

Non-chemical control options against grey mould (*Botrytis cinerea*) on strawberry (*Fragaria x ananassa*)

Možnosti nekemičnega zatiranja sive plesni (*Botrytis cinerea*) na žlahtnem jagodnjaku (*Fragaria x ananassa*)

Klara ŠAVLI¹ (✉), Janja ZAJC ŽUNIČ¹, Franci Aco CELAR²

¹ Agricultural Institute of Slovenia, Hacquetova street 17, 1000 Ljubljana, Slovenia

² University of Ljubljana, Biotechnical Faculty, Department of Agronomy, Jamnikarjeva street 101, 1000 Ljubljana, Slovenia

✉ Corresponding author: klara.savli@kis.si

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ABSTRACT

Phytopathogenic fungi cause significant crop losses every year. Plant protection products have a negative and long-lasting impact on the environment and, in addition, the fungus *Botrytis cinerea* is known to be resistant to several types of fungicides. In the last decade, more emphasis has been placed on the discovery and use of new antagonistic species of microorganisms that could control plant pests and inhibit the growth of pathogenic diseases. The necrotrophic pathogenic fungus *B. cinerea* causes grey mould of strawberries, which leads to considerable yield losses in the strawberry harvest. Some antagonistic filamentous fungi and yeast-like fungi inhibit the growth of the fungus *B. cinerea* on plants and on fruits after harvest, by mechanisms such as parasitism and attachment to the surface of the pathogenic fungus, competition for environmental sources and reduction of available nutrients, antibiosis and secretion of metabolites (antibiotics, lytic enzymes, toxins, polysaccharides, siderophores, etc.). Bacteria express their antifungal effect through the production of extracellular hydrolytic enzymes, acids, antibiotics and other substances. Other effective non-chemical control methods include biofumigation, the use of essential oils and plant extracts, and the application of recommended agrotechnical measures. Products based on biological control agents may have some disadvantages (more complex instructions for use and lower control efficacy). The simultaneous use of these preparations has been proven to be more effective and holds great potential for further research.

Keywords: *Botrytis cinerea*, *Fragaria x ananassa*, biological control, antagonistic fungi, antagonistic bacteria, secondary metabolites

IZVLEČEK

Fitopatogene glive povzročajo vsako leto znatne izgube pridelka. Fitofarmacevtska sredstva imajo negativne in dolgotrajne vplive na okolje, poleg tega je za glivo *Botrytis cinerea* znana odpornost proti nekaterim vrstam fungicidov. V zadnjem desetletju je poudarek na odkrivanju in uporabi novih antagonističnih vrst mikroorganizmov, ki bi lahko zatirali rastlinske škodljivce in zavirali rast patogenih bolezni. Nekrotrofna patogena gliva *B. cinerea* povzroča sivo plesen jagod, ki povzroči zmanjšanje tržnega pridelka na žlahtnem jagodnjaku. Nekatere antagonistične filamentozne glive in kvasovkam podobne glive zavirajo rast glive *B. cinerea* na rastlinah in na plodovih po spravi preko mehanizmov inhibicije, kot so parazitiranje in pritrjevanje na površje patogene glive, kompeticija za okoljske vire in zmanjšanje dostopnosti hranil, antibioza in izločanje metabolitov (antibiotikov, litičnih encimov, toksinov, polisaharidov, sideroforov itd.). Bakterije izražajo protiglivično delovanje preko tvorbe zunajceličnih hidrolitičnih encimov, kislin, antibiotikov in drugih snovi. Drugi učinkoviti nekemični načini zatiranja so biofumigacija, uporaba esencialnih olj in rastlinskih izvlečkov ter upoštevanje priporočenih agro tehničnih ukrepov. Uporaba pripravkov na osnovi biotičnih agensov ima lahko nekatere pomanjkljivosti (zahtevnejša navodila uporabe in manjša učinkovitost). Sočasna uporaba takih pripravkov se je izkazala za učinkovitejšo in predstavlja velik potencial za nadaljnja raziskovanja.

