Article

Efficacy of Postharvest Application of *Aureobasidium pullulans* to Control White Haze on Apples and Effect on the Fruit Mycobiome

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Abstract: White haze, an emerging disorder caused by extensive fungal growth on the apple surface, results in a compromised fruit quality and decreased marketability. The use of biological control agents could be an interesting tool to reduce its development. This work aimed to test the efficacy of two *Aureobasidium pullulans* strains (AP2 and PL5) in controlling white haze on stored apples. An *in vivo* trial was conducted by inoculating fruits with white haze causal agents (*Entyloma belangeri*, *Golubevia pallescens*, *Tilletiopsis washingtonensis*) and treating them with the antagonistic yeasts. Three commercial biofungicides were also included in the trial. Both *A. pullulans* strains, along with the *Metschnikowia fructicola*-based product, reduced white haze incidence after 110 days of storage at $1 \pm 1\,^\circ\mathrm{C}$ and after 7 days of shelf life. Furthermore, the effect of *A. pullulans* application on the fruit fungal microbiome was assessed. A significant impact of apple matrix and treatment on the mycobiome composition was observed. Analyses showed a good colonization of *A. pullulans* on the treated apples, both epiphytically and endophytically. A decrease in white haze-related fungi abundance was observed in the treated fruits. Additionally, a reduction of *Ramularia* spp. and modifications in the abundance of other fungal genera were detected after storage and shelf life.

Keywords: biocontrol agents; biological control; *Malus × domestica*; metabarcoding; cold storage

1. Introduction

Apple (*Malus × domestica*) ranks among the most cultivated fruit crops in the world, with an estimated annual production of over 95 million tons in 2022 [1]. After harvest, apples can be stored for several months at low temperatures, making them available on the market all year round. In recent years, with the introduction of new apple varieties and the reduction of pesticide treatments, new diseases and disorders are appearing, negatively affecting the fruit quality and reducing the commercial value [2,3]. Among them, white haze, together with dry lenticel rot, are emerging issues in several apple-producing areas, including Italy [4–6].

White haze appears as a white to pale-grey layer of fungal growth on the fruit skin, first described on cold-stored apples [7] and later observed also on fruits in orchard [4,8]. This disorder has been reported in European apple-producing areas, particularly in the Netherlands [7], Italy [4,8], Germany [9] and Croatia [10]. Currently, 11 species belonging to five basidiomycetous genera have been associated with white haze: *Entyloma belangeri*, *E. davenportii*, *E. elstari*, *E. mali*, *E. randwijkense*, *Gjaerumia minor*, *Golubevia heteromorpha*, *Golubevia mali*, *Golubevia pallescens*, *Jamesdicksonia mali* and *Tilletiopsis washingtonensis* [4,7–9,11]. Species distribution varies geographically. In the Netherlands and northern Germany, *G. heteromorpha* is the most widespread species, although many others have been identified [7,9].

In Croatia, only *G. pallescens* was reported [10]. In northern Italy, Guarnaccia et al. [4] identified six species associated with white haze, with *E. belangeri*, *G. pallescens* and *T. washingtonensis* being the most widespread.

Knowledge about the biology of white haze-related fungi is limited, and their epidemiology is currently being clarified [2]. The presence of the fungal growth on the fruit surface compromises fruit quality and marketability, leading to considerable economic losses [12,13]. No effective management strategies, either preventive or curative, have been identified. Higher white haze incidence is favored by high relative humidity during the cropping season, due to increased rainfall, longer periods of leaf wetness and the use of hail protection nets. Additionally, low temperatures and foliar fertilization can favor the disorder [8,13]. Adoption of proper agronomic practices, aimed at reducing moisture retention in the orchard and avoiding foliar fertilizers, are the only solutions currently adopted for white haze control [14]. Therefore, it is of great importance to develop effective management strategies.

Biological control is an emerging alternative to synthetic fungicides for managing fungal pathogens on fruits [15,16]. Yeasts and yeast-like fungi deserve special attention as biocontrol agents (BCAs), since they are able to colonize the fruit surface and survive in stressful environmental conditions that occur before and after harvest (wide range of relative humidity, low temperatures, low oxygen levels, pH fluctuations and UV radiations). Additionally, they are adapted to the fruit microenvironment, characterized by high sugar concentration, high osmotic pressure and low pH, and they do not produce allergens or mycotoxins harmful for human health [15,17]. Many yeasts or yeast-like fungi have been shown to effectively control various apple postharvest diseases, such as blue mold caused by *Penicillium expansum* [18,19], grey mold caused by *Botrytis cinerea* [20], black rot caused by *Alternaria* spp. [21] and bitter rot caused by *Colletotrichum acutatum* [22]. However, no studies have yet been conducted on the use of BCAs to control white haze. *Aureobasidium pullulans*, a yeast-like fungus commonly isolated from fruit surfaces from early development to maturity [23], is a well-known BCA, effective against many fungal pathogens [22,24–27].

Several yeast-based products have reached advanced stages of development and commercialization. Among those registered in Europe for the control of postharvest diseases of fruit, there are Nexy® (*Candida oleophila*, Agrauxine, Beaucouzé, France), Boni Protect® (*Aureobasidium pullulans*, Manica, Rovereto, Italy) and Noli® (*Metschnikowia fructicola*, Koppert, Berkel en Rodenrijs, The Netherlands) [28].

The aim of this study was to evaluate the efficacy of two *Aureobasidium pullulans* strains in the control of white haze on apples during storage. *Entyloma belangeri*, *Golubevia pallescens* and *Tilletiopsis washingtonensis* were selected for apple inoculation, as they are the most prevalent species associated with white haze in Italy [4]. Three commercial biofungicides registered in Europe to control postharvest diseases of fruits were also included in the trials. Finally, the effect of *A. pullulans* treatments on the epiphytic and endophytic apple mycobiome was evaluated.

2. Materials and Methods

2.1. Microorganisms and Culture Conditions

The white haze causal agents *Entyloma belangeri* strain FR4B (Accession n° PQ220222), *Golubevia pallescens* strain FR22 (Accession n° PQ220223), and *Tilletiopsis washingtonensis* strain FR23 (Accession n° PQ220224) were used for fruit inoculation. The strains were isolated from 'Ambrosia' apples grown in Piedmont (Northern Italy) showing white haze symptoms and preserved in the collection of the Department of Agricultural, Forest and Food Sciences (DISAFA), University of Turin, Italy. The strains were cultured on malt extract agar (MEA; Merck, Darmstadt, Germany), amended with 25 mg/L of streptomycin sulphate, for 21 days at 25 ± 1 °C. Once conidial fructification was obtained, 10 mL of a 1% Tween-20 solution were added to each plate and conidia were collected using a L-shaped spatula. Conidial suspensions were filtered through a four-layer sterile gauze.

Quantifications were carried out using a haemocytometer and the three fungal strain suspensions (3×10^6 conidia/mL per fungal strain) were pooled to obtain a final concentration of 1×10^6 conidia/mL [4].

Two strains of *Aureobasidium pullulans*, belonging to the Turin University Culture Collection (TUCC), and three commercial biofungicides were used for the treatments. *A. pullulans* strains AP2 (TUCC00001040) and PL5 (TUCC00000491) were previously isolated from 'Fuji' apples and 'Angeleno' plums, respectively, grown in Piedmont (Northern Italy). The strains were previously identified by amplification of the ITS region and the sequences were deposited in GenBank (accession n° PQ220221 and FJ919775). The strains were grown in Erlenmeyer flasks containing 30 mL of potato dextrose broth (PDB; Merck, Darmstadt, Germany), placed on a rotary shaker (100 rpm) at 25 ± 1 °C. After 48 h, 5 mL of the cell suspensions were transferred to Erlenmeyer flasks containing 300 mL of PDB, placed on a rotary shaker for 48 h at 25 ± 1 °C. Yeast cells were collected by centrifugation at $4000 \times g$ for 20 min, washed and resuspended in sterile Ringer solution (Merck, Darmstadt, Germany). Quantification was performed by direct countingthe cell suspensions using a haemocytometer. Cell concentration was adjusted to 1×10^8 cells/mL, as previously indicated by [29]. The commercial biofungicides Nexy® (Agrauxine, *Candida oleophila*, strain O), Noli® (Koppert, *Metschnikowia fructicola*, strain NRRL Y-27328), and Boni Protect® (Manica, *A. pullulans*, strains DSM 14940 and DSM 14941) were prepared at 1×10^7 cfu/mL, 1×10^9 cfu/mL and 5×10^8 cfu/mL, respectively, as indicated for commercial use.

2.2. In Vivo Antagonistic Assay under Storage Conditions

The efficacy of the antagonistic yeasts in the control of white haze was assessed *in vivo* on apples cv. Ambrosia, harvested in Saluzzo (CN), Northern Italy. Three replicates of 50 fruits, selected without wounds and rots, were prepared for each treatment. Apples were disinfected in 1% sodium hypochlorite for 2 min, rinsed in tap water and air-dried. Inoculation was performed by dipping the fruits for 1 min in the conidial suspension of the white haze causal agents, prepared as described previously. After 3 h, antagonistic yeasts and commercial biofungicides were applied by dipping the fruits in the cell suspensions for 1 min. Three controls were included. Chemical control was represented by fruits inoculated with white haze causal agents and treated with thiabendazole (Tecto® SC, Syngenta Italia S.p.A., Milan, Italy, a.i.: 19.7%, 30 g a.i. 100 L^{-1}). Apples inoculated and not treated were used as an inoculated control, while fruits not inoculated and not treated served as a healthy control. After drying, apples were placed in plastic boxes and stored at 1 ± 1 °C and 95% relative humidity (RH) in storage chambers for 110 days, and then transferred to shelf life at 11 ± 1 °C for 7 days. The storage took place in experimental cabinets placed in storage rooms, built by Isolcell (Laives, BZ, Italy). White haze incidence, evaluated at the end of storage and shelf life, was expressed as a percentage of fruits showing white haze on their skin.

