activity

The anti-*Botrytis* activity of the 58 *A. pullulans* strains was evaluated by in vitro assays using the commercial product Botector® as a positive reference control. The antagonistic activity was assessed by measuring the gap between the streak of *A. pullulans* culture and the edges of *B. cinerea*'s colony on PDA medium after 4–8 and 14 days of incubation at 25 °C (Figure 2).

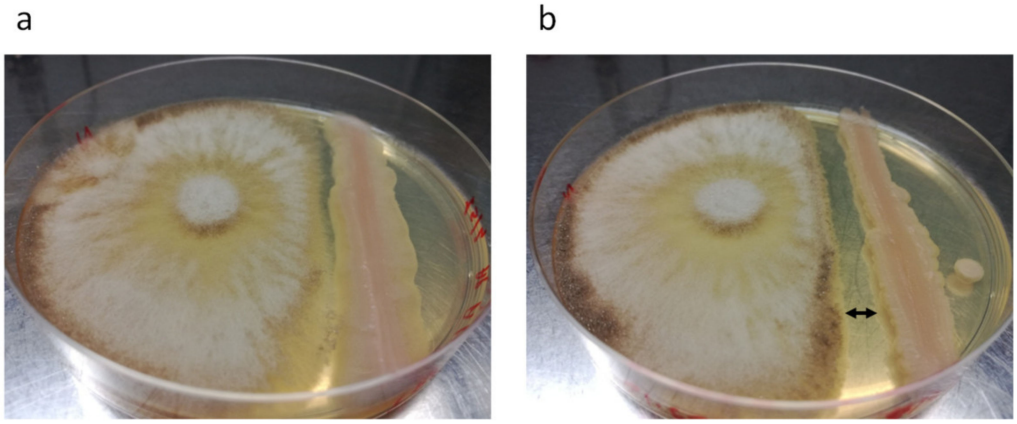

Figure 2. Inhibitory effect of *Aureobasidium pullulans* (pink strike) against *Botrytis cinerea* (white mould) in dual culture on potato dextrose agar: (**a**) strain without activity; (**b**) *A. pullulans* strain AP159-18 possessing the highest inhibition activity (black arrow).

Measures after 14 days of incubation were not different from those displayed after 8 days. Eight *A. pullulans* strains did not show any anti-*Botrytis* activity, 40 strains exhibited an activity lower than 6%, whereas 10 strains showed an activity ranging from 6 to 22% (Figure 3).

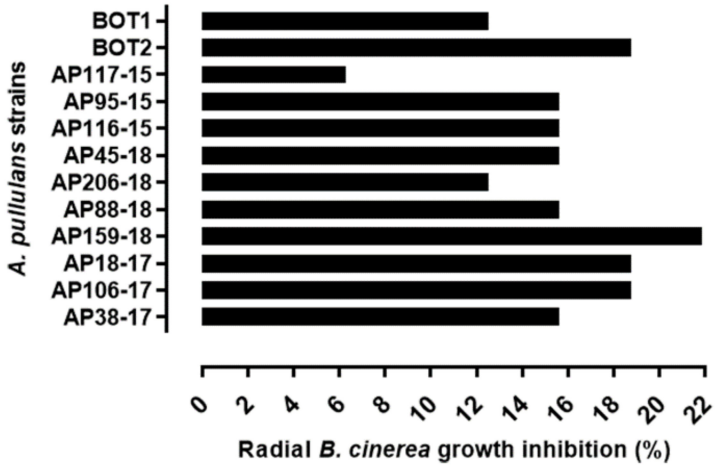

Figure 3. Radial *B. cinerea* growth inhibition (%) by 10 indigenous *A. pullulans* strains in comparison to the two strains (BOT1 and BOT2) contained in the commercial product Botector® (values are the mean of triplicate tests).