Ključne besede: *Botrytis cinerea*, *Fragaria x ananassa*, biotično varstvo, antagonistične glive, antagonistične bakterije, sekundarni metaboliti

INTRODUCTION

Phytopathogenic fungi are important harmful organisms that cause significant crop losses worldwide every year (González-Fernández et al., 2010). According to the FAO (2019), plant pathogens and pests are estimated to cause up to 40% of annual crop losses in economically important crops. The damage caused by plant diseases is estimated at 220 billion euros annually. The most common species of pathogenic fungi, which are among the main causes of diseases in agricultural production, include *Magnaporthe oryzae*, *Botrytis cinerea*, *Puccinia* spp., *Fusarium graminearum*, *Fusarium oxysporum*, *Blumeria graminis*, *Mycosphaerella graminicola*, *Colletotrichum* spp., *Ustilago maydis* and *Melampsora lini* (Dean et al., 2012). Due to the negative and long-lasting effects of pesticides on the environment and the increasing emergence of fungicide resistance in fungi (El-Baky and Amara, 2021), interest in the discovery of potential antagonistic species of microorganisms and their ability to control plant pests and diseases has increased in recent decades (Ayaz et al., 2023). Previous research has shown that some fungal species and their metabolites can reduce the impact of economically important diseases in major crops and fruit species (Abbey et al., 2019; Chen et al., 2018; Roca-Couso et al., 2021; Santos et al., 2004). Antagonism of microbial or fungal species is typically a direct interaction between microorganisms that share the same ecological niche. Direct interactions can be categorised into three main types according to their characteristics. Parasitism of pathogenic fungi is an interaction in which microorganisms parasitise by producing lytic enzymes that break down the cell wall of fungal hyphae and feed on their contents. Antibiosis is an interaction in which the growth of the organism is inhibited by antibiotic substances secreted by antagonists. The third type of direct interaction is competition for environmental resources, especially for space and food (Alabouvette et al., 2006; Elad, 1995, 1996).

Several types of diseases are economically important and frequently occur on strawberry plants. These include strawberry grey mould (*B. cinerea*), red and white leaf

spot (*Diplocarpon fragariae* and *Mycosphaerella fragariae*), strawberry powdery mildew (*Podosphaera aphanis*) and black spot of strawberry (*Colletotrichum acutatum*) (Mykhailenko et al., 2022). *B. cinerea*, the causal agent of grey mould of strawberry, is a filamentous necrotrophic pathogenic fungus with a wide range of host plants (Elad et al., 2007). The fungus causes significant crop losses in strawberry plants and many others (Mykhailenko et al., 2022). The rarer sexual form (teleomorph) of the anamorph *B. cinerea* is *Botryotinia fuckeliana* (Faretra et al., 1988). The symptoms depend on the plant species and the type of plant tissue. Soft rot often occurs on softer fruits, but it also infects other parts of the plant (flowers, leaves and flower stalks, and stems). Due to the production of enzymes, the parenchymal tissue collapses after infection, and a grey mass of conidia soon appears on the tissue (Williamson et al., 2007).

Given the limitations of chemical control methods and the need for sustainable plant protection strategies, the use of non-chemical control options for controlling the growth of *B. cinerea* on naturally occurring and cultivated plants might become the only reasonable option in the future. This review aims to explore and evaluate established and innovative, non-chemical approaches, with emphasis on biological control agents, for managing *B. cinerea* in strawberry cultivation. By presenting promising antagonistic microorganisms and their mechanisms of action and other non-chemical control options, this review seeks to support the development of effective biocontrol strategies as viable alternatives to chemical fungicides.

