

Article

Reducing Postharvest Losses in Organic Apples: The Role of Yeast Consortia Against *Botrytis cinerea*

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Abstract: Grey mould caused by *Botrytis cinerea* presents significant challenges to apple production including organic farming. Biocontrol yeasts and their consortia can limit fungal diseases. This study evaluates the efficacy of selected yeast isolates and their consortia in suppressing *B. cinerea* in stored apples. The yeast strains tested—*Wickerhamomyces anomalus* 114/73, *Naganishia albidosimilis* 117/10, and *Sporobolomyces roseus* 117/67—were assessed at 4 °C and 23 °C, individually and in consortia. The results demonstrate the superior efficacy of a consortium combining all three isolates, which achieved the highest reduction in spore germination and disease severity. A two-strain consortium of isolates 114/73 and 117/10 also showed substantial biocontrol activity, outperforming single-strain treatments. These combinations effectively suppressed *B. cinerea* growth and displayed rapid colonization of apple wounds. The study highlights the potential of yeast isolates and their consortia to manage postharvest fungal decay, addressing a critical need for sustainable, eco-friendly solutions in organic apple production.

Keywords: yeast; biocontrol; consortia; organic farming; apple; storage; *Botrytis cinerea*; grey mould



Academic Editor: Filippo De Curtis

Received: 16 January 2025

Revised: 27 February 2025

Accepted: 10 March 2025

Published: 11 March 2025

Citation: Krzysińska, J.; Kowalska, J.

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Organic Apples: The Role of Yeast

Consortia Against *Botrytis cinerea*.*Agriculture* **2025**, *15*, 602.[https://doi.org/10.3390/](https://doi.org/10.3390/agriculture15060602)[agriculture15060602](https://doi.org/10.3390/agriculture15060602)**Copyright:** © 2025 by the authors.

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1. Introduction

Agriculture and food storage have been among the most essential practices for humanity to meet global food demands and sustain communities worldwide. Conventional methods, while successfully protecting post-harvest crops, heavily rely on chemical pesticides that can pose health risks to humans through chemical residues. In response to these issues, biocontrol methods including the use of beneficial microorganisms offer a viable alternative for managing plant diseases and can ensure proper food storage while minimizing losses.

For organic production, the range of plant protection products is more restricted than for conventional produce and a defined set of rules must be followed (i.e., Regulation 2021/1165 in the European Union or National Organic Program in the United States). Other protection strategies that are alternative to chemical protection are essential for maintaining plant and yield health. Maintaining high yields and quality is a major challenge in organic production. An additional difficulty is the problem of storage in warehouses, which is why methods are sought to extend the durability of crops, e.g., fruit. Decay in stored apples can cause losses of up to 80% [1]. It mostly results from preharvest pathogens infection which further develops during storage causing the loss in apple quality and its marketable value [2]. One of the diseases being a particular problem in post-harvest storage of apples is grey mould caused by *Botrytis cinerea* Pers. [3,4]. This destructive pathogen causes losses to hundreds of crop species worldwide, mostly attacking plants' soft tissues such as fruits and

flowers [5]. It is considered one of the most economically important plant pathogens [6]. By synthesizing a large number of toxins and enzymes, it infects a broad spectrum of host plants and can also overcome the plant defences [7].

Solutions such as essential oils and basic substances were described, for example, lavender essential oil [8], clove and thyme essential oils combined with chitosan [9], chitosan-based edible coatings containing ascorbic or acetic acid, and sea buckthorn or grape seed essential oils [10], cinnamon essential oil [11], or sodium bicarbonate [12,13]. Another solution to limit the development of fungal plant pathogens is to use biological control by applying microorganisms or their metabolic products. Biocontrol yeasts are a recognized group of microorganisms limiting fungal diseases by such mechanisms as competing for space and nutrients, lytic enzymes and antifungal metabolites production, biofilm formation, or the involvement in oxidative stress [14–16]. Some commonly studied yeast genera showing potential in controlling postharvest pathogens include *Saccharomyces* [17,18] and among non-*Saccharomyces* yeast *Candida* [19–21], *Pichia* [22–24], *Aureobasidium* [25,26] or *Metschnikowia* [27–29].

The growing interest in biocontrol solutions has led to an increase in studies exploring the use of yeast isolates to manage postharvest fungal decay. As the need for safer and more sustainable alternatives to chemical fungicides continues to rise, research into novel formulations with biocontrol organisms becomes necessary. For organic apple producers, finding effective, eco-friendly methods to reduce postharvest losses is crucial, as they must adhere to strict guidelines that prohibit synthetic fungicide use. Microorganisms' antagonistic expression, reaching threshold population, or strain establishment can be limited by various biotic and abiotic factors [30,31] and treatment with a single strain may not be efficient. Moreover, not all microorganisms (specific species or even strains) are effective against all plant pathogens. Combining two or multiple biocontrol agents may potentially enhance their efficacy compared to single-strain applications due to various mechanisms of specific organisms, their synergistic interactions, and various resistance to biotic and abiotic stress. Microbial consortia can further reduce the disease severity compared to single microorganisms or even being able to suppress it when single strains are ineffective [32].