2.3. Statistical Analysis

Statistical analysis was performed using R Studio version 4.3.1 [30]. After verifying normality and homoscedasticity using Shapiro–Wilk and Levene's tests, data were subjected to one-way analysis of variance (ANOVA). Statistical significance was assessed at the level of $p < 0.05$. Duncan's Multiple Range Test was used for pairwise comparisons among treatments.

2.4. Microbiome Sampling, Sequencing, and Bioinformatics

Apple microbiome was sampled at harvest, after 110 days of storage at 1 ± 1 °C and after 7 days of shelf life at 11 ± 1 °C. Sampling was conducted on apples treated with the *A. pullulans* strains AP2 and PL5 as well as on the healthy, chemical, and inoculated controls. Five replicates of 10 fruits were analyzed for each treatment. Epiphytic and endophytic microbiome sampling and DNA extraction were performed following the protocol reported in Schiavon et al. [31].

Library preparation, pooling, and sequencing were performed at the Genomics and Microbiome Core Facility (GMCF) of Rush University (Chicago, IL, USA). Amplicons were generated using a two-stage PCR amplification protocol [32]. Genomic DNA was amplified with primers ITS3-KYO2 (GATGAAGAACGYAGYRAA) and ITS4 (TCCTCCGCT-TATTGATATGC) targeting the fungal ITS2 region [33]. First-stage PCR amplifications were performed in 96-well plates in 10 µL reactions, with the repliQa HiFi ToughMix (Quantabio, Qiagen, Beverly, MA, USA). Each primer was used at 300 nM concentration. A blocking oligonucleotide (ATTGATATGCTTAAATTCAGCGGGTAACCCCGCCTGACCTGGGGTC GCGTT-C3 spacer) [33] was added to the mastermix at 1 µM concentration to reduce amplification of the plant host DNA. Thermal cycler conditions were 98 °C for 2 min, followed by 28 cycles of 98 °C for 10 s, 78 °C for 1 s, 55 °C for 1 s and 68 °C for 1 s. Subsequently, a second PCR amplification was performed in 10 µL reactions in 96-well plates using repliQa HiFi ToughMix. Each well received a separate primer pair with a unique 10-base barcode, obtained from the Access Array Barcode Library for Illumina (Fluidigm, San Francisco, CA, USA). One microliter of PCR product from the first stage amplification was used as a template for the second stage, without clean-up. Cycling conditions were 98 °C for 2 min, followed by 8 cycles of 98 °C for 10 s, 60 °C for 1 s and 68 °C for 1 s. Libraries were then pooled and sequenced with a 15% phiX spike-in on a MiSeq V3 flow cell (2 × 300 paired-end reads).

Bioinformatic analyses were performed using the QIIME2 pipeline [34], as described in Schiavon et al. [31], with minor modifications. Briefly, contamination from sequencing adapters was removed using the Cutadapt plugin [35], with default settings. The resulting sequences were filtered and denoised using the DADA2 plugin [36], with forward and reverse truncation values set at 235 and 190 bp, respectively. Generated ASVs were classified using a Naive Bayes classifier trained on the UNITE database, version 8.3 global [37]. Classification results were then used to remove all plant contaminations, together with all ASVs which were detected in less than 5 samples and presented a cumulative frequency of less than 0.05%. For alpha and beta diversity analyses, sample depth was normalized at 5200 with the Scaling with Ranked Subsampling (SRS) approach implemented in SRS plugin [38]. Shannon index, number of observed features and Pielou evenness were chosen as alpha diversity metrics, while for beta diversity the Bray–Curtis Dissimilarity metric was selected. Statistical analyses for alpha diversity were performed with the non-parametric Kruskal–Wallis test, followed by a Dunn post-hoc test. For beta diversity, the overall effect of investigated parameters was tested by means of a Permutational Multivariate Analysis of variance (PERMANOVA) with the Adonis plugin [39,40]. For each parameter, significant differences among groups were detected by mean of a pairwise PERMANOVA and by means of pairwise Permutation Analysis of Dispersion (PERMDISP) [41], to detect possible spurious differences produced by centroid dispersion. Dimensionality reduction with the Principal Coordinate Analysis (PCoA) and representation of beta diversity was performed using the PCoA plugin [42]. Compositional analyses were performed with custom python scripts. ASV frequencies were collapsed together based on genera and converted to relative frequencies, which were mediated based on treatment, sampling timepoint and apple matrix. Genera present in less than 1% of all samples were collapsed in the "Other" category.

3. Results

3.1. In Vivo Antagonistic Assay under Storage Conditions

The efficacy of the treatments in reducing white haze on apples was assessed after 110 days of cold storage (1 ± 1 °C) and after a further 7 days of shelf life at 11 ± 1 °C (Figure 1).
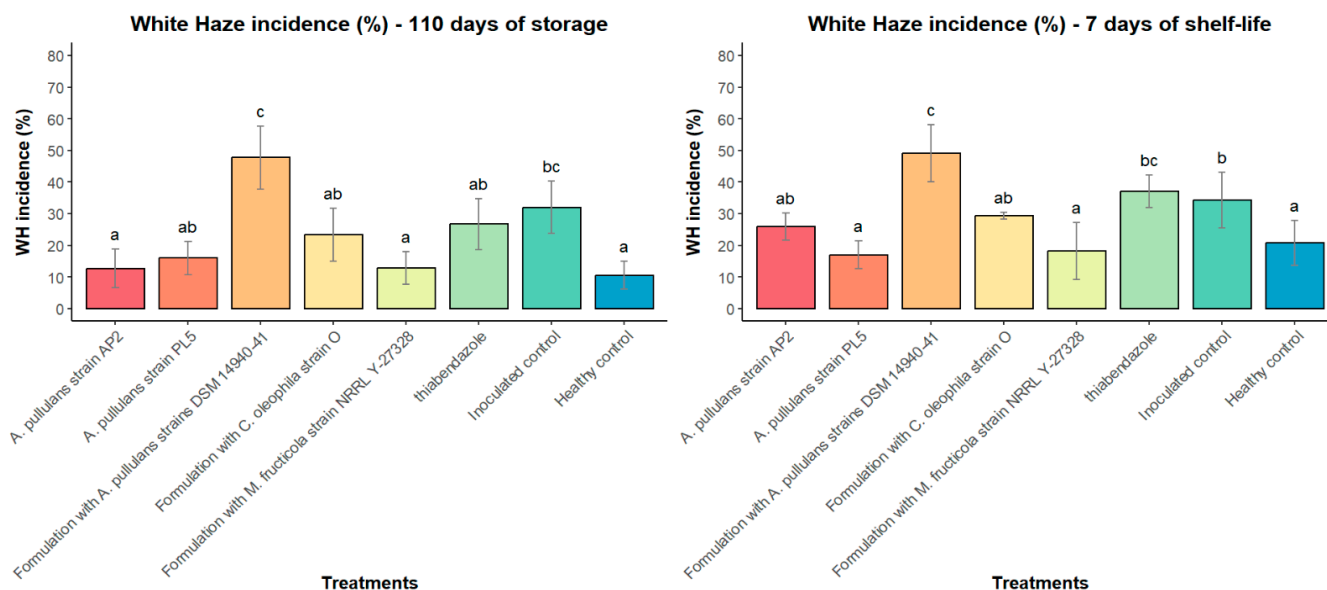
Figure 1. White haze incidence on 'Ambrosia' apples treated with antagonistic yeasts after 110 days of storage at $1 \pm 1\,°C$ (**left**) and after subsequent exposure to shelf life at $11 \pm 1\,°C$ for 7 days (**right**). Values at the same timepoint, followed by the same letter, are not significantly different by Duncan's Multiple Range Test.

After storage, apples treated with both *A. pullulans* strains showed a low white haze incidence (13% and 16% for AP2 and PL5, respectively). Notably, AP2 treatment resulted in a significantly lower incidence than the inoculated control (32%), comparable to the healthy control (11%). Commercial biofungicides exhibited variable efficacy. Treatment with *Metschnikowia fructicola* strain NRRL Y-27328 was the most effective, as the fruits showed a significantly lower white haze incidence (13%) than the inoculated control, comparable to the healthy control. Treatments with *Candida oleophila* strain O and *A. pullulans* strains DSM 14940–DSM 14941 resulted in incidence values of 23% and 48%, respectively, the latter comparable to the value reported for the inoculated control. Apples treated with thiabendazole also showed a white haze incidence (27%) not significantly different from the inoculated control.

After shelf life, treatment with the PL5 strain maintained a low white haze incidence (17%), comparable to the healthy control (21%) and significantly lower than the inoculated control (34%). A low incidence value (26%) was also observed for apples treated with the AP2 strain, although no longer significantly different from the inoculated control. Among the commercial products, the *M. fructicola* strain NRRL Y-27328 maintained a significantly lower white haze incidence (18%) than the inoculated apples, while the value reported for fruits treated with the *C. oleophila* strain O increased to 29%. Treatment with thiabendazole was still ineffective by the end of the trial (37% incidence), comparable to the inoculated control. Apples treated with *A. pullulans* strains DSM 14940–DSM 14941 maintained the highest white haze incidence (49%).