GREY MOULD

The genus *Botrytis* comprises 30 species that differ greatly in terms of biology, ecology, morphological characteristics, and hosts. The fungi *Botrytis* spp. infect numerous plant species from 170 families of wild and cultivated plants (Elad et al., 2016). The fungus *B. cinerea* occurs on host species in tropical and cooler regions, in dry and humid climates, in closed and stored spaces, as well

as in greenhouses and outdoors (Elad et al., 2016). The fungus *B. cinerea* is classified in the phylum Ascomycota, the subphylum Pezizomycotina, the class Leotiomycetes, the order Helotiales, the family Sclerotiniaceae and the genus *Botrytis* (MYCOBANK database, 2024). The life cycle of *B. cinerea* consists of several stages: vegetative mycelium, producing asexual conidia (mainly macroconidia, characteristic of anamorphic forms of *Botrytis* spp.), microconidia (spermatia) and sclerotia. Sclerotia, a common overwintering form of the fungus, usually germinate to form mycelium and conidia, but under suitable environmental conditions and in the presence of nutrient sources, can germinate to form apothecia (fruiting bodies), which are characteristic of the teleomorphic form of *B. fuckeliana*. Apothecia form asci with sexual ascospores, which are formed during the meiosis process (Beever and Weeds, 2007), thus increasing the genetic diversity of the species. Chlamydiospores are viable asexual spores, found mainly in older cultures of the fungus. They are formed by the transformation of terminal or intercalary cells of hyphae in the vegetative mycelium and are released after the decay of the hyphae (Holz et al., 2007). The fungus is characterised by structures on macroconidiogenous hyphae, on which terminal clusters of hydrophobic conidia are synchronously formed (Card, 2005; Willets, 1997). The appearance of the pathogenic fungus on the surface of the growth medium in a Petri dish is light grey and fluffy, becoming darker later on. The hyphae are septate and have dark-coloured cell walls. The darker colour of the older fungal culture is attributed to mature conidiophores with branched growth. Under favourable growth conditions, *B. cinerea* can form a secondary mycelial phase, sclerotia, consisting of a dark mass of hyphae (Card, 2005; Jarvis, 1977; Williamson et al., 2007). The fungus *B. cinerea* produces a number of secondary metabolites. The metabolites botrydial and botcinic acid are phytotoxic and necessary for the necrotrophic survival mode of the fungus (Elad et al., 2016). Most species of the genus *Botrytis* are usually spread through the air by large amounts of conidia (Elad et al., 2016).

GREY MOULD ON STRAWBERRY

The fungus *B. cinerea* is one of the main causes of disease and crop failure in strawberry plants (Mykhailenko et al., 2022). The first infections with *B. cinerea* usually occur on the dying petals, followed by the spread of soft rot to the developing fruits. On the petals, the disease symptoms can vary depending on environmental conditions; we can observe small spots or a fully developed soft rot (Williamson et al., 2007).

Primary infections of *B. cinerea* on strawberry fruits can occur through infection of open flowers, allowing the fungal hyphae to grow into the inflorescence itself. Sources of primary infections can be conidia, overwintering sclerotia or mycelia from infected neighbouring plants (Bristow et al., 1986; Petrasch et al., 2022). Secondary infections often occur very quickly on injured fruit, older leaves and flower parts. The time from inoculation to infection can be up to 16 hours, and after 48 hours, the fungal biomass increases significantly. Early responses of strawberry fruits to fungal infection include increased expression of defence genes that encode the production of chitinases. Late stages of infection are characterised by lower expression of the reference gene FaDBP (a gene that binds to DNA sequences), indicating extensive cell death (Bristow et al., 1986; Holz et al., 2007; Mehli et al., 2005).

Nagpala et al. (2016), Haile et al. (2019) and Blanco-Ulate et al. (2016) found that the degree of ripeness of strawberries influences their susceptibility to infection with *B. cinerea*. Strawberry fruits are resistant to infection, especially at the immature stage, which is due to the chemical composition of the fruits and the different gene expressions. The plants show a stronger defence reaction to infection with the fungus in unripe fruits. In such fruits, there is an increased activity of genes that encode the production of pathogenesis-related proteins (PRPs), and genes responsible for the synthesis of the various cell wall components and thus for their strengthening.