The objective of this study was to evaluate the effectiveness of yeast isolates used alone or/and as consortia in controlling *B. cinerea* in apples during storage.

2. Materials and Methods

2.1. Yeast Isolates and Consortia

Three selected yeast isolates—114/73 (*Wickerhamomyces anomalus* E.C. Hansen of the genus *Wickerhamomyces* in the family *Wickerhamomycetaceae*), 117/10 (*Naganishia albidosimilis* Vishniac and Kurtzman of the genus *Naganishia* in the family *Filobasidiaceae*), and 117/67 (*Sporobolomyces roseus* Kluyver and C.B. Nielwere of the genus *Sporobolomyces* in the family *Sporidiobolaceae*) isolated from leaves of Virginia mallow (*Sida hermaphrodita* (L.) Rusby) plantation in Winna Góra, Poland (52°12'21"N 17°26'49"E), identified using API® ID 32 C yeast identification system (bioMérieux, Marcy-l'Étoile, France) and positively assessed for antagonistic abilities against *B. cinerea* in vitro [33]. These yeast isolates were freeze-dried and stored at room temperature and reactivated in 2024 to conduct the experiment. To activate them they were rehydrated by adding 1 mL of 0.9% NaCl, after 30 min pouring the suspension into Petri dishes containing solidified PDA (Potato Dextrose Agar, Sigma Chemical Co, St. Louis, MO, USA) and incubating at 23 °C. Isolates were subcultured twice and centrifuged to collect yeast cells, which were suspended in sterile saline solution. Cell concentrations were determined using a hemocytometer and adjusted as needed.

To prepare yeast consortia with different yeast isolates, isolates were grown separately on PDA medium (pH 6.5) at 23 °C for three days. Then, yeast cells were flooded with sterile 0.9% NaCl, adjusted to 2×10^7 CFU/mL (density chosen based on preliminary in vitro tests) with a hemacytometer, and mixed in the 1:1 or 1:1:1 ratio. Each pair or group of yeast strains was checked in an in vitro assay for compatibility.

2.2. Selected Apple Pathogen Inoculum

A *B. cinerea* strain obtained from the IPP-NR, Poznań, Poland Bank of Pathogens collection (2305) (where it was identified) isolated from infected apples was used. For conidial production, it was cultured on PDA (pH 6.5) at 20–25 °C until the mycelium appeared, and then at 15 °C to induce sporulation. Two-week-old PDA cultures were flooded with sterile distilled water containing 0.1% (*v/v*) Tween 80 to obtain a spore suspension. The concentration of spores was determined with a hemacytometer (Thoma counting chamber, Hirschmann, Eberstadt, Germany) and adjusted with sterile distilled water to 10^6 spores mL^{−1}.

2.3. Fruit

Organic apples (*Malus domestica* Borkh, cv Jonatan) at commercial maturity, without mechanical damage or infection, and uniform in size and colour were used. Apples were stored at 4 °C. To disinfect the surface, apples were first immersed in 0.1% sodium hypochlorite (as a standard disinfection method) for two minutes, then washed in running tap water, and finally air-dried at room temperature. Apples were uniformly wounded with sterile corkborer (diameter 5 mm, depth 5 mm).

2.4. Wound Colonization by Yeast Isolates

Each wound on apples (fruit wounded as described above) was inoculated with 15 µL of yeast isolate suspension (1×10^7 cells mL^{−1}). Apples were incubated at 4 °C and 23 °C. Yeast cells were recovered 1 h after treatment and then every 24 h for fourteen days after incubation at 4 °C and for seven days after incubation at 23 °C. Fruit tissue was removed with a sterile corkborer (5 mm diameter) to a depth of 10 mm, macerated in 5 mL of sterile 0.9% NaCl solution with a glass rod, diluted and plated on PDA medium. Plates were incubated at 23 °C for two days and colonies were counted. Yeast population was expressed as mean log₁₀ CFU (colony forming unit) per wound. There were three replicates of each treatment, and the experiment was repeated twice, so the results are the means of both experiments.