The indigenous *A. pullulans* strain 159-18 exhibited the highest biocontrol activity in the in vitro bio-assay (22%) and it was greater compared to the strains of the Botector® product, which separately showed a radial *B. cinerea* growth inhibition of 13% and 19%. Since, as reported by several authors [10,16,32], the main mechanism by which *A. pullulans* was effective in its biocontrol ability was due to the competition for nutrients and space, the isolates with the best biocontrol potential were expected to possess a rapid growth rate. Indeed, the *A. pullulans* strain 159-18 was fully grown after 4 days of incubation, inducing the maximum inhibition distance, too.

3.3. A. pullulans Population Dynamics in Vineyard in the 2019 Vintage

In the vintage of 2019, the *A. pullulans* strain 159-18, which possessed the highest activity in vitro against *B. cinerea*, was sprayed as a biocontrol agent in a vineyard of the winery where it was isolated and compared with the commercial product Botector® according to experimental design (Figure 1 and Table 1). Furthermore, a pre-flowering defoliation was carried out to compare the effectiveness of this practice in preventing the development of *B. cinerea* with the biocontrol treatments. In fact, according to recent studies, pre-flowering leaf removal led to lower bunch compactness, and were more effective than fungicide application [33].

Since temperature, relative humidity, and rainfall regime, in addition to affecting *B. cinerea* development, were evaluated as key factors in determining biocontrol agent efficacy [33,34], daily meteorological variables recorded during experimental trials, are shown in Figure 4.

In the period between budding (BBCH9, 27 March) and full flowering (BBCH65, 9 June), the 2019 vintage was characterised by high relative humidity, abundant rainfall, and low temperatures. Then, from 31 May until the veraison the mean temperature increased (+2.2 °C compared to the historical average) while mean rainfall was lower than the historical average (17.6 mm vs. 67.6 mm), despite two significant rainfall events occurring in July (79.2 mm). Finally, in the period between veraison and harvest (27 September), rainfall was mainly concentrated in September and high values of leaf wetness (343 h) were recorded. In conclusion, the 2019 weather conditions were quite favourable for the development of *B. cinerea*. Indeed, high rainfall favoured grey mould regardless of when it occurred, either before or during the grape ripening period [2]. Moreover, grey mould could also occur in the absence of water, if the RH was high [35].

With regard to the quantification of *A. pullulans* populations on inflorescence or grape berries during the vineyard experiments, results are summarised in Figure 5.

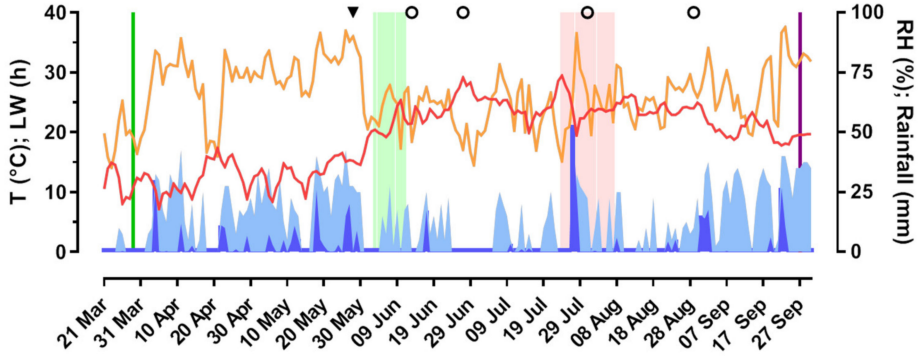

Figure 4. Daily meteorological variables recorded during the experimental time: mean temperature (°C, red line); mean relative humidity (%, orange line); mean leaf wetting (h, light blue bar); mean rainfall (mm, dark blue bar). Vertical bars represent budbreak (dark green bar); flowering (light green bar); veraison (pink bar); harvest (purple bar). Black triangle indicates the time of the defoliation treatment; black circles indicate the time of treatment with Botector® and *Aureobasidium pullulans* AP159-18 strain solution. T: temperature; LW: leaf wetting; RH: relative humidity.