NON-CHEMICAL CONTROL OPTIONS FOR *B. cinerea* ON STRAWBERRY PLANTS

The problem of fungicide resistance

The extensive and long-term use of pesticides has a negative impact on the environment and can disrupt its ecological balance. In addition, the use of single-site chemical pesticides in large quantities can lead to resistance of *B. cinerea* to fungicides, which reduces their protective effect (Brent and Hollomon, 2007; Leurox, 2007; Rupp et al., 2017; Weber and Hahn, 2019). As there are no authorised broad-spectrum fungicides, chemical control of *Botrytis* spp. is mainly based on fungicides with a specific mode of action (Rupp et al., 2017). A pathogenic organism that develops resistance to one fungicide often simultaneously develops resistance to other fungicides that have a similar chemical structure and the same mode of action. This phenomenon is known as cross-resistance (Brent and Hollomon, 2007), an example of which is simultaneous resistance to dicarboximides and fludioxonil (Leroux et al., 1999). However, resistance to two or more unrelated fungicides can also occur in *B. cinerea*. This phenomenon is known as multiresistance. An example of this is the resistance of *B. cinerea* to benzimidazole derivatives and dicarboximides. Weber and Petridis (2023) found that the occurrence of mass resistance of different isolates of *Botrytis* sp. to a number of fungicides, such as fenhexamid, trifloxystrobin, boscalid, fludioxonil and cyprodinil, is increasing in Germany and Denmark. Mass resistance of *Botrytis* sp. to fungicides has also been confirmed in Slovenia (Marolt and Žerjav, 2019). A rarer form of resistance is negative cross-resistance, in which increased resistance to one or more active substances reduces the resistance of the pathogenic fungus to other active substances (Brent and Hollomon, 2007). An example of negative cross-resistance occurs between benzimidazoles (e.g. carbendazim) and phenylcarbamates (e.g. dietofencarb) (Leroux, 2007).

Use of antagonistic fungi to control grey mould

Some genera of antagonistic fungi that have been proven effective in the control of grey mould

in the past are filamentous fungi from the genera *Trichoderma*, *Gliocladium*, *Ulocladium* and yeasts from the genera *Pichia*, *Candida*, *Aureobasidium*, *Rhodotorula* and *Metschnikowia* (Di Francesco et al., 2023; Elad and Stewart, 2007; Freimoser et al., 2019; Sansone et al., 2005). Biological agents have different mechanisms of action to inhibit the growth of *Botrytis* spp. One control mechanism is attachment to the surface of pathogenic organisms, e.g. attachment of the yeast *Rhodotorula glutinis* and *Cryptococcus albidus* to the surface of *B. cinerea* conidia by the fibrous substances formed (Elad, 1996; Elad and Stewart, 2007). An important mode of control is competition between the biological agent and the pathogenic organism for nutrients and habitat. The reduction in the concentration of available nutrients results in a lower germination rate of the pathogenic organism's spores and slower growth of the germ tube, which consequently means a lower infection rate and a smaller area of dead plant tissue (Alabouvette et al., 2006; Elad and Stewart, 2007). *Botrytis* spp. are more susceptible to the inhibitory mechanism of competitive inhibition due to their dependence on external nutrient sources required for conidia germination, germ tube growth and host infection (Elad and Stewart, 2007). Antibiosis is a method of inhibiting the growth of one organism by inhibitory substances produced by another organism. The fungi *Trichoderma* spp. and *Gliocladium* spp. often produce antibiotic substances (antibiotics). The conidia and germ tubes of the fungus *Botrytis* spp. on the surface of plant tissue are very sensitive to the activity of antibiotics and lytic enzymes secreted by antagonistic microorganisms, leading to inhibition of their germination and lysis of the germ tubes (Elad, 1995; Elad and Stewart, 2007). The parasitism of pathogenic fungi by various microorganisms is based on the production of enzymes that degrade the cell wall of the fungus. The best known mycoparasites are fungi and fungus-like organisms from the genera *Trichoderma*, *Gliocladium* and *Pythium* (Elad, 1995). Some other mechanisms for inhibiting the growth of pathogenic organisms by biological control agents are reduction of pathogenicity (virulence) of the organism (by interfering with the pathogenesis process), reduction of

pathogen inoculum formation, induced plant resistance and combinations of different mechanisms (Elad and Stewart, 2007).