2.5. Effect of Selected Yeast Isolates and Consortia on the Rate of Spore Germination of *B. cinerea* In Vitro

The *B. cinerea* spore suspension (adjusted to 1×10^6 spores mL^{−1}) was inoculated into 50 mL of potato dextrose broth (PDB, A&A Biotechnology, Gdańsk, Poland). Then, 1 mL of yeast isolate or yeast consortium suspension (2×10^7 cells mL^{−1}) or sterile 0.9% NaCl (control) was added. After 24 h of incubation at 28 °C on a rotary shaker (75 rpm) in 100 mL conical flasks, the germination rate of 100 spores of *B. cinerea* was observed with a microscope. The test was conducted in two independent experiments in three repetitions, and the results of the two experiments were used as six replications.

2.6. Efficacy of Yeast Isolates and Consortia in Inhibiting Grey Mould Decay of Apples at 4 °C and at 23 °C

Apples were wounded as described above. Each wound was inoculated with 15 µL of yeast isolate suspension (1×10^7 cells mL^{−1}, 2×10^7 cells mL^{−1}, 4×10^7 cells mL^{−1}), yeast consortium suspension (2×10^7 cells mL^{−1}) or sterile 0.9% NaCl (control). After 24 h,

apples were inoculated with 20 μL of a conidial suspension of *B. cinerea* at 10^6 spores mL^{-1} . Fruits were stored at 4 °C (chosen as a cold storage temperature) or at 23 °C (chosen as room temperature for storage) in enclosed plastic trays. Apples were observed after 7, 14, and 21 days of storage for cold storage or after 7, 10, and 14 days of room temperature storage, and lesion diameter was measured. The mean of 10 apples as samplings and the mean of five (cold storage) or three (room temperature storage) repetitions (with errors) were used.

2.7. Statistical Analysis

All data were tested for normality and homoscedasticity (Shapiro–Wilk normality test, Levene’s Test). Data were subjected to a two-way analysis of variance (ANOVA) and a post hoc Fisher test at the significance level of $p \leq 0.05$ and are presented as mean $\pm$ standard deviation (SD).

3. Results

3.1. Wound Colonization by Yeast Isolates

All three yeast isolates (14/73, 117/10, and 117/67) grew rapidly in apple wounds at 4 °C and 23° days, especially for the first 24 h at 4 °C and 48 h at 23 °C. At 4 °C the population of isolates 114/73 and 117/10 reached its maximum after 12 days (1.57×10^6 and 1.84×10^6 CFU per wound, respectively) and decreased, but remained over 1.5×10^6 CFU per wound for the next two days. The population of isolate 117/67 reached its maximum on day 11 (1.18×10^6 CFU per wound) and decreased, but maintained over 1×10^6 CFU per wound for the following three days (Figure 1).

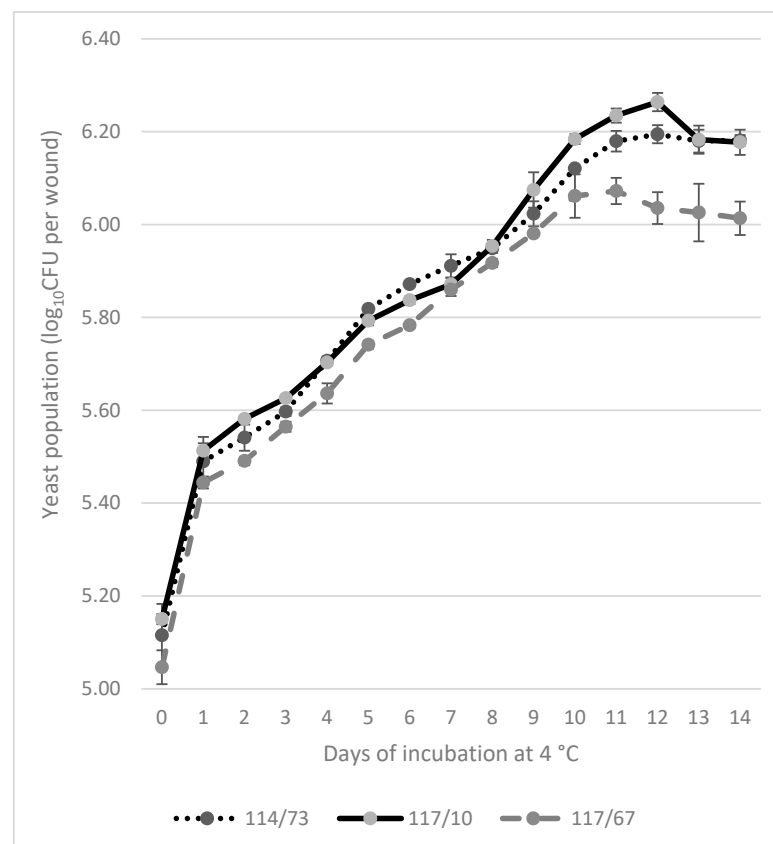


Figure 1. Dynamics of yeast population (isolates 114/73, 117/10, and 117/67) colonization in wounds (diameter 5 mm, depth 5 mm) in apples at 4 °C from 0 days (1 h after inoculation) to 14 days post-inoculation of the yeast. Yeast population expressed by log₁₀ CFU per wound.