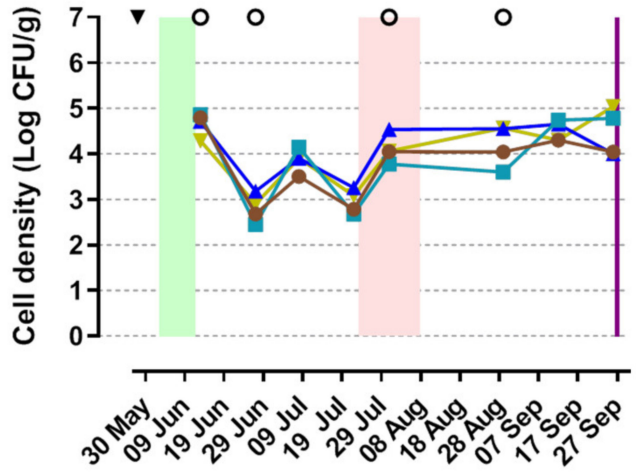

Figure 5. Population dynamics of *Aureobasidium pullulans* occurring on inflorescence/grape berries of the different trials during the experimental time: control (brown line); defoliation (light blue line); Botector® (dark blue line); indigenous *A. pullulans* (yellow line). Vertical bars indicate flowering (light green bar); veraison (pink bar) and harvest (purple bar). Black triangle indicates the time of the defoliation treatment; black circles indicate the time of treatment with Botector® and *A. pullulans* AP159-18 strains.

After the first application of Botector® or the indigenous strain AP159-18 (13 June) at the end of flowering, *A. pullulans* populations were around 4 Log CFU/g, independent of the treatment, including the early defoliation as well as the untreated control. These results demonstrated that indigenous *A. pullulans* were widespread in vineyards at flowering. To our knowledge, these are the first data on the occurrence of *A. pullulans* at this vine phenological stage. In the period between flowering and veraison, the *A. pullulans* populations showed significant variations related more to the meteorological conditions than the different treatments. In fact, *A. pullulans* populations attained higher cell density when an increase in leaf wetness and relative humidity occurred before the sampling, with a decrease in the presence of warmer and drier conditions.

Subsequently, from full veraison (31 July) to the harvest, *A. pullulans* populations maintained similar levels except in the defoliation trial where cell density ranged from Log 3.6 to Log 4.6. Finally, in the sampling close to the harvest (26 September), the control and

grapes treated with the Botector®, or those that were defoliated, showed populations of the order of 4 Log CFU/g, while grapes treated with the indigenous strain AP159-18 had 5 Log units. Other ecological studies performed on different wine grape varieties [3,36,37] found that, at harvest, *A. pullulans* populations numbered around 10^4 CFU/g, while populations at a higher cell density (10^5 CFU/g) were present only in damaged or withered grapes [3,37]. In our study, grapes from the defoliated trial treated with an indigenous AP1598-18 strain showed lower *B. cinerea* incidence with respect to other trials. Therefore, the greater cell densities could be due to different strains constituting the *A. pullulans* populations and their physiological characteristics, as it has been demonstrated that phenotypic heterogeneity in fungi populations can increase their fitness or survival when exposed to particular environments [38].

3.4. Biodiversity of the A. pullulans Population

The intraspecific biodiversity of the *A. pullulans* population was evaluated by analyzing 250 isolates derived from the grape samples of the four trials, and a total of 31 different indigenous strains were detected. In Figure 6 the isolation frequency of each indigenous strain and of strains DSM14940 and DSM 14941 contained in the commercial Botector® product, are reported. The grapes of the control were characterised by 22 indigenous strains of which only eight were detected in this trial. When calculating the ratio between the number of different patterns (22) and the number of isolates (64), as a polymorphism index of the *A. pullulans* population occurring on untreated grapevines, a value of 0.34 was obtained. Rathnayake et al. [10], by using three PCR primers, different from our primers, to characterise *A. pullulans* populations, measured a polymorphism index of 0.15 and 0.24, according to the primer used. Other authors [21], by applying RAPD-PCR technique and OPD 20 primers to study *A. pullulans* isolates from the surface of several fruits and vegetables attained higher values (0.83). The early defoliation (DEF) affected the *A. pullulans* populations, but only immediately after this treatment; indeed, only the first two sampling points presented a limited number of indigenous strains. On the contrary, the following sampling points showed 17 indigenous strains, of which five were isolated only from the grapes of this trial. In the remaining trials (BOT and AP), the application with Botector® and the AP158-19 strain decreased the biodiversity of the indigenous population. BOT2 and AP158-19 strains became dominant in the respective trials from the third sampling point onwards (08 July). Finally, the *A. pullulans* population showed a lower biodiversity in the trials with Botector® than in the AP trial; in fact the first showed a total of six indigenous strains, while the second showed 14 strains.