The use of preparations, based on biological control agents, has some disadvantages, such as mandatory observance of proper storage and expiry dates, greater sensitivity of the preparations to environmental conditions, and fewer and inconsistent efficacy. In addition, many of these preparations are more effective in inhibiting the growth of pathogens when used in combination with other biological control agents or fungicides, with lower risks (Abbey et al., 2019). Guetsky et al. (2001) tested the efficacy of inhibiting the growth of *B. cinerea* on strawberry leaves by applying the yeast *Pichia guilhermondii* and the bacterium *Bacillus mycoides*, individually and in combination. In addition to testing the effectiveness of the bacteria in inhibiting fungal growth and the differences between the various treatments, they were also interested in whether the simultaneous use of biological control agents increased the range of environmental factors (e.g. temperature and relative humidity) in which the control was effective. They found that in the treatments where the biological control agents were used separately, the inhibitory effect varied greatly (from 38% to 98%); the coefficient of variation ranged from 9.7% to 75%. The simultaneous use of *B. mycoides* and *P. guilhermondii* effectively suppressed the growth of *B. cinerea* under all environmental conditions (from 80% to 99%); the coefficient of variation was significantly lower, between 0.4% and 9%. They found that using more than one biological agent to control grey mould is a more reliable control method in certain cases. In their study, Cruz et al. (2018) investigated whether the use of one or more biological agents to control *B. cinerea* has an impact on the microbial community naturally present on strawberry plants. Using sequencing of microbial communities (bacteria and fungi), they found that a combination of two or more biological control agents had a greater and better impact on the composition and functional properties of the microbial communities. They concluded that this could influence the more effective functioning of such preparations.

Yeasts are also a group of fungi that have the ability to act antagonistically against some plant pathogens. Due to their mode of action, ease of cultivation, applicability and stress resistance, they are promising for the development of preparations for biological plant protection. The best known yeasts that have been used to control phytopathogenic fungi are *Candida oleophila*, *Aureobasidium pullulans*, *Metschnikowia fruticola*, *Cryptococcus albidus* and *Saccharomyces cerevisiae* (Freimoser et al., 2019). In their study, Santos et al. (2004) found that the yeast *Pichia membranifaciens* secretes the so-called "killer" toxin, a secondary metabolite that successfully inhibits the growth of the fungus *B. cinerea* on inoculated apple fruits. It has an antifungal effect, can inhibit calcium absorption, impair glucanase activity and damage the membranes of pathogenic fungi. The metabolite »pulcherrimin«, of the yeast *Metschnikowia pulcherrima*, inhibits the growth of pathogenic fungi through the process of iron binding. The metabolite binds iron ions, and due to the immobility or unavailability of iron, the growth of pathogenic fungi is prevented (Saravanakumar et al., 2008). One of the metabolites of the yeast *A. pullulans* that shows antifungal activity against *B. cinerea* on strawberries is aureobasidin, an exopolysaccharide that inhibits the enzyme inositol phosphoryl ceramide synthase, which catalyses the formation of sphingolipids (Prasongsuk et al., 2018).

In their study, Iqbal et al. (2023) confirmed the efficacy of *A. pullulans*-based preparations for suppressing the growth of *B. cinerea*. The preparations were sprayed on flowering strawberry plants planted outdoors. The highest suppression efficiency (57%) was observed at a higher concentration of *A. pullulans* conidia (10^7 CFU/mL). The strawberry yield was 52% higher and the storage time was extended by 35%. Huang et al. (2011) determined the effectiveness of suppressing *B. cinerea* with volatile organic compounds produced by the yeast *Candida intermedia*. Using gas chromatography and mass spectrometry, 49 volatile compounds (esters, alcohols, alkanes, alkenes, organic acids, etc.) were identified. The results of the study show that the growth of *B. cinerea* on strawberry fruits was inhibited up to 75% due to