3.2. Microbial Diversity and Composition

3.2.1. Alpha Diversity

By considering the alpha diversity, the comparison between epiphytes and endophytes (Figure 2) showed significant differences for all the considered metrics, with lower values for the endophytic communities. On the other hand, no significant differences were found for any of the considered metrics considering the sampling timepoint (Figure 3). Concerning treatments, significant differences were observed for the Shannon Index and the Pielou evenness. Both treatments with *A. pullulans* strains resulted in lower values compared to

the healthy, inoculated, and chemical controls. No significant differences were found either between *A. pullulans* treatments or among the controls (Figure 4).
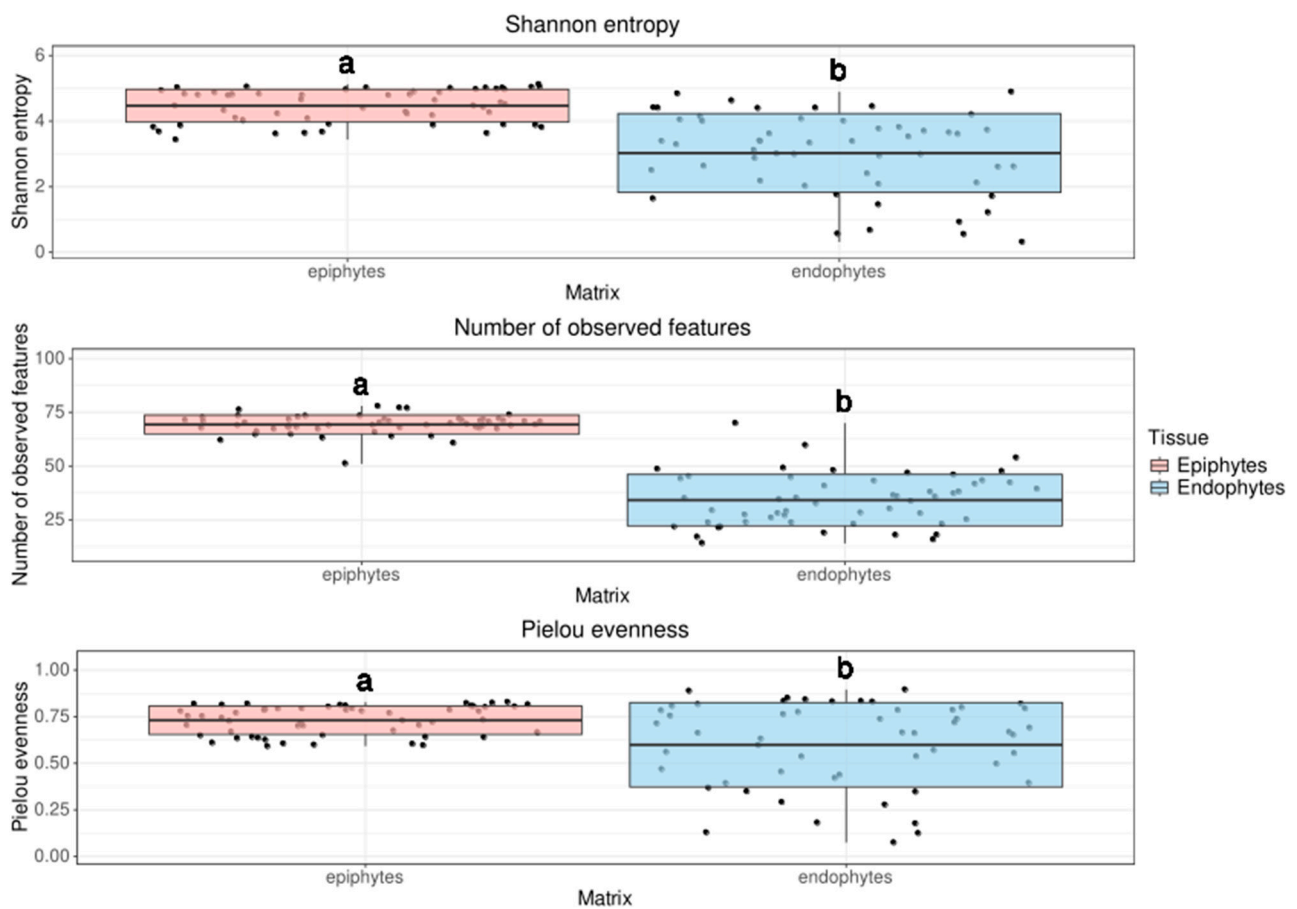

Figure 2. Box and whisker plots of the alpha diversity values of the analyzed microbial communities, based on the apple matrix. Middle line of each box coincides with the mean, while upper and lower box bounds are placed at one standard deviation from the mean. Whiskers above and below the box extend to the highest and lowest values of the distribution, respectively. Comparison of sample groups and assignation to significance groups was performed by means of a Kruskal–Wallis test followed by a Dunn post-hoc test, with the q-value (FDR adjusted *p*-value) rejection threshold set at 0.05.

3.2.2. Beta Diversity

Adonis analysis (Table 1) showed that treatment had the highest impact on the total variance (22.5%), followed by apple matrix (15%). Pairwise PERMANOVA and PER-MDISP (Table S1) highlighted significant differences in beta diversity between *A. pullulans* treatments (AP2 and PL5) and controls. Moreover, significant differences were observed between AP2 and PL5 treatments. At the same time, no significant differences were found for centroid dispersion, which indicates the presence of a real effect based on treatment, except for the comparison between AP2 treatment and healthy control.

Table 1. Adonis analysis results for the considered experimental parameters and combination of parameters. The column F.model contains the test statistic, the column R2 is the percentage of variance explained by the associated parameter/combination of parameters and the column Pr(>F) is the FDR (false discovery rate) adjusted *p*-value using the Benjamini–Hochberg procedure. Values in bold indicate statistically significant results, based on a cutoff threshold of 0.05.

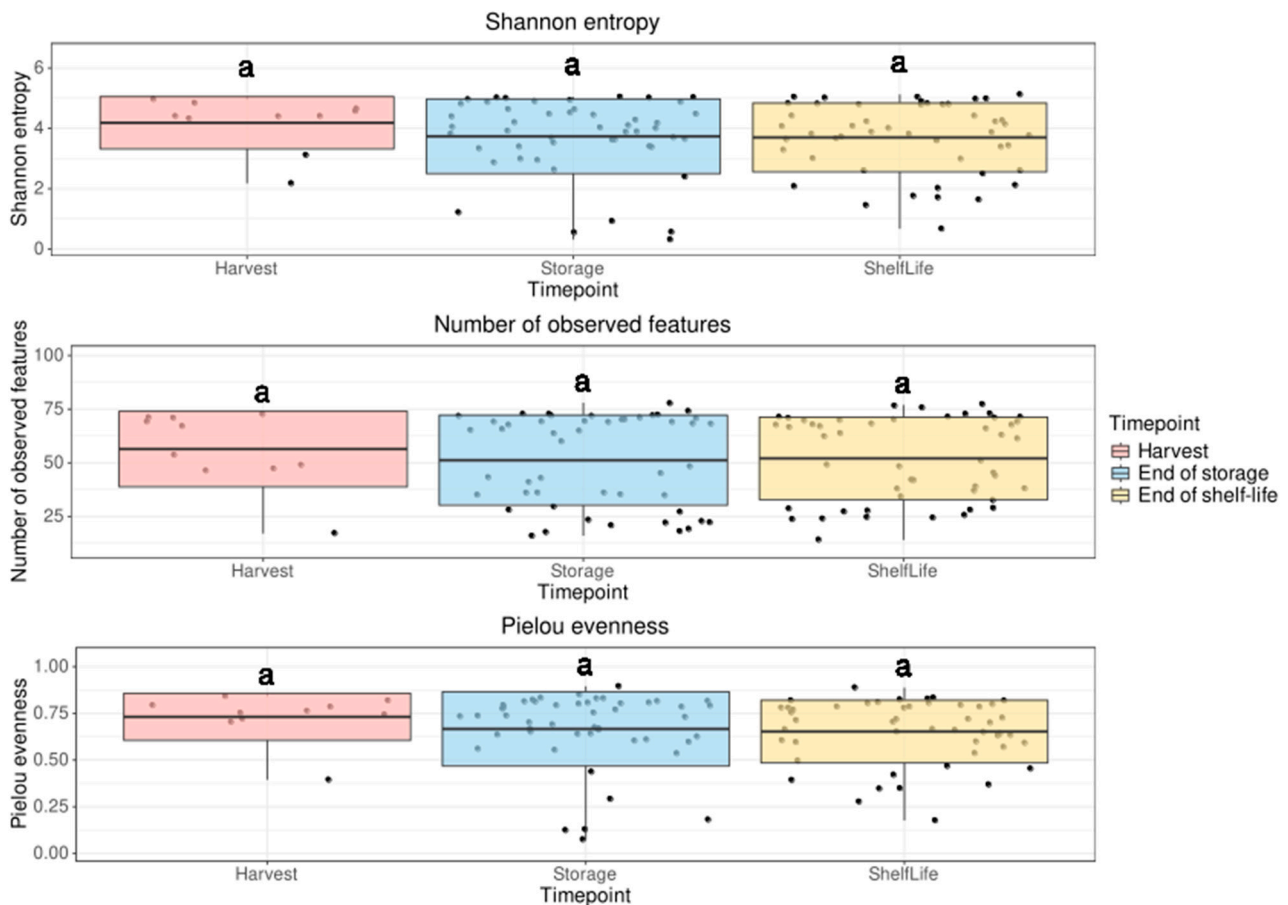| Parameter	F.Model	R2	Pr(>F)
Treatment	9.75	22.5%	**0.001**
Matrix	25.17	15%	**0.001**
Timepoint	1.75	2.5%	**0.012**
Treatment × Matrix	1.04	2%	0.391
Treatment × Timepoint	1.25	3%	0.066
Matrix × Timepoint	1.73	2.5%	**0.017**
Treatment × Matrix × Timepoint	1.12	3%	0.158
Residuals	NA	49.5%	NA
Total	NA	100%	NA