By comparing the *A. pullulans* indigenous strains detected in the 2019 vintage with indigenous strains found during previous vintages (2015–2018), three strains (A= AP117-15, B = AP38-17, C = AP88-18) were recurrent, demonstrating the occurrence of resident indigenous strains probably more adapted to environmental conditions. In this regard, it is interesting to analyse *A. pullulans* strains which occurred on grapes from the four trials sampled on 13 June, before the beginning of treatments with Botector® or with the indigenous AP159-18 strain. Actually, in all of these samples, the strain C was present at frequencies from 14% to 33% and the BOT2 strain occurred in the grapes from trials coded "BOT" and "AP". These results indicated the ability of the strain C isolated in 2015 to survive in the vineyard ecosystem, and that the strain BOT2 showed a higher persistence capability than the strain BOT1 included in the commercial product, as the vineyards were treated with Botector® in the 2015 vintage. The dominance of the strain BOT2 also occurred in all grape samples treated with Botector® in the 2019 vintage. Finally, independent of the trial, the *A. pullulans* strain D was widespread in the vineyard, being present at different percentages in almost all grape samples. This indigenous *A. pullulans* strain might be further investigated to assess its suitability for use as a biocontrol agent against *B. cinerea.*

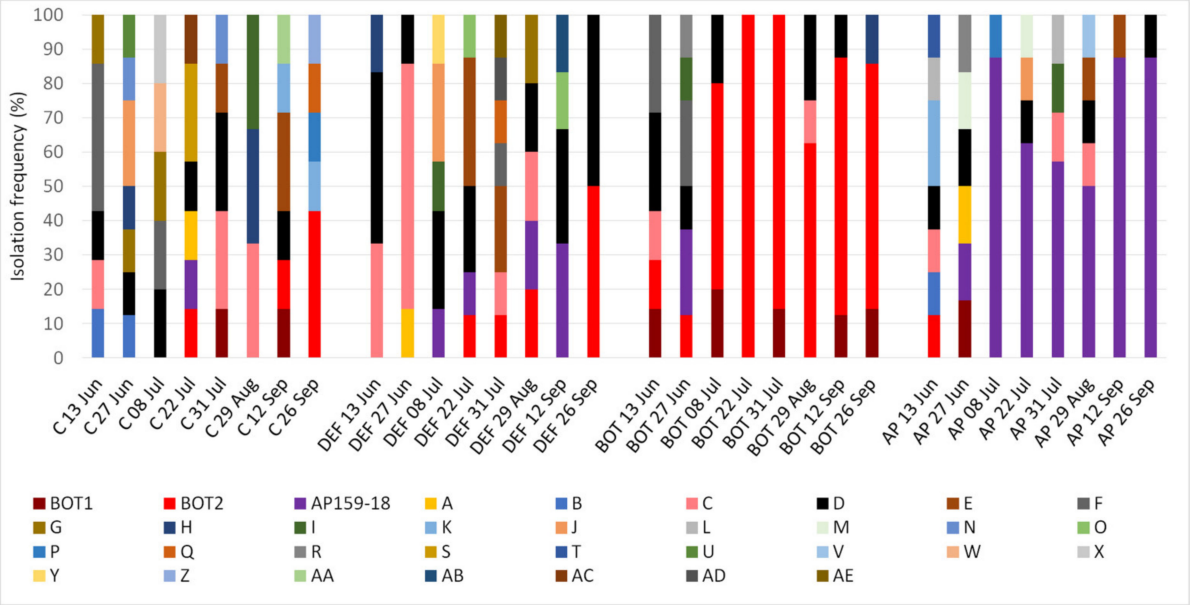

Figure 6. *Aureobasidium pullulans* strains isolation frequencies (%) from grape berry samples of the different trials: C (control); DEF (early defoliation); BOT (Botector® treatment); AP (*A. pullulans* strain AP159-18 treatment); BOT1: Botector® *A. pullulans* strain 1; BOT2: Botector® *A. pullulans* strain 2; A-AE: 31 different indigenous *A. pullulans* strains.

3.5. Assessment of Biocontrol Activity against B. cinerea

At the harvest, the incidence of *B. cinerea* on the berries of the different trials showed average values ranging between 7 and 16%. The lowest value was observed for the grapes treated with the AP158-19 strain, even if no statistically significant differences were found due to the high standard deviations observed for each of the trials (Figure 7). In fact, as reported by various authors, many variables can influence the susceptibility of the grapes to gray rot and this affected the effectiveness of the