At 23 °C, after the rapid growth during the first 48 h, the population of each yeast isolate stabilized. The population of the isolate reached its maximum on day 7 (2.98×10^7 CFU per wound). The population of isolate 117/10 reached its peak on day 3 (3.15×10^7 CFU per wound) and of isolate 116/67, which grew at the slowest rate, on day 7 (2.93×10^7 CFU per wound) (Figure 2).

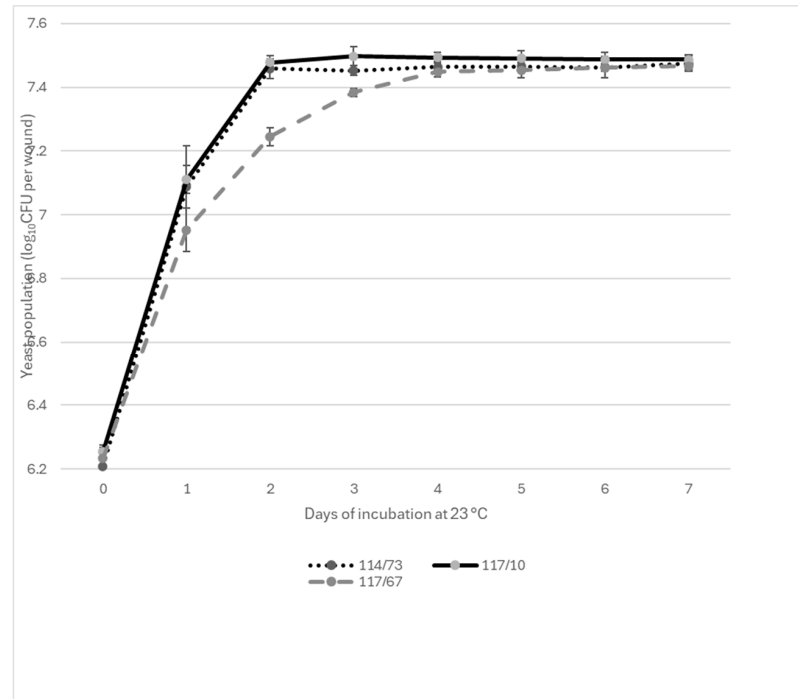


Figure 2. Dynamics of yeast population (isolates 114/73, 117/10, and 117/67) colonization in wounds (diameter 5 mm, depth 5 mm) in apples at 23 °C from 0 days (1 h after inoculation) to 7 days post-inoculation of the yeast. Yeast population expressed by log₁₀ CFU per wound.

3.2. Effect of Yeast Isolates and Consortia on the Rate of Spore Germination of *B. cinerea* In Vitro

After 24 h, the spore germination rate was significantly reduced by all combinations except the isolate 117/67. The combinations with yeast consortia consisting of isolates 114/73 and 117/10 and all three isolates spore germination dropped the most (to 4.17% and 1.83%, respectively) (Figure 3).

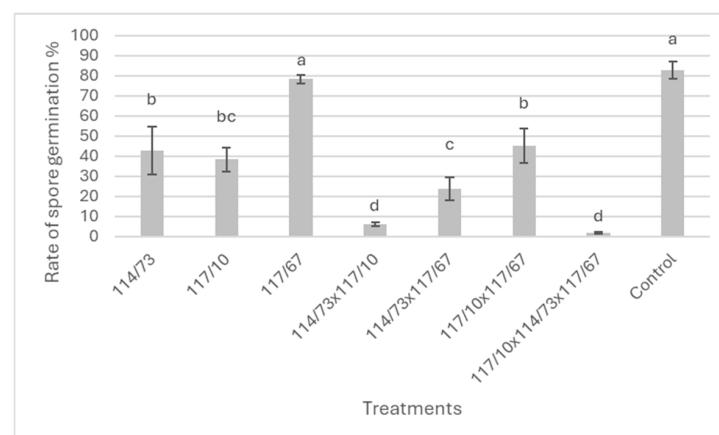


Figure 3. Effect of yeast isolates and yeast consortia at a concentration of 2×10^7 CFU/mL on the rate of spore germination (%) of *B. cinerea* in vitro measured by microscope after 24 h of incubation at 28 °C, 75 rpm in PDB. Means with different letters have a significant difference at $p < 0.05$ based on Fisher LSD tests. Control treated with sterilized normal saline.