inoculation with the yeast *C. intermedia* and exposure to the volatile organic compounds produced by it. Yeasts of the genus *Rhodotorula* secrete siderophores or rhodotorulic acid (small molecules that chelate iron ions and thus prevent other microorganisms from using them), which successfully inhibit the growth of *B. cinerea* strains that are sensitive to the fungicide iprodione (Sansone et al., 2005). Sansone et al. (2005) found that the combination of the fungus *R. glutinis* and additional rhodotorulic acid, produced in the laboratory, successfully suppressed the germination of *B. cinerea* spores on inoculated apple fruits. The effectiveness of the rhodotorulic acid was attributed to its ability to inhibit the activity of the enzymes polygalacturonase and laccase. Huang et al. (2012) investigated the antagonistic potential of the yeast *Sporidiobolus pararoseus*, strain YCXT3, for biological control of *B. cinerea* on strawberry fruits. The yeast secretes a volatile organic compound that inhibits spore germination and mycelial growth. The results showed that the yeast did not affect the growth of the *B. cinerea* mycelium in a dual culture test. In contrast, inoculation of fruits with a spore suspension of *S. pararoseus* successfully inhibited the growth of *B. cinerea* (96 to 100%). Chen et al. (2018) conducted a screening test of yeast antagonists with different efficacy to control of *B. cinerea* on strawberry fruits. They found that the enzyme chitinase, from the yeast strain *Galactomyces candidum* JYC1146, has the potential to inhibit the growth of *B. cinerea*. The chitinase was shown to be effective *in vitro*, inhibiting the growth of *B. cinerea* by 70%, and furthermore effectively inhibited the growth of *B. cinerea* mycelium on strawberry fruits. It was pointed out that the yeast *G. candidum* has been identified as a cause of acid rot in some fruits and vegetables. Although it is not thought to be pathogenic for strawberry, the risk of using this fungus for biological control of grey mould should be considered (François et al. 2022).

Many species of *Trichoderma* fungi (e.g. *T. harzianum*, *T. viride*, *T. atroviride*, *T. asperellum*) are known to inhibit the growth of various pathogenic fungi. They secrete a number of metabolites and enzymes with antifungal activity (e.g. trichodermol, alkenes, alcohols, proteases,

chitinases, etc.) (Brunner et al., 2005; Fan et al., 2024; Vos et al., 2015; Yassin et al., 2021). The fungus *T. asperellum* produces highly effective volatile organic compounds that inhibit the growth of *B. cinerea*. Fan et al. (2024) found that volatile metabolites of *T. asperellum* inhibited the growth of *B. cinerea* on strawberry fruits and in the detached leaf assay (DLA) by 76 to 100%. It is assumed that they effectively inhibit growth by damaging the cell membranes of *B. cinerea* and thereby inhibiting mycelial growth and germination of the conidia. The fungal species *Gliocladium* spp. have known antagonistic potential to inhibit the growth of many pathogenic fungi. Some metabolites and enzymes isolated from *Gliocladium* spp. with antifungal activity are gliotoxin, endochitinase and glucanase (Di Pietro et al., 1993; Roca-Couso et al., 2021).

Thomidis et al. (2015) tested the efficacy of the commercial preparation BOTRY-Zen® based on the soil fungus *Ulocladium oudemansii* to control *B. cinerea* on strawberry fruits. They indicated that the preparation can effectively inhibit the growth of *B. cinerea* at a concentration of 6 g/L; at lower concentrations, no differences were observed between treated and control plants. They also found that the efficacy of the preparation was significantly worse compared to the chemical fungicide Switch 25/37.5 WG (based on fludioxonil and cyprodinil), which was used at a concentration of 1 g/L. The biological preparation reduced fruit rot by 50% compared to the control, while the fungicide almost completely inhibited the growth of grey mould. They pointed out that preparations based on *U. oudemansii* could be used in integrated pest management. Weiss et al. (2014) investigated the effectiveness of the preparation Boni Protect forte®, which contains the yeast *A. pullulans*, in inhibiting the growth of grey mould on strawberry plants under field conditions. After a two-year trial, they found that the effectiveness of the preparation in controlling grey mould on strawberry plants was up to 63%.

Use of antagonistic bacteria to control grey mould

Many bacterial species are used for the biological control of pathogenic fungi that cause plant diseases.