biocontrol agents [13,34,36]. On the other hand, considering the average values of BBR incidence, the treatment with *A. pullulans* strain AP159-18 reduced the incidence by 55% compared to the control. Hence, despite the limitations due to the experiments carried out in the vineyard, the choice of using indigenous *A. pullulans* strains could be a useful strategy that deserves more in-depth investigations. The commercial product seemed scarcely effective in the conditions which occurred in the vineyard, supported by the results obtained in other oenological areas, [13] although, under certain conditions, it showed efficacy [36].

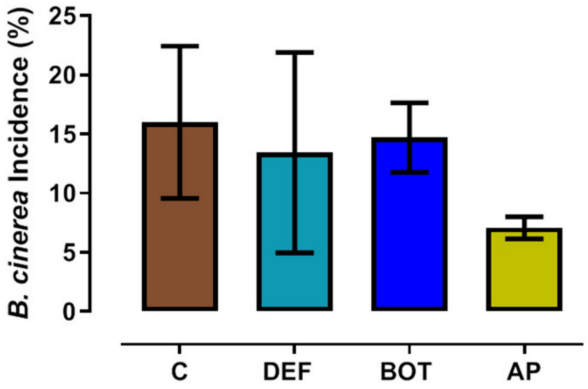

Figure 7. *Botrytis cinerea* incidence (%) at the harvest time for the four different theses: C (control); DEF (defoliation); BOT (Botector®); AP (*A. pullulans* strain AP159-18).

4. Conclusions

With a sustainable management approach for the *Botrytis cinerea* control, an indigenous selected *Aureobasidium pullulans* strain was used as a biocontrol agent and compared with a commercial product (Botector®) using the early-defoliation practice. Due to the large biodiversity of the *A. pullulans* populations, 58 indigenous strains were screened to select the best-performing strain for the biocontrol (AP159-18). The high polymorphism was confirmed by the microbiological and molecular monitoring of *A. pullulans* strains on grape berries during the experiment time, especially for defoliation and untreated trials. Despite the in vitro biocontrol potential, both for AP159-18; the commercial strains, DSM14940 and DSM 14941; and the usual application of Botector® as a biocontrol agent, no significant differences ($p < 0.05$) in the *B. cinerea* incidence on bunches were detected according to different treatments. The efficacy of the biocontrol agent was affected more by meteorological conditions than by the defoliation practice. Nonetheless, considering the lowest incidence values shown in the grape berries treated with the indigenous *A. pullulans* strain and the large biodiversity strain richness, the approach of using an autochthonous strain to *B. cinerea* could be further exploited. This approach is likely to be a more environmentally sustainable way to control these diseases than chemically based fungicides, as recently reported by other authors [21,39].

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