3.3. Efficacy of Yeast Isolates and Consortia in Inhibiting Grey Mould Decay of Apples at 4 °C

All three yeast isolates were effective in controlling decay caused by *B. cinerea* in all tested concentrations, by causing a reduction in the mean diameter of lesions, compared to the untreated control. The higher the concentration of yeast isolates, the lower the diameter of grey mould lesions. In particular, the isolate 117/10 at the concentrations 2×10^7 CFU/mL and 4×10^7 CFU/mL showed the highest control (mean lesion diameter 6.90 and 3.09 mm, respectively) (Table 1). For isolates, 114/73 and 117/10 mean lesion size was statistically the same when concentrations 2×10^7 CFU/mL and 4×10^7 CFU/mL were used and, for the isolate 117/67, using concentrations 1×10^7 CFU/mL and 2×10^7 CFU/mL caused statistically same level of decay control throughout the whole experiment (Figure 4). Fruit treated with the isolate 117/10 in concentrations 2×10^7 CFU/mL and 4×10^7 CFU/mL showed symptoms after 21 days (and some treated fruit did not have any symptoms till the end of the experiment on the 28th day), while among control fruit and other treatments, lesions were visible after seven days of storage.

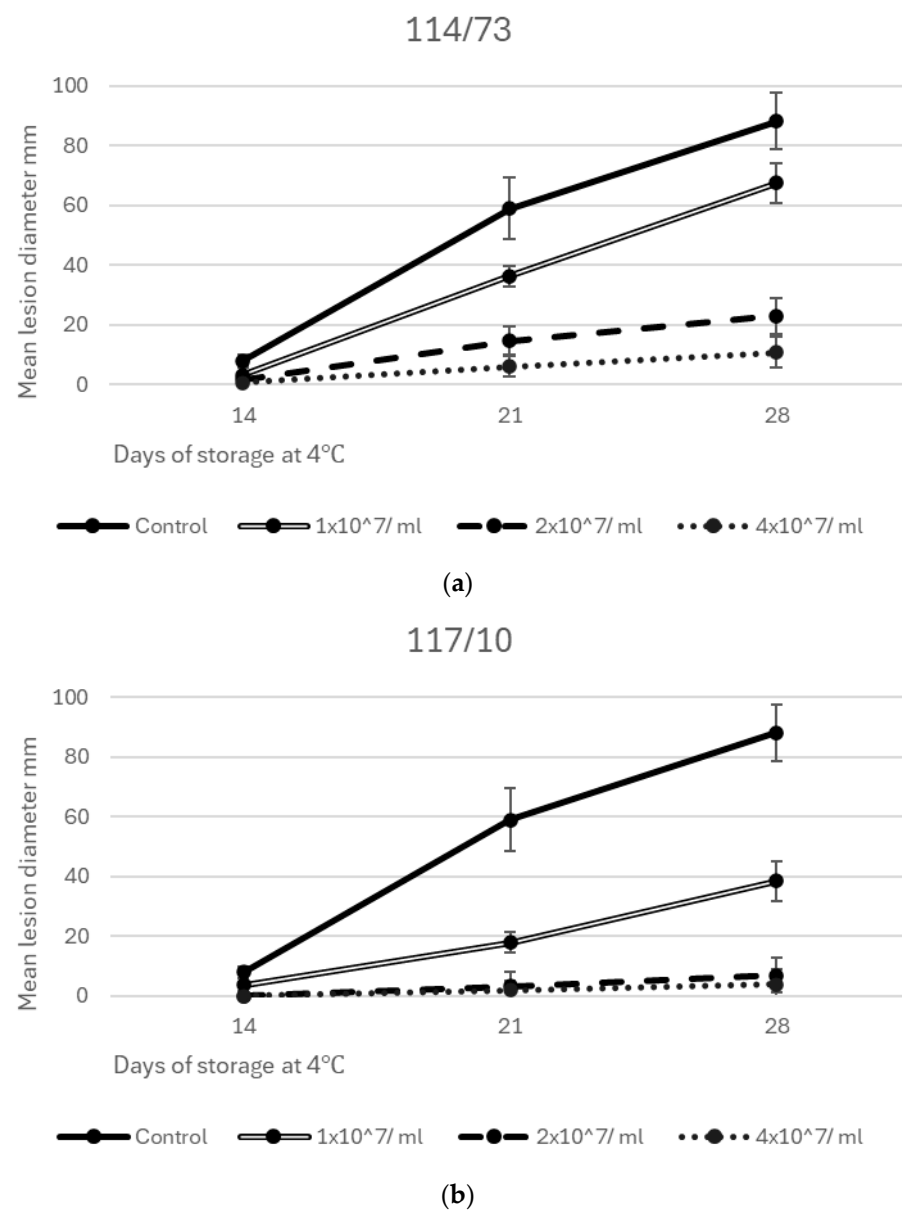


Figure 4. Cont.