Figure 3. Box and whisker plots of alpha diversity values of analyzed microbial communities, based on the sampling timepoint. Middle line of each box coincides with the mean, while upper and lower box bounds are placed at one standard deviation from the mean. Whiskers above and below the box extend to the highest and lowest values of the distribution, respectively. Comparison of sample groups and assignation to significance groups was performed by means of a Kruskal–Wallis test followed by a Dunn post-hoc test, with the q-value (FDR adjusted *p*-value) rejection threshold set at 0.05.
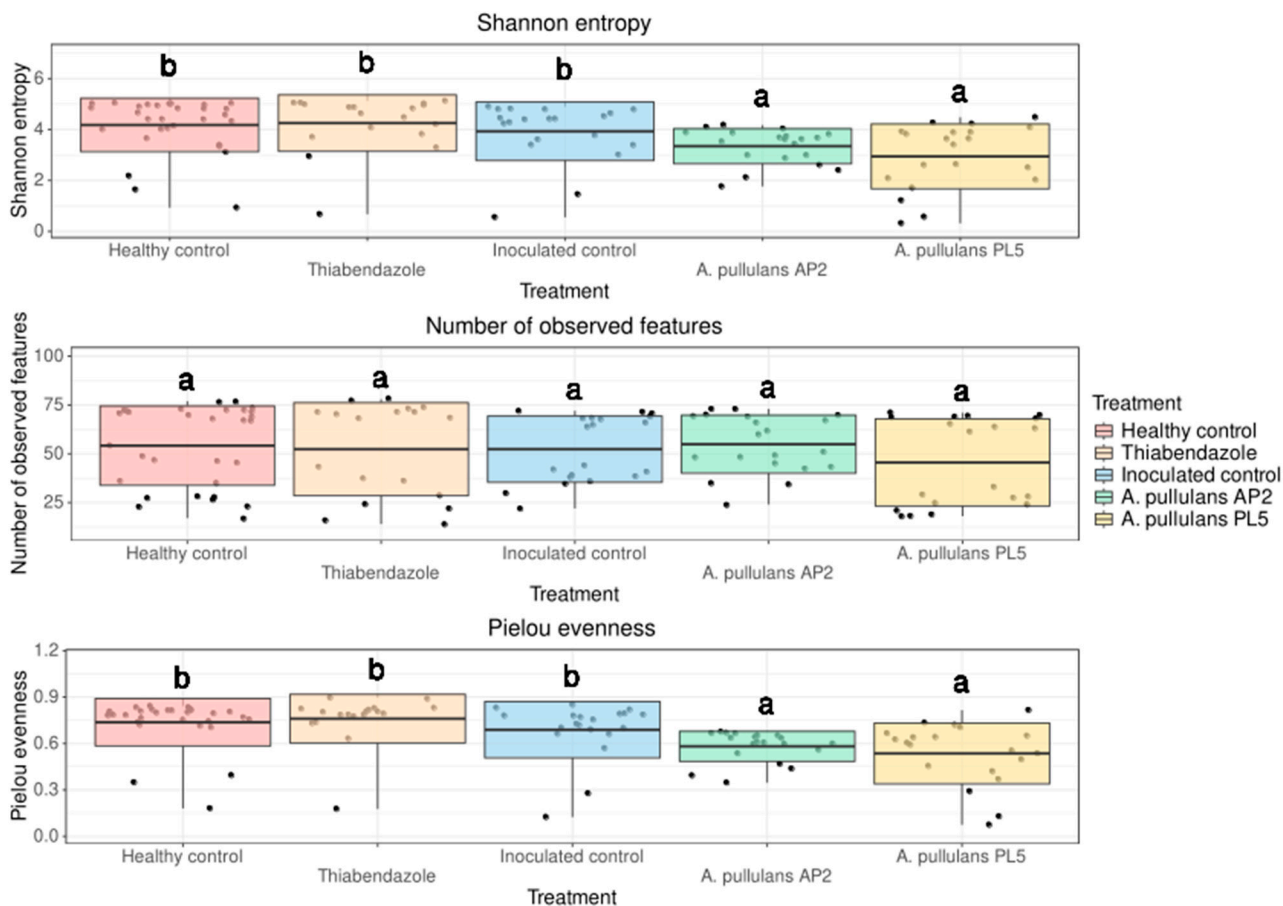
Figure 4. Box and whisker plots of alpha diversity values of analyzed microbial communities, based on treatment. Middle line of each box coincides with the mean, while upper and lower box bounds are placed at one standard deviation from the mean. Whiskers above and below the box extend to the highest and lowest values of the distribution, respectively. Comparison of sample groups and assignation to significance groups was performed by means of a Kruskal–Wallis test followed by a Dunn post-hoc test, with the q-value (FDR adjusted *p*-value) rejection threshold set at 0.05.

Considering the apple matrix, i.e., epiphytic or endophytic population, pairwise PERMANOVA and PERMDISP (Table S1) showed significant differences for both centroid effect and centroid dispersion. Thus, the effect of dispersion in the presence of a significant difference could not be ruled out.

The sampling timepoint effect, while significant, constitutes 2.5% of the total explained variance, comparable to the interaction between apple matrix and timepoint. PERMANOVA and PERMDISP analyses (Table S1) highlighted a significant difference for the centroid effect, but not for the centroid dispersion. Overall, almost half of the total observed variance (49.5%) was not explained by the considered parameters.

A significant effect of treatment and apple matrix on the compositional variance of microbial communities was shown also by the PCoA dimensionality reduction plot (Figure 5).
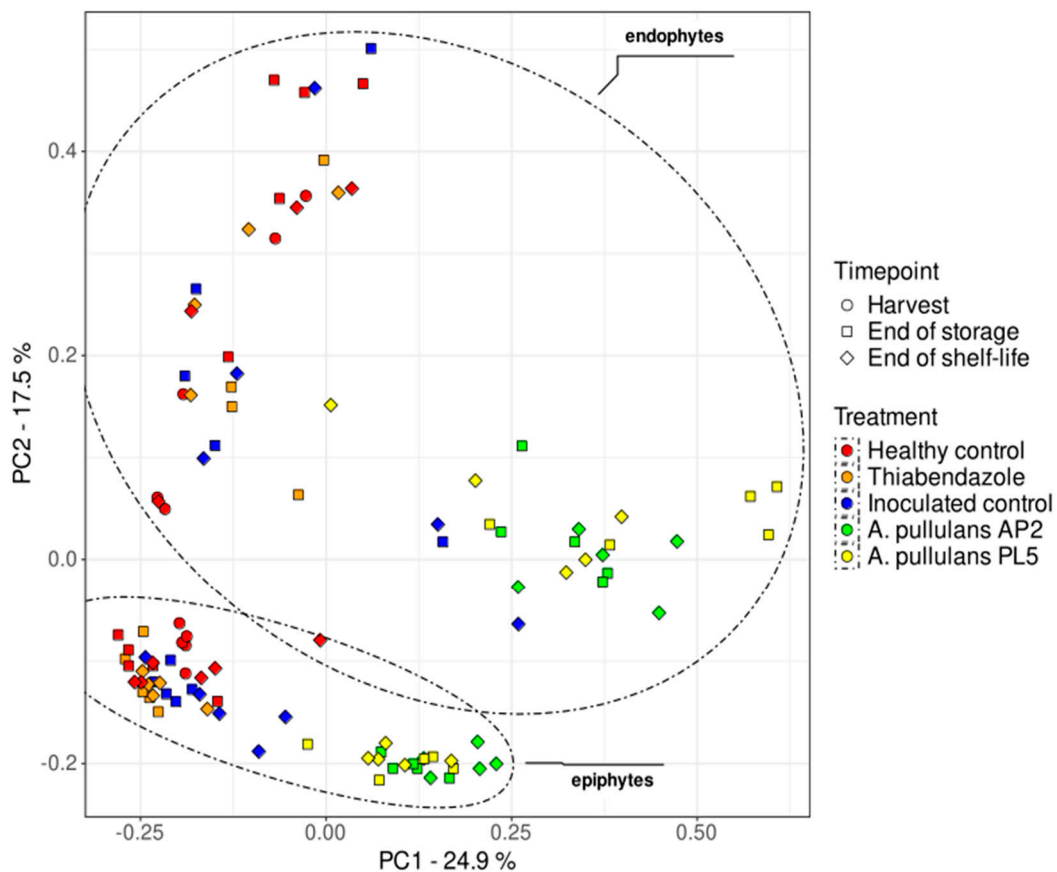
Figure 5. Principal Coordinate Analysis (PCoA) plot based on the Bray–Curtis distance matrix results. Each point is associated with a sample. Point shape is based on sampling timepoint, while fill colors are based on treatments. Two ellipses have been drawn to represent sample grouping based on apple matrix. Percentages shown on each axis indicate the fraction of microbial variance explained by each Principal Coordinate (PC).

3.2.3. Compositional Analysis of Microbial Population

The compositional analysis of the epiphytic communities (Figure 6) revealed that the predominant genera at harvest were *Diplodia* (22.6%), *Aureobasidium* (7.9%), *Cladosporium* (7.5%), *Nigrospora* (6.7%), *Symmetrospora* (6.5%), *Vishniacozyma* (4.8%), *Septobasidium* (4.1%), *Buckleyzyma* (3.3%), *Ramularia* (2.9%) and *Cystobasidium* (2.7%). White haze-related genera *Entyloma*, *Golubevia* and *Tilletiopsis* were also present, but with a relative abundance lower than 1%.

By the end of storage, *Aureobasidium* spp. abundance was higher in samples treated with AP2 and PL5 strains (35.2% and 34.4%, respectively). In contrast, it remained similar to the abundance at harvest in both healthy (8.0%) and inoculated controls (8.3%), with a slight decrease in thiabendazole-treated apples (6.3%). This pattern persisted after shelf life, where *Aureobasidium* spp. abundance was higher in fruits treated with AP2 (39.1%) and PL5 (32.9%) compared to the healthy, inoculated, and chemical controls (7.9%, 14.1%, and 8.8%, respectively).

White haze-related genera were present in all treatments after storage and shelf life, with differences in relative abundance. A reduction was observed in AP2- and PL5-treated apples at all sampling timepoints. Specifically, after storage, *Tilletiopsis* spp. was found at 1.4% and 0.7%, *Entyloma* spp. at 2.6% and 2.8%, and *Golubevia* spp. at 2.5% and 2.6% in AP2- and PL5-treated fruits, respectively. After shelf life, the abundance of *Tilletiopsis* spp. was 0.9% and 1.3%, *Entyloma* spp. was 1.3% and 3.0%, and *Golubevia* spp. was 3.3% and 2.9% in AP2- and PL5-treated apples, respectively. Higher abundances were detected in healthy, chemical and inoculated control fruits.