Bacteria can express antifungal activity through the production of extracellular lytic enzymes, siderophores, salicylic acid, antibiotics, and various volatile metabolites such as hydrocyanic acid (Manwar et al., 2004; Nagarajkumar et al., 2004).

Essghaier et al. (2009) investigated a number of effective halophilic bacteria for their antagonistic activity against grey mould. Bacteria inhibit the growth of fungi by secreting extracellular hydrolytic enzymes (chitinases, glucanases, proteases, cellulases), non-volatile and volatile metabolites. Most of the effective bacterial isolates were classified into the genera *Bacillus* and *Staphylococcus*. Strawberry fruits were treated with a bacterial suspension and inoculated with a suspension of *B. cinerea* conidia. They found that about thirty strains of the tested moderately halophilic bacteria exhibited active antifungal activity and reduced the proportion of infected fruits by 50% to 90% after three days under controlled conditions. Ni et al. (2023) isolated a secondary metabolite macrolactin R from the bacterium *Bacillus siamensis*. In an *in vitro* test, it inhibited spore germination, germ tube elongation, and mycelial growth of *B. cinerea*. Macrolactin R affected the structure of the cell membranes of *B. cinerea* and led to increased membrane permeability and leakage of soluble proteins and nucleic acids from the cytoplasm, resulting in cell death. Helbig and Bochow (2001) successfully suppressed grey mould under laboratory conditions using the bacterium *Bacillus subtilis* (isolate 25021). To test the inhibition of conidia germination of the fungus, they used a suspension of strawberry pulp with a certain concentration of conidia and an added bacterial suspension. The growth of germ tubes of conidia was strongly inhibited by the presence of the antagonistic bacteria, but no inhibitory effect on the germination of conidia themselves was observed. They found that the presence of the antagonistic bacteria reduced the presence of germ tubes longer than 100 µm by about 95% within 24 hours, compared to the control. Wafaa et al. (2013) tested the efficacy of strains of two types of bacteria, *Bacillus brevis* and *Bacillus polymyxa*, to inhibit the growth of *B. cinerea* under laboratory conditions. They found that the bacterial cells of both

strains and their filtrates significantly inhibited the growth of the fungus *B. cinerea*. Through analyses using liquid chromatography with mass spectrometry, they found that the most effective bacterial culture filtrates produced larger amounts of antifungal compounds: the peptides polymyxin B and gramicidin S. Zamani-Zadeh et al. (2014) tested the efficacy of different combinations of the bacterium *Lactobacillus plantarum* with essential oil of thyme (*Thymus vulgaris*) and cumin (*Cuminum cyminum*) to inhibit the growth of *B. cinerea* mycelium. To perform the *in vitro* experiment, a liquid culture of *L. plantarum* was added to potato dextrose agar, to which a small square of filter paper was placed, soaked with a certain amount of essential oils and then inoculated with a 3 mm diameter *B. cinerea* mycelium disc. To test the efficacy of the combinations on strawberry fruits, intentionally injured fruits were sprayed with a bacterial suspension of *L. plantarum* and then inoculated with a suspension of *B. cinerea* spores. It was found that mycelial growth was successfully inhibited at all concentrations of added thyme essential oil (0.5 to 20 µL/mL), while higher concentrations were required when cumin essential oil was used (3 to 20 µL/mL). The inhibition of mycelial growth of *B. cinerea* on strawberry fruits was significantly more effective (up to 90%) with the combination of the bacterium *L. plantarum* and thyme essential oil (100 µL/L) and the combination of *L. plantarum* and cumin essential oil (50 µL/L), compared to the efficacy of the individual applications of the bacterial suspension or the essential oils.

Zhang et al. (2015) tested the effect of the natural compound phenazine-1-carboxamide (PCN), which was isolated from metabolites of the antagonistic bacterium *Pseudomonas aeruginosa*, on the growth of *B. cinerea* mycelium. The strongest inhibitory effect of PCN on the growth of *B. cinerea* under *in vitro* conditions on strawberry fruits was about 75%, at a concentration of 700 µg/mL. At the same concentration, the compound PCN strongly inhibited the germination of spores and sclerotia of the fungus *B. cinerea*; the inhibition was between 77% and 82%, respectively. The growth of the fungal mycelium was inhibited by up to 87%.