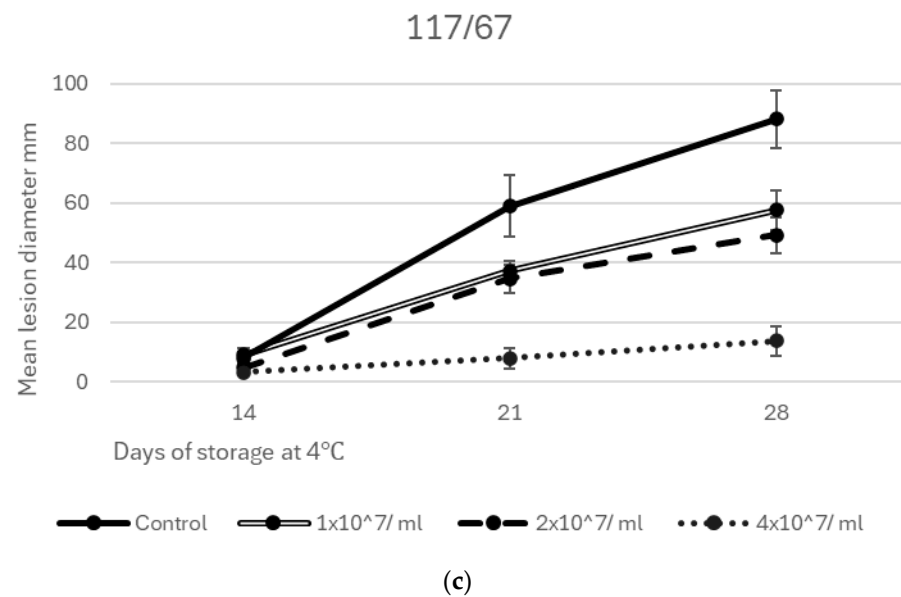


Figure 4. Growth dynamics of *B. cinerea* lesions growth on apples stored at 4 °C and treated with yeast water suspension; (a) isolate 114/73, (b) isolate 117/10, (c) isolate 117/67 at concentration 1×10^7 CFU/mL, 2×10^7 CFU/mL, 4×10^7 CFU/mL.

Table 1. Efficacy of yeast isolates applied as water suspension treatment in inhibiting decay caused by *B. cinerea* in apples stored at 4 °C for 4 weeks. Mean diameters of grey mould lesions (mm). Control treated with sterilized normal saline.

Yeast Concentration	Yeast Isolate			Control
	114/73	117/10	117/67	
1×10^7 CFU/mL	67.50 ± 6.58 b *	38.40 ± 6.41 de	57.60 ± 4.46 bc	88.10 ± 9.52 a
2×10^7 CFU/mL	23.00 ± 5.95 ef	6.90 ± 1.22 g	49.20 ± 7.82 cd	
4×10^7 CFU/mL	10.80 ± 4.98 fg	3.90 ± 3.40 g	13.70 ± 2.72 fg	

* Data expressed as mean $\pm$ standard error. Means with different letters have a significant difference at $p < 0.05$ based on Fisher LSD tests.

All yeast consortia were effectively reducing mean diameters of grey mould lesions at 4 °C. After four weeks combined isolates 114/73 and 117/10 and all three isolates were the most effective (Table 2).

Table 2. Efficacy of yeast consortia (at concentration 2×10^7 CFU/mL) applied as water suspension treatment in inhibiting decay caused by *B. cinerea* in apples stored at 4 °C for 2–4 weeks. Mean diameters of grey mould lesions (mm). Control treated with sterilized normal saline.

Combination	Day		
	14	21	28
114/73 $\times$ 117/10	0.00 ± 0.0 b	1.60 ± 0.58 c	1.90 ± 0.92 c
114/73 $\times$ 117/67	1.20 ± 0.68 b	14.50 ± 2.76 b	22.00 ± 3.59 b
117/10 $\times$ 117/67	1.00 ± 0.89 b	11.40 ± 3.61 bc	20.30 ± 2.36 b
117/10 $\times$ 114/73 $\times$ 117/67	0.00 ± 0.0 b	0.50 ± 0.45 c	1.20 ± 0.51 c
Control	7.90 ± 2.15 a	58.90 ± 11.85 a	88.10 ± 9.52 a

Data expressed as mean $\pm$ standard error. This means that in columns with different letters, there is a significant difference at $p < 0.05$ based on Fisher LSD tests.