Ramularia spp., the causal agent of dry lenticel rot, showed a significant presence throughout the experiment. At the end of storage, it was most abundant in the inoculated fruits (12.9%), followed by thiabendazole-treated apples (8.1%) and healthy control (7.4%). In contrast, it showed a reduction in AP2- (4.5%), and PL5 (3.6%)-treated fruits. After shelf life, its abundance remained lower in *A. pullulans* treated apples (4.0% for AP2 and 3.4% for PL5) compared to the chemical (8.4%), healthy (9.1%) and inoculated (10.7%) controls.

Additionally, other apple pathogens were detected, although sparingly or with lower abundance. Specifically, *Neofabraea* spp., the causal agent of bull's eye rot, was found with a significant presence in the inoculated control at the end of storage (5.5%).
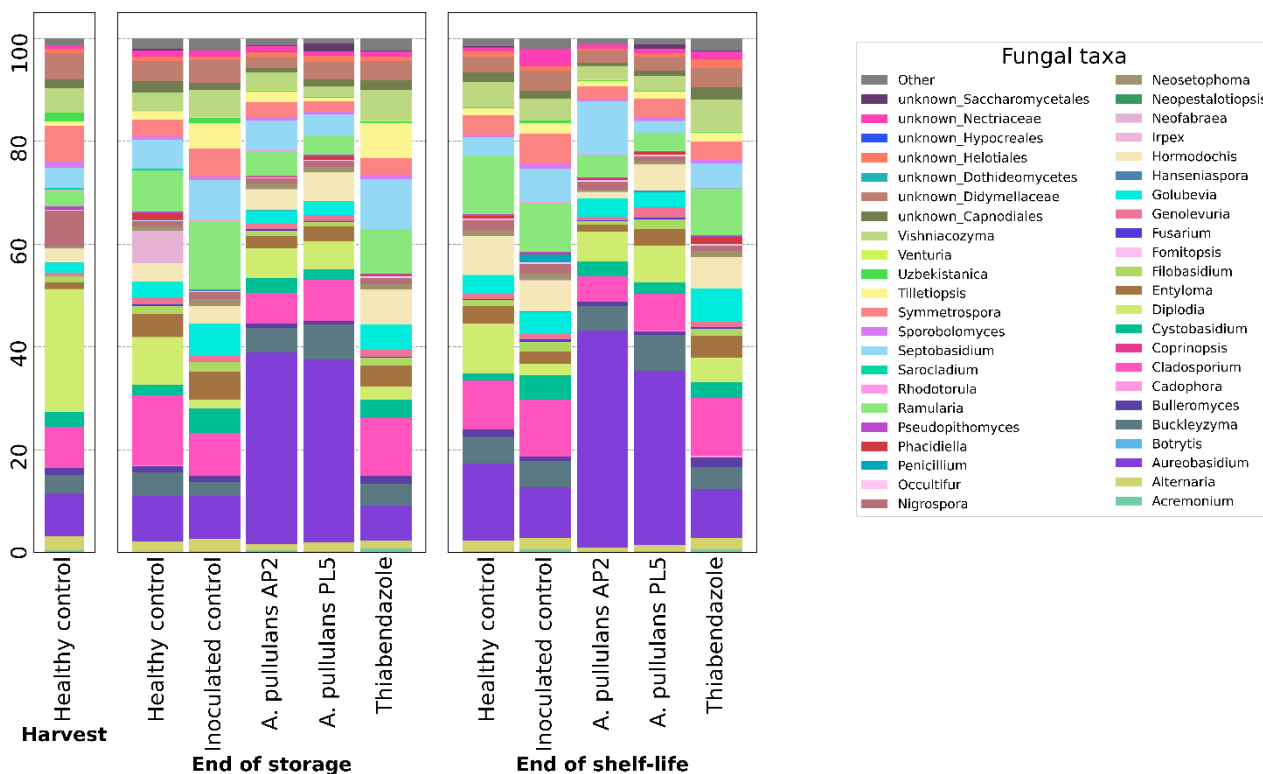

Figure 6. Taxa composition of epiphytic fungal communities. The "Other" category includes all taxa with less than 1% relative abundance in all considered groups.

Focusing on the endophytic communities (Figure 7), the main detected genera at harvest were *Venturia* (13.6%), *Cladosporium* (9.8%), *Alternaria* (7.7%), *Nigrospora* (6.2%), *Ramularia* (5.7%), *Aureobasidium* (3.9%), *Vishniacozyma* (3.8%), *Symmetrospora* (3.1%) and *Fusarium* (2.7%). *Entyloma*, *Golubevia* and *Tilletiopsis* spp. were detected, even though in small amounts (below 1.4%). As observed in the epiphytic communities, by the end of storage, the genus *Aureobasidium* showed the highest abundance in fruits treated with AP2 (44.3%) and PL5 (66.9%) strains, while it was lower in the healthy (2.5%), inoculated (5.8%) and chemical (6.2%) controls. Its abundance was still significantly higher in the treated apples after shelf life (43.1% for AP2 and 39.4% for PL5, respectively), compared to the controls (5.2%, 16.8% and 4.9% for healthy, inoculated and chemical controls, respectively).

White haze-related genera were less abundant in endophytic samples compared to the epiphytic ones. *Golubevia* spp. abundance was less than 0.7% in all treatments, except for the thiabendazole-treated fruits (3.5% and 4.6% at the end of storage and shelf life, respectively). Abundance of the genus *Entyloma* was below 0.7% in most samples, except for the chemical control after storage (1.5%) and the healthy control at the end of shelf life (1.3%). *Tilletiopsis* spp. abundance was lower than 0.6% in most treatments, except for the healthy (2.2%) and chemical (2.2%) controls at the end of storage, and for the healthy control (2.3%) after shelf life.
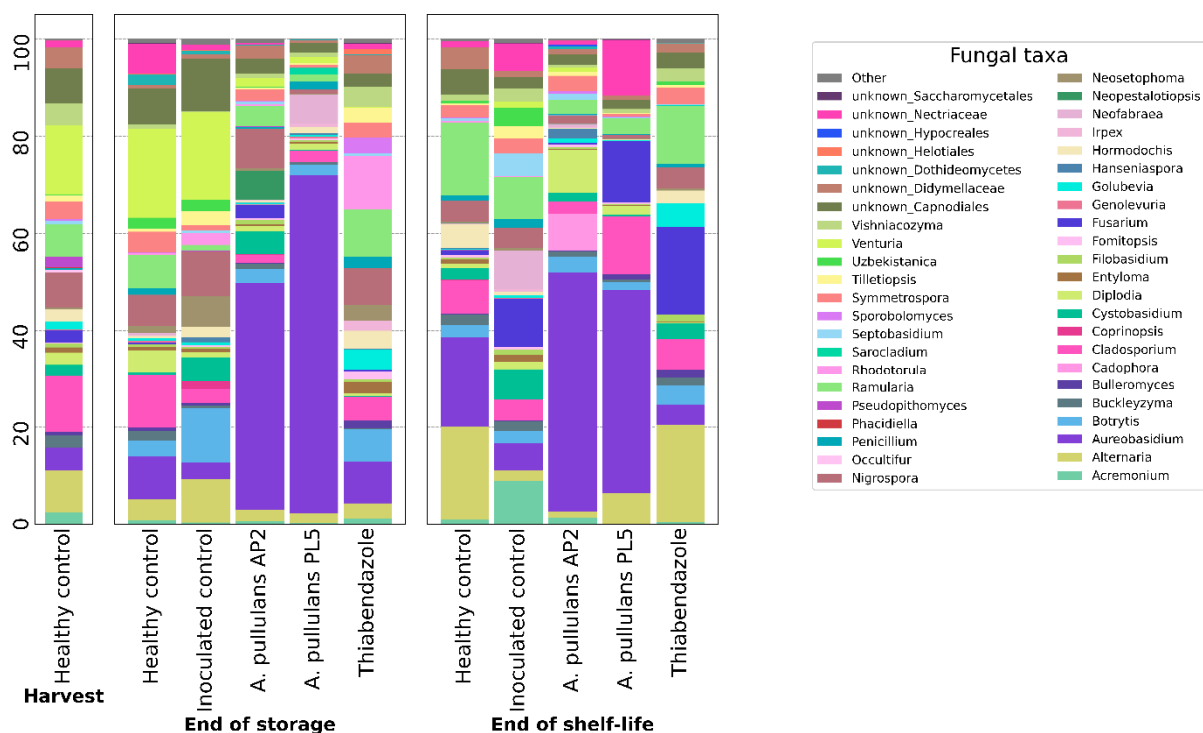
Figure 7. Taxa composition of endophytic fungal communities. The "Other" category includes all taxa with less than 1% relative abundance in all considered groups.

The genus *Ramularia* was detected in all treatments and controls, as for the epiphytes. Its abundance at the end of storage was highest in the inoculated (15.5%) and thiabendazole-treated apples (8.8%), followed by treatments with AP2 (4.0%), PL5 (1.3%) and the healthy control (1.0%). The same pattern persisted after shelf life: *Ramularia* spp. abundance was highest in the inoculated (13.9%) and chemical (10.1%) controls and lowest in the healthy control (8.0%), AP2 (2.5%) and PL5 (3.3%)-treated fruits.

Presence of other genera of phytopathological interest was observed in endophytic samples. The genus *Penicillium* was detected at the end of storage in the inoculated (1.4%) and chemical (1.4%) controls, as well as in PL5 treatment (1.6%). At the end of shelf life, it was present in the healthy (1.2%) and inoculated (1.2%) controls, and with a lower abundance in thiabendazole (0.8%), AP2 (0.7%) and PL5 (0.3%)-treated apples. *Botrytis* spp. was consistently detected in endophytic samples, with higher abundance in the healthy control at the end of storage (8.7%) and in the chemical control after shelf life (4.3%). The genus *Venturia* was reported at the end of storage mainly in the healthy (17.7%), inoculated (18.1%) and chemical (14.3%) controls, with a lower presence in AP2 (1.8%) and PL5 (1.2%)-treated fruits. *Neofabraea* spp. was present in the PL5 treatment at the end of storage (6.1%) and in the healthy control at the end of shelf life (7.4%).