The efficacy of the strain *Serratia rubidaea* for biological control of *B. cinerea* on strawberry plants was investigated by Alijani et al. (2022). Bacterium *S. rubidaea* secretes the pigment prodigiosin, which is thought to have an antifungal effect. From the results obtained, they concluded that the bacterial strain effectively inhibited the growth of the *B. cinerea* mycelium by 72% in the double culture method. Volatile compounds isolated from the fungus inhibited the growth of the mycelium and the germination of *B. cinerea* conidia by 65 to 71%. The efficacy of inhibition of *B. cinerea* growth by the secondary metabolite prodigiosin was 94% at the highest concentration tested (420 µg/mL). Yong et al. (2022) investigated the efficacy of the actinomycete *Streptomyces* sp. sdu1201 for the biological control of *B. cinerea*. The bacteria live in symbiosis with fungi in the gut of the termite *Odontotermes formosanus*, from which they were isolated. They found that the bacteria have a significant antifungal effect on the growth of the fungus *B. cinerea*. They isolated four compounds, which they identified as actinomycins. Actinomycin D had the strongest inhibitory effect against *B. cinerea*, with an EC_{50} value of 7.65 µg/mL. The inhibitory effect of actinomycin D on the growth of *B. cinerea* mycelium in the detached leaf and fruit test, on fermentation medium, was 78%. They also observed an inhibition of the growth of germinating spores. They found that the actinomycin D-producing bacterium *Streptomyces* sp. sdu1201 could be a potential biocontrol agent for suppressing the growth of the fungus *B. cinerea* on strawberries. Maung et al. (2021) investigated the efficacy of *B. amyloliquefaciens* strain Y1 in controlling grey mould on strawberry plants in a greenhouse. The seedlings were treated several times with a bacterial suspension, and after 19 weeks, the plants were sprayed with a suspension of *B. cinerea* spores at a concentration of 10^6 spores/mL. They found that the bacterium successfully inhibited the growth of the fungus *B. cinerea* on flowers and fruits under *in vivo* conditions by 56%. The bacterial suspension stimulated a defence reaction in the plants, which was determined by a higher amount of the expressed enzyme, β -1,3-glucanase in the treated plants, compared to the control plants.

Other non-chemical control options

To minimise the damage caused by *B. cinerea*, it is advisable to consider several agrotechnical measures. To reduce the spread of infection and ensure sufficient air circulation in the room, it is important to maintain adequate crop rotation and plant spacing, which helps to reduce moisture retention and thus reduce the risk of infection (Datta et al., 2022). In addition, it is important to regularly remove infected plant material, maintain an adequate water balance and good lighting, avoid unnecessary wetting of the leaves, and use mulch around the lower part of the plant (hay, plastic film, etc.) (Alabouvette et al., 2006; Datta et al., 2022; Weber and Petridis, 2023). A variety of natural products are commercially available that are labelled as plant strengtheners or plant defence stimulants. They are intended to prevent/reduce infections with pathogenic organisms. They may contain plant extracts such as *Azadirachta indica* oil (neem), algae extracts (laminarin), secondary metabolites produced by microorganisms (e.g. harpin) or essential oils (e.g. geraniol, menthol) (Alabouvette et al., 2006).

One of the non-chemical methods for combating phytopathogenic fungi is biofumigation or biological soil disinfection. In this method, volatile organic compounds derived from natural sources are used to control pathogenic organisms, which is why these compounds are considered safe (Tian et al., 2023). The volatile compounds can inhibit pathogenic organisms without direct contact with crops, and in addition, the slow release of the compounds into the crop environment has an inhibitory effect on pathogens that may appear after harvest (El-Baky and Amara, 2021; Medina-Romero et al., 2017; Tian et al., 2023). One of the compounds used for the antimicrobial effect of