3.4. Efficacy of Yeast Isolates and Consortia in Inhibiting Grey Mould Decay of Apples at 23 °C

All combinations of yeast isolates and yeast consortia at the concentration 2×10^7 CFU/mL effectively reduced mean diameters of grey mould lesions at 23 °C after

two weeks of the experiment, compared to the untreated control, except for the isolate 177/67 which significantly reduced the lesions just for the first ten days. In particular, the combination of all three yeast isolates significantly reduced the lesion diameters throughout the experiment (from 94.00 mm for the control to 23.50 mm for the treatment on day 14); however, even if there was a trend towards the beneficial effect of using a consortium, it was not more effective than the isolate 117/10 on its own. The combination of 114/73 and 117/10 was more effective than individually used isolate 114/73, but not 117/10. Combined isolates 114/73 and 1116/67 and 117/10 and 117/67 were no more effective than those isolates used on their own (Table 3).

Table 3. Efficacy of yeast isolates water suspension treatment (2×10^7 CFU/mL) in inhibiting decay caused by *B. cinerea* in apples stored at 23 °C for 2 weeks. Yeast isolates were used separately and as consortia. Mean diameters of grey mould lesions (mm). Control treated with sterilized normal saline.

Combination	Day		
	7	10	14
114/73	20.00 ± 3.61 c	31.17 ± 6.54 bc	61.17 ± 4.37 b
117/10	21.83 ± 6.15 c	32.33 ± 11.07 bc	48.17 ± 21.22 bcd
117/67	37.17 ± 6.64 b	51.00 ± 7.33 b	71.17 ± 5.78 ab
114/73 × 117/10	20.17 ± 2.13 c	23.67 ± 0.27 bc	28.67 ± 3.28 cd
114/73 × 117/67	36.67 ± 1.16 b	46.00 ± 4.23 b	52.83 ± 2.79 bc
117/10 × 117/67	31.67 ± 5.33 b	47.67 ± 7.01 b	55.67 ± 2.37 b
117/10 × 114/73 × 117/67	17.50 ± 4.99 c	20.67 ± 4.81 c	23.50 ± 4.89 d
Control	55.83 ± 5.47 a	81.67 ± 5.33 a	94.00 ± 3.61 a

Data expressed as mean ± standard error. This means that in columns with different letters, there is a significant difference at $p < 0.05$ based on Fisher LSD tests.

4. Discussion

B. cinerea is known to affect a wide range of horticultural plants and is particularly harmful to fruit crops postharvest, such as apples, strawberries, grapes, or blueberries [34]. It infects apples both during growth in the orchard and after harvest, leading to a decrease in the crop's quantity and quality [35–38]. Many microorganisms were reported in control *B. cinerea*, including antagonistic yeast, which demonstrated significant inhibition of *B. cinerea* strains on apples, both in vitro and in vivo. For example, *Pichia kluyveri* Bedford Y64 achieved 57% growth inhibition against *B. cinerea* achieving slightly better results than a commercial fungicide [39]. In another experiment [40], yeast isolated from apple surface (isolates YP16, YP24, YP25) reduced grey mould incidence on apples to 8.3% in comparison to 100% incidence in control. In our study, most yeast isolates and their consortia were effectively reducing grey mould infection in apples at both 4 °C and 23 °C. Commercial yeast-based products are readily available in the market, including strains specifically registered for mitigating the effects of *B. cinerea* on plants and stored fruit. Examples of such yeast species, products, and countries they are registered in include *Candida oleophila* (Aspire® registered in the United States and Israel, Nexy® registered in Belgium), *Aureobasidium pullulans* (Botector® registered in Poland and New Zealand), or *Candida sake* (Candifruit® registered in Spain).

In this study, all three yeast isolates, i.e., 114/73 (*W. anomalus*), 117/10 (*N. albidosimilis*) and 117/67 (*S. roseus*) rapidly colonized wounds of the apples maintained a stable population for 7 days at 23 °C and for 14 days at 4 °C. The populations could be stable for a wider interval than the duration of the experiments. At 4 °C all isolates reached their maximum after 11–12 days and their population slightly decreased after that time. At 23 °C, the population of isolate 117/10 reached its maximum the fastest (on day 3) and isolate 117/67 the slowest (on day 7). Being able to compete for space and nutrients by quickly

adapting to the surface environment is one of the main mechanisms of antagonistic yeast. Similarly, a study by Zhao et al. [41] showed that *W. anomalus* strain CCTCC M 2,018,053 maintained stable growth both on wounded apples and on the apple surface. *W. anomalus* was also able to colonize other fruit surfaces and wounds like peach, [42], kiwi [43], and cherry tomato [44,45]. Those yeasts grow quickly and thus can compete with pathogens for space and nutrition [42]. Yeast belonging to the genus *Naganishia* is also known for being able to colonize the yeast surface and wounds. For example, *Nagashinia albida* (Saito) (synonym *Cryptococcus albidus*) was found to be able to colonize such fruits as apples [46], strawberries [47] or grapes [48]. *S. roseus* was also found to colonize apples [49,50], sour and sweet cherries [51], or peaches [52].