4. Discussion

White haze, caused by extensive fungal growth on the apple surface, is an emerging disorder that compromises fruit quality and reduces the commercial value. An effective control against white haze is currently not achievable due to the limited knowledge about the epidemiology and the efficacy of fungicides. Additionally, significant restrictions in pesticide use, driven by concerns about human health and environmental risks, along with the development of fungicide-resistant strains, have shifted research towards alternative strategies, including biological control [15,43]. In this study, the efficacy of two *Aureobasidium pullulans* strains (AP2 and PL5) to control white haze on apples during storage was evaluated. Three commercial biofungicides were included in the trial. Both *A. pullulans* strains reduced white haze incidence at the end of storage and shelf life. Notably, AP2

was more effective at the end of the storage, whereas PL5 showed the lowest white haze incidence after shelf life. Although the application of biological control agents (BCAs) in white haze management has not been previously documented, our results are consistent with studies demonstrating the efficacy of *A. pullulans* in controlling several apple postharvest diseases. Specifically, *A. pullulans* showed efficacy against *Penicillium expansum* [22,44], *Botrytis cinerea* [22,45], *Colletotrichum acutatum* [22] and *Alternaria alternata* [21]. The biocontrol activity of *A. pullulans* is ascribed to several mechanisms of action. These include competition for nutrients and space [46,47], production of siderophores [48,49], aureobasidin A [50] and biofilm formation [49,51]. The secretion of lytic enzymes, including exo- and endochitinase, β-1,3-glucanase and protease, also contribute to the biocontrol activity [49,52,53]. Additionally, volatile organic compounds produced by *A. pullulans* have demonstrated antifungal activity against apple postharvest pathogens both *in vitro* and *in vivo* [18,21,54].

The PL5 strain, isolated from plums, previously showed to be effective against *Monilinia laxa* on stone fruits and *B. cinerea* and *P. expansum* on apples when applied at 10^8 cells/mL, the same concentration used in this study. This strain has been shown to produce lytic enzymes such as β-1,3-glucanase, exochitinase and endochitinase, which contribute to its antifungal activity [29]. Moreover, the efficacy of a protease derived from PL5 was evaluated on apples, showing efficacy in controlling *Monilinia fructicola*, *B. cinerea* and *P. expansum* [55].

Treatment with the commercial product based on *A. pullulans* strains DSM 14940 and DSM 14941 was not effective in this experiment. This result confirm that the antagonistic activity is strain specific. Zajc et al. [49] analyzed the biocontrol characteristics of 20 *A. pullulans* strains with known antagonistic activity against postharvest fruit pathogens, including two strains re-isolated from Boni Protect. Differences in salt tolerance, biofilm formation and enzyme production were found among the analyzed strains, which may be related to their different biocontrol activities.

Among the commercial biofungicides, *Metschnikowia fructicola* strain NRRL Y-27328 [56] demonstrated significant efficacy in the control of white haze. Several studies demonstrated the effectiveness of *M. fructicola* in controlling fruit postharvest diseases [57,58]. Similarly to other BCAs, *M. fructicola* acts as an antagonist through various mechanisms of action, including competition for nutrients and space [59], production of superoxide anions [60] and chitinase activity [61].

However, a more in-depth knowledge of the mechanisms of action used by BCAs against white haze is required.

Thiabendazole was included as chemical control, as it is commonly used in postharvest disease management. However, apples treated with thiabendazole showed a white haze incidence comparable to the inoculated control, both at the end of storage and after shelf life. Few studies are available on the efficacy of fungicides in controlling white haze. Angeli et al. [62] observed a reduction of fungal growth in vitro using captan, dodine, penconazole and fosetyl aluminium applied at the label concentration. Further trials, however, are needed to confirm their efficacy in orchard.

The effect of *A. pullulans* treatments on the epiphytic and endophytic fungal microbiome of apples during storage was evaluated. Significant differences in alpha diversity were observed between epiphytes and endophytes, with epiphytic communities exhibiting higher values for all considered metrics. This indicates that epiphytes are richer in microbial taxa and exhibit greater uniformity, probably due to the different conditions of the epicuticular and subcuticular regions. The subcuticular region is known to have lower oxygen levels [63] and high sugar concentrations [64], which could provide a strong selective pressure in the establishment of microbial communities. Beta diversity analysis further supported these findings, with significant differences between epiphytes and endophytes indicated by PERMANOVA and distinct clustering in the PCoA plot.

When considering sampling timepoint, no significant differences in alpha diversity metrics were found. Beta diversity analysis revealed significant differences in PER-

MANOVA, which, however, did not lead to distinct clustering groups in the PCoA analysis. These findings are consistent with previous studies on several fruits, which reported stable diversity values of fungal communities during storage at low temperatures [65,66]. Additionally, significant shifts in the apple mycobiome composition were only observed after two months of storage [67], suggesting a greater inertia of the microbial community to low temperatures.

Regarding treatment effects, significant differences were observed in the Shannon index and Pielou evenness, with lower values in apples treated with AP2 and PL5 compared to the controls. Even beta diversity analyses revealed significant differences between *A. pullulans*-treated fruits and controls, as reflected in the PCoA plot. These differences can be attributed to the higher abundance of *A. pullulans* in treated fruits, leading to a non-specific reduction in other fungal taxa. In this regard, Biasi et al. [68] reported a similar reduction in fungal community richness on stored apples treated with *M. fructicola*.

Compositional analysis showed that the application of *A. pullulans* did not significantly alter the overall composition of the apple microbiome, but it rather affected the relative abundance of some taxa. A higher presence of the genus *Aureobasidium* was observed in AP2 and PL5-treated apples, both in the epiphytic and endophytic communities, indicating successful colonization by the BCAs. These findings align with previous studies investigating fruit microbiome composition following the application of yeast-based BCAs, which also reported a high persistence of the yeasts on the treated fruits during storage [68,69]. Additionally, the high abundance of *Aureobasidium* spp. in the endophytic community of the treated fruits may indicate an internalization of the BCA. Alternatively, the application of *Aureobasidium* strains AP2 and PL5 promoted the growth of native endophytic strains of *Aureobasidium* spp. A BLAST analysis was performed on the obtained ASVs of *Aureobasidium* and it showed for all the ASVs, 100% identity to the strains CBS 584.75 and CBS 100280 of the species *A. pullulans*. White haze causal agents were predominantly detected in the epiphytic communities. Both after storage and shelf life, the presence of *Entyloma*, *Golubevia* and *Tilletiopsis* spp. decreased in fruits treated with *A. pullulans* AP2 and PL5. Furthermore, the presence of these genera was also detected on asymptomatic apples at harvest, confirming that white haze-associated fungi are common inhabitants of the carposphere, as previously reported using both metabarcoding [2] and microbiological methods [4,7]. Environmental conditions leading to their overgrowth causing symptoms on fruits needs to be clarified.

Interesting results were observed regarding other genera of phytopathological interest. In both *A. pullulans* treatments, the abundance of *Ramularia* spp., the causal agent of dry lenticel rot, was lower compared to the controls in the epiphytic and endophytic communities, both after storage and shelf life. Conversely, higher abundance was always associated with the control inoculated with white haze causal agents. These results suggest a complex interplay between *Aureobasidium* spp., white haze-related fungi and *Ramularia* spp., as partially observed in Garello et al. [2].

Venturia spp. was only detected in the endophytic samples and mainly at the end of storage, where its presence was lower in fruits treated with AP2 and PL5 compared to the controls. Although no studies evaluated the effect of *A. pullulans* on *Venturia* spp. development, *in vitro* tests have shown that another species, *Aureobasidium microstictum*, had a strong inhibitory effect on this pathogen [70]. Finally, the genus *Botrytis* was consistently detected in endophytic samples, but not in the epiphytic ones. This observation, along with the absence of visible symptoms, confirms previous reports of *Botrytis* spp. adopting an endophytic lifestyle without causing rot [71].

5. Conclusions

To our knowledge, this is the first study considering the management of apple white haze in postharvest using biocontrol agents. *In vivo* trials showed efficacy of two *Aureobasidium pullulans* strains, as well as of a commercial formulate based on a strain of *Metschnikowia fructicola*, after both storage and shelf life. Microbiome analyses showed that *A. pullulans*

had a good colonization ability on the fruit surface and could also be internalized into the flesh.

Overall, our results confirm that metabarcoding can be used to better understand the interactions between applied BCAs and the epiphytic and endophytic mycobiome of apple fruit. The effect of *A. pullulans* treatment also resulted in a lower abundance of other postharvest pathogens, including *Ramularia* spp. However, this aspect needs to be further clarified. Future research should focus on understanding the mechanism of action of BCAs against white haze causal agents, as well as focusing on optimizing application methods, considering a pre-harvest application, and exploring synergistic effects with other management practices to improve efficacy.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10090927/s1, Table S1: Results of the pairwise PERMANOVA (Permutational Analysis of Variance) and PERMDISP (Permutational Dispersion Analysis) on the beta diversity distance matrix for all apple matrix/treatment/sampling timepoint combinations. Pseudo-F and F-value are the PERMANOVA's and PERMDISP's statistics, respectively. Q-value is the FDR (false discovery rate) adjusted *p*-value.

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References

1. Food and Agriculture Organization. FAOSTAT. Available online: https://www.fao.org/faostat/en/?#data/QCL (accessed on 27 June 2024).
2. Garello, M.; Piombo, E.; Prencipe, S.; Schiavon, G.; Berra, L.; Wisniewski, M.; Droby, S.; Spadaro, D. Fruit microbiome: A powerful tool to study the epidemiology of dry lenticel rot and white haze–emerging postharvest diseases of apple. *Postharvest Biol. Technol.* **2023**, *196*, 112163. [CrossRef]
3. Reddy, K.; Spadaro, D.; Lore, A.; Gullino, M.L.; Garibaldi, A. Potential of patulin production by *Penicillium expansum* strains on various fruits. *Mycotoxin Res.* **2010**, *26*, 257–265. [CrossRef] [PubMed]
4. Guarnaccia, V.; Remolif, G.M.; Nari, L.; Gualandri, V.; Angeli, D.; Oettl, S.; Dijksterhuis, J.; Boekhout, T.; Spadaro, D. Characterization of fungal species involved in white haze disorder on apples in Northern Italy and description of *Golubevia mali* sp. nov. and *Entyloma mali* sp. nov. *Postharvest Biol. Technol.* **2024**, *209*, 112678. [CrossRef]
5. Prencipe, S.; Valente, S.; Nari, L.; Spadaro, D. A quantitative real-time PCR assay for early detection and quantification of *Ramularia mali*, an emerging pathogen of apple causing dry lenticel rot. *Plant Dis.* **2023**, *107*, 1399–1407. [CrossRef]
6. Primisser, S.; Spadaro, D.; Deltedesco, E.; Oettl, S. *Ramularia mali* associated with symptoms of dry lenticel rot, an emerging postharvest disease on apples in Italy. *Plant Dis.* **2024**, *108*, 2579. [CrossRef] [PubMed]
7. Boekhout, T.; Gildemacher, P.; Theelen, B.; Müller, W.H.; Heijne, B.; Lutz, M. Extensive colonization of apples by smut anamorphs causes a new postharvest disorder. *FEMS Yeast Res.* **2006**, *6*, 63–76. [CrossRef]
8. Baric, S.; Lindner, L.; Marschall, K.; Dalla Via, J. Haplotype diversity of *Tilletiopsis* spp. causing white haze in apple orchards in Northern Italy. *Plant Pathol.* **2010**, *59*, 535–541. [CrossRef]

9. Weber, R.; Zabel, D. White haze and scarf skin, two little-known cosmetic defects of apples in northern Germany. *Eur. J. Hortic. Sci.* **2011**, *76*, 45.

10. Prencipe, S.; Spadaro, D.; Fruk, G.; Jemric, T. First report of *Tilletiopsis pallescens* causing white haze on apple in Croatia. *Plant Dis.* **2016**, *100*, 225. [CrossRef]

11. Richter, C.; Yurkov, A.M.; Boekhout, T.; Stadler, M. Diversity of *Tilletiopsis*-like fungi in Exobasidiomycetes (Ustilaginomycotina) and description of six novel species. *Front. Microbiol.* **2019**, *10*, 2544. [CrossRef]

12. Lindner, L.; Baric, S. White haze: A new disorder of apples caused by fungi colonizing the fruit surface [*Malus pumila* Mill.; South Tyrol]. *Inf. Fitopatol.* **2006**, *56*, 60–63.

13. Spadaro, D.; Torres, R.; Errampalli, D.; Everett, K.; Ramos, L.; Mari, M. Pome fruits. In *Postharvest Pathology of Fresh Horticultural Produce*; CRC Press: Boca Raton, FL, USA, 2019; pp. 55–110.

14. Angeli, D.; Gualandri, V.; Oliveira Longa, C.M.; Zaffoni, M. Patina bianca, una nuova emergenza in melicoltura. *Inf. Agrar.* **2021**, *77*, 50–54.

15. Droby, S.; Wisniewski, M.; Teixidó, N.; Spadaro, D.; Jijakli, M.H. The science, development, and commercialization of postharvest biocontrol products. *Postharvest Biol. Technol.* **2016**, *122*, 22–29. [CrossRef]

16. Spadaro, D.; Droby, S. Development of biocontrol products for postharvest diseases of fruit: The importance of elucidating the mechanisms of action of yeast antagonists. *Trends Food Sci. Technol.* **2016**, *47*, 39–49. [CrossRef]

17. Liu, J.; Sui, Y.; Wisniewski, M.; Droby, S.; Liu, Y. Utilization of antagonistic yeasts to manage postharvest fungal diseases of fruit. *Int. J. Food Microbiol.* **2013**, *167*, 153–160. [CrossRef] [PubMed]

18. Cignola, R.; Zucchinali, S.; Firrao, G.; Di Francesco, A. Aspects of the biocontrol activity of *Aureobasidium* spp. strain against *Penicillium expansum* of apple. *Ann. Appl. Biol.* **2024**, *184*, 307–313. [CrossRef]

19. Usall, J.; Teixidó, N.; Fons, E.; Vinas, I. Biological control of blue mould on apple by a strain of *Candida sake* under several controlled atmosphere conditions. *Int. J. Food Microbiol.* **2000**, *58*, 83–92. [CrossRef]

20. Zhang, D.; Spadaro, D.; Garibaldi, A.; Gullino, M.L. Potential biocontrol activity of a strain of *Pichia guilliermondii* against grey mold of apples and its possible modes of action. *Biol. Control* **2011**, *57*, 193–201. [CrossRef]

21. Tulukoğlu-Kunt, K.S.; Özden, M.; Di Francesco, A. Exploring wild and local fruits as sources of promising biocontrol agents against *Alternaria* spp. in apples. *Horticulturae* **2023**, *9*, 1156. [CrossRef]

22. Mari, M.; Martini, C.; Spadoni, A.; Rouissi, W.; Bertolini, P. Biocontrol of apple postharvest decay by *Aureobasidium pullulans*. *Postharvest Biol. Technol.* **2012**, *73*, 56–62. [CrossRef]

23. Janisiewicz, W.; Kurtzman, C.; Buyer, J. Yeasts associated with nectarines and their potential for biological control of brown rot. *Yeast* **2010**, *27*, 389–398. [CrossRef] [PubMed]

24. Di Francesco, A.; Calassanzio, M.; Ratti, C.; Mari, M.; Folchi, A.; Baraldi, E. Molecular characterization of the two postharvest biological control agents *Aureobasidium pullulans* L1 and L8. *Biol. Control* **2018**, *123*, 53–59. [CrossRef]

25. Mari, M.; Martini, C.; Guidarelli, M.; Neri, F. Postharvest biocontrol of *Monilinia laxa*, *Monilinia fructicola* and *Monilinia fructigena* on stone fruit by two *Aureobasidium pullulans* strains. *Biol. Control* **2012**, *60*, 132–140. [CrossRef]

26. Zhang, D.; Spadaro, D.; Garibaldi, A.; Gullino, M.L. Selection and evaluation of new antagonists for their efficacy against postharvest brown rot of peaches. *Postharvest Biol. Technol.* **2010**, *55*, 174–181. [CrossRef]

27. Schena, L.; Nigro, F.; Pentimone, I.; Ligorio, A.; Ippolito, A. Control of postharvest rots of sweet cherries and table grapes with endophytic isolates of *Aureobasidium pullulans*. *Postharvest Biol. Technol.* **2003**, *30*, 209–220. [CrossRef]

28. EU Pesticides Database. Available online: https://food.ec.europa.eu/plants/pesticides/eu-pesticides-database_en (accessed on 28 June 2024).

29. Zhang, D.; Spadaro, D.; Garibaldi, A.; Gullino, M.L. Efficacy of the antagonist *Aureobasidium pullulans* PL5 against postharvest pathogens of peach, apple and plum and its modes of action. *Biol. Control* **2010**, *54*, 172–180. [CrossRef]

30. R Core Team. R: A Language and Environment for Statistical Computing. Available online: https://www.R-project.org/ (accessed on 13 June 2024).

31. Schiavon, G.; Garello, M.; Prencipe, S.; Meloni, G.R.; Buonsenso, F.; Spadaro, D. Essential oils reduce grey mould rot of apples and modify the fruit microbiome during postharvest storage. *J. Fungi* **2022**, *9*, 22. [CrossRef]

32. Naqib, A.; Poggi, S.; Wang, W.; Hyde, M.; Kunstman, K.; Green, S.J. Making and sequencing heavily multiplexed, high-throughput 16S ribosomal RNA gene amplicon libraries using a flexible, two-stage PCR protocol. In *Gene Expression Analysis: Methods and Protocols*; Humana Press: New York, NY, USA, 2018; pp. 149–169.

33. Abdelfattah, A.; Freilich, S.; Bartuv, R.; Zhimo, V.Y.; Kumar, A.; Biasi, A.; Salim, S.; Feygenberg, O.; Burchard, E.; Dardick, C. Global analysis of the apple fruit microbiome: Are all apples the same? *Environ. Microbiol.* **2021**, *23*, 6038–6055. [CrossRef]

34. Bolyen, E.; Rideout, J.R.; Dillon, M.R.; Bokulich, N.A.; Abnet, C.C.; Al-Ghalith, G.A.; Alexander, H.; Alm, E.J.; Arumugam, M.; Asnicar, F.; et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. *Nat. Biotechnol.* **2019**, *37*, 852–857. [CrossRef]

35. Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J.* **2011**, *17*, 10–12. [CrossRef]

36. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.; Holmes, S.P. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **2016**, *13*, 581–583. [CrossRef] [PubMed]

37. Abarenkov, K.; Zirk, A.; Piirmann, T.; Pöhönen, R.; Ivanov, F.; Nilsson, R.H.; Kõljalg, U. UNITE QIIME Release for Eukaryotes 2. Version 10.05.2021. Available online: https://doi.org/10.15156/BIO/1264861 (accessed on 20 June 2024).

38. Beule, L.; Karlovsky, P. Improved normalization of species count data in ecology by scaling with ranked subsampling (SRS): Application to microbial communities. *Peer J.* **2020**, *8*, e9593. [CrossRef] [PubMed]

39. Anderson, M.J. A new method for non-parametric multivariate analysis of variance. *Austral Ecol.* **2001**, *26*, 32–46.

40. Oksanen, J.; Simpson, G.L.; Guillaume Blanchet, F.; Kindt, R.; Legendre, P.; Minchin, P.R.; O'Hara, R.B.; Solymos, P.; Stevens, M.H.H.; Szoecs, E.; et al. Vegan: Community Ecology Package. R Package Version 2.5–3. 2018. Available online: https://CRAN.R-project.org/package=vegan (accessed on 20 June 2024).

41. Anderson, M.J. Distance-based tests for homogeneity of multivariate dispersions. *Biometrics* **2006**, *62*, 245–253. [CrossRef] [PubMed]

42. Halko, N.; Martinsson, P.-G.; Shkolnisky, Y.; Tygert, M. An algorithm for the principal component analysis of large data sets. *SIAM J. Sci. Comput.* **2011**, *33*, 2580–2594. [CrossRef]

43. Wisniewski, M.; Droby, S.; Norelli, J.; Liu, J.; Schena, L. Alternative management technologies for postharvest disease control: The journey from simplicity to complexity. *Postharvest Biol. Technol.* **2016**, *122*, 3–10. [CrossRef]

44. Ippolito, A.; El Ghaouth, A.; Wilson, C.L.; Wisniewski, M. Control of postharvest decay of apple fruit by *Aureobasidium pullulans* and induction of defense responses. *Postharvest Biol. Technol.* **2000**, *19*, 265–272. [CrossRef]

45. Schena, L.; Ippolito, A.; Zahavi, T.; Cohen, L.; Nigro, F.; Droby, S. Genetic diversity and biocontrol activity of *Aureobasidium pullulans* isolates against postharvest rots. *Postharvest Biol. Technol.* **1999**, *17*, 189–199. [CrossRef]

46. Bencheqroun, S.K.; Bajji, M.; Massart, S.; Labhilili, M.; El Jaafari, S.; Jijakli, M.H. *In vitro* and *in situ* study of postharvest apple blue mold biocontrol by *Aureobasidium pullulans*: Evidence for the involvement of competition for nutrients. *Postharvest Biol. Technol.* **2007**, *46*, 128–135. [CrossRef]

47. Di Francesco, A.; Ugolini, L.; D'Aquino, S.; Pagnotta, E.; Mari, M. Biocontrol of *Monilinia laxa* by *Aureobasidium pullulans* strains: Insights on competition for nutrients and space. *Int. J. Food Microbiol.* **2017**, *248*, 32–38. [CrossRef]

48. Vero, S.; Garmendia, G.; González, M.B.; Garat, M.F.; Wisniewski, M. *Aureobasidium pullulans* as a biocontrol agent of postharvest pathogens of apples in Uruguay. *Biocontrol Sci. Technol.* **2009**, *19*, 1033–1049. [CrossRef]

49. Zajc, J.; Cernosa, A.; Di Francesco, A.; Casteria, R.; De Curtis, F.; Lima, G.; Badri, H.; Jijakli, H.; Ippolito, A.; Gostincar, C. Characterization of *Aureobasidium pullulans* isolates selected as biocontrol agents against fruit decay pathogens. *Fungal Genom. Biol.* **2020**, *10*, 163.

50. Liu, X.; Wang, J.; Gou, P.; Mao, C.; Zhu, Z.-R.; Li, H. In vitro inhibition of postharvest pathogens of fruit and control of gray mold of strawberry and green mold of citrus by aureobasidin A. *Int. J. Food Microbiol.* **2007**, *119*, 223–229. [CrossRef]

51. Di Francesco, A.; Di Foggia, M.; Corbetta, M.; Baldo, D.; Ratti, C.; Baraldi, E. Biocontrol activity and plant growth promotion exerted by *Aureobasidium pullulans* strains. *J. Plant Growth Regul.* **2021**, *40*, 1233–1244. [CrossRef]

52. Castoria, R.; De Curtis, F.; Lima, G.; Caputo, L.; Pacifico, S.; De Cicco, V. *Aureobasidium pullulans* (LS-30) an antagonist of postharvest pathogens of fruits: Study on its modes of action. *Postharvest Biol. Technol.* **2001**, *22*, 7–17. [CrossRef]

53. Banani, H.; Spadaro, D.; Zhang, D.; Matic, S.; Garibaldi, A.; Gullino, M.L. Biocontrol activity of an alkaline serine protease from *Aureobasidium pullulans* expressed in *Pichia pastoris* against four postharvest pathogens on apple. *Int. J. Food Microbiol.* **2014**, *182*, 1–8. [CrossRef]

54. Di Francesco, A.; Ugolini, L.; Lazzeri, L.; Mari, M. Production of volatile organic compounds by *Aureobasidium pullulans* as a potential mechanism of action against postharvest fruit pathogens. *Biol. Control* **2015**, *81*, 8–14. [CrossRef]

55. Zhang, D.; Spadaro, D.; Valente, S.; Garibaldi, A.; Gullino, M.L. Cloning, characterization, expression and antifungal activity of an alkaline serine protease of *Aureobasidium pullulans* PL5 involved in the biological control of postharvest pathogens. *Int. J. Food Microbiol.* **2012**, *153*, 453–464. [CrossRef]

56. Kurtzman, C.P.; Droby, S. *Metschnikowia fructicola*, a new ascosporic yeast with potential for biocontrol of postharvest fruit rots. *Syst. Appl. Microbiol.* **2001**, *24*, 395–399. [CrossRef]

57. Karabulut, O.; Tezcan, H.; Daus, A.; Cohen, L.; Wiess, B.; Droby, S. Control of preharvest and postharvest fruit rot in strawberry by *Metschnikowia fructicola*. *Biocontrol Sci. Technol.* **2004**, *14*, 513–521. [CrossRef]

58. Spadaro, D.; Lorè, A.; Garibaldi, A.; Gullino, M.L. A new strain of *Metschnikowia fructicola* for postharvest control of *Penicillium expansum* and patulin accumulation on four cultivars of apple. *Postharvest Biol. Technol.* **2013**, *75*, 1–8. [CrossRef]

59. Piombo, E.; Sela, N.; Wisniewski, M.; Hoffmann, M.; Gullino, M.L.; Allard, M.W.; Levin, E.; Spadaro, D.; Droby, S. Genome sequence, assembly and characterization of two *Metschnikowia fructicola* strains used as biocontrol agents of postharvest diseases. *Front. Microbiol.* **2018**, *9*, 593. [CrossRef]

60. Macarisin, D.; Droby, S.; Bauchan, G.; Wisniewski, M. Superoxide anion and hydrogen peroxide in the yeast antagonist–fruit interaction: A new role for reactive oxygen species in postharvest biocontrol? *Postharvest Biol. Technol.* **2010**, *58*, 194–202. [CrossRef]

61. Banani, H.; Spadaro, D.; Zhang, D.; Matic, S.; Garibaldi, A.; Gullino, M.L. Postharvest application of a novel chitinase cloned from *Metschnikowia fructicola* and overexpressed in *Pichia pastoris* to control brown rot of peaches. *Int. J. Food Microbiol.* **2015**, *199*, 54–61. [CrossRef]

62. Angeli, D.; Turrini, L.; Zeni, F.; Longa, C.; Gualandri, V.; Roman, T. Controlling white haze disease under in vitro controlled conditions. In Proceedings of the VI International Symposium on Postharvest Pathology: Innovation and Advanced Technologies for Managing Postharvest Pathogens 1363, Limassol, Cyprus, 29 May–2 June 2022; pp. 95–100.

63. Rajapakse, N.C.; Banks, N.H.; Hewett, E.W.; Cleland, D.J. Development of oxygen concentration gradients in flesh tissues of bulky plant organs. *J. Am. Soc. Hortic. Sci.* **1990**, *115*, 793–797. [CrossRef]

64. Butkeviciute, A.; Viskelis, J.; Viskelis, P.; Liaudanskas, M.; Janulis, V. Changes in the biochemical composition and physicochemical properties of apples stored in controlled atmosphere conditions. *Appl. Sci.* **2021**, *11*, 6215. [CrossRef]

65. Olimi, E.; Kusstatscher, P.; Wicaksono, W.A.; Abdelfattah, A.; Cernava, T.; Berg, G. Insights into the microbiome assembly during different growth stages and storage of strawberry plants. *Environ. Microbiome* **2022**, *17*, 21. [CrossRef] [PubMed]

66. Wang, J.; Shi, C.; Fang, D.; Che, J.; Wu, W.; Lyu, L.; Li, W. The impact of storage temperature on the development of microbial communities on the surface of blueberry fruit. *Foods* **2023**, *12*, 1611. [CrossRef]

67. Zhimo, V.Y.; Kumar, A.; Biasi, A.; Abdelfattah, A.; Sharma, V.K.; Salim, S.; Feygenberg, O.; Bartuv, R.; Freilich, S.; Whitehead, S.R. Assembly and dynamics of the apple carposphere microbiome during fruit development and storage. *Front. Microbiol.* **2022**, *13*, 928888. [CrossRef]

68. Biasi, A.; Zhimo, V.Y.; Kumar, A.; Abdelfattah, A.; Salim, S.; Feygenberg, O.; Wisniewski, M.; Droby, S. Changes in the fungal community assembly of apple fruit following postharvest application of the yeast biocontrol agent *Metschnikowia fructicola*. *Horticulturae* **2021**, *7*, 360. [CrossRef]

69. Zhao, Q.; Shi, Y.; Ngea, G.L.N.; Zhang, X.; Yang, Q.; Zhang, Q.; Xu, X.; Zhang, H. Changes of the microbial community in kiwifruit during storage after postharvest application of *Wickerhamomyces anomalus*. *Food Chem.* **2023**, *404*, 134593. [CrossRef] [PubMed]

70. Ebrahimi, L.; Hatami Rad, S.; Etebarian, H.R. Apple endophytic fungi and their antagonism against apple scab disease. *Front. Microbiol.* **2022**, *13*, 1024001. [CrossRef] [PubMed]

71. Van Kan, J.A.; Shaw, M.W.; Grant-Downton, R.T. *Botrytis* species: Relentless necrotrophic thugs or endophytes gone rogue? *Mol. Plant Pathol.* **2014**, *15*, 957–961. [CrossRef] [PubMed]