In comparison to individual cultures, microbial consortia can exhibit broader effectiveness in disease suppression. Multiple yeast strains within the consortia or beneficial yeast and filamentous fungi or bacteria, which have various modes of action and growth characteristics, can perform specific functions and the biocontrol effect can be enhanced [53–55], providing that they do not compete against each other and are compatible [56–58]. Mechanisms such as competition for nutrients, secreting volatile and non-volatile inhibitory compounds, activation of the plant's defence systems, and inhibiting germination and conidial damage to pathogenic fungi can augment each other and result in increased disease suppression [59,60]. These consortia are also more stable under changing environmental conditions [61,62].

In this study, competition for space and inhibiting spore germination were determined to occur as such mechanisms. The isolate 117/10 exhibited the fastest growth rate and was as efficient against *Botrytis* decay on its own as a part of a consortium. The ability to compete for space is a frequent mechanism occurring in biocontrol yeast. Species such as *Meyerozyma guilliermondii* and *M. caribbica* [62], *Aureobasidium pullulans*, *Rhodotorula minuta* [63–66] or *Bullera alba* [67] were reported as such in recent publications, including yeast mixtures [58]. It is considered one of the primary mechanisms of antagonistic yeast action together with competing for nutrients [68]. Yeasts display a high rate of reproduction and can colonize plant surfaces quickly, especially in wounded areas [69,70]. Furthermore, two of the isolates—117/10 and 114/73 were found to lower the *B. cinerea* spore germination rate used as single isolates. This ability was further enhanced when those isolates were used as a consortium. A few mechanisms may be responsible for slowing or completely inhibiting spore germination. Fungi tend to produce self-inhibitory volatile compounds when spores are abundant in proximity [71]. Yeast may produce volatile organic compounds, enzymes, and antibiotic-like compounds similarly inhibiting the growth and development of pathogenic fungi spores [72]. Recently yeast such as *Saccharomyces cerevisiae* [73] or *Scheffersomyces spartinae* [74] were reported to produce volatile organic compounds. Yeast can also deplete the amount of available nutrients required by spores to germinate [75].

The data presented in this study identifies yeast isolates and their consortia that can be effective under organic standards, demonstrating the effectiveness of yeast isolates 114/73 (*W. anomalus*), 117/10 (*N. albidosimilis*), and 117/67 (*S. roseus*) in colonizing apple wounds and preventing *Botrytis* decay. The three-isolate consortium showed the greatest reduction in spore germination, followed by the yeast consortia comprising isolates 114/73 and 117/10, which demonstrated the second-highest efficiency. These isolate combinations were also highly effective in suppressing *B. cinerea* growth on apples under both room and storage temperatures. Additionally, the isolates exhibited rapid growth in apple wounds. These findings highlight the potential of these yeast strains, individually and as a consortium, in managing postharvest fungal decay in apples and contribute to the development of new yeast-based formulations aimed at reducing postharvest losses in apples and other fruits and advancement of yeast research as biocontrol agents.

Our findings can also contribute to further research into yeast consortia as a flexible and customizable strategy for managing multiple postharvest pathogens beyond *B. cinerea*. Ultimately, this work adds to the growing body of literature that supports organic and sustainable agricultural practices, encourages reduced chemical use, promotes environmental health, and aligns with consumer demand for organically produced fruit.

Author Contributions: Conceptualization, J.K. (Joanna Krzysińska) and J.K. (Jolanta Kowalska); methodology, J.K. (Joanna Krzysińska); validation, J.K. (Jolanta Kowalska); formal analysis, J.K. (Jolanta Kowalska); investigation, J.K. (Joanna Krzysińska); resources, J.K. (Joanna Krzysińska); data curation, J.K. (Joanna Krzysińska); writing—original draft preparation, J.K. (Joanna Krzysińska); writing—review and editing, J.K. (Jolanta Kowalska); visualization, J.K. (Joanna Krzysińska); supervision, J.K. (Jolanta Kowalska); project administration, J.K. (Jolanta Kowalska); funding acquisition, J.K. (Jolanta Kowalska). All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Data Availability Statement: Data are available on request due to restrictions. The data presented in this study are available on request from the corresponding author. The data are not publicly available to allow for commercialization of research findings.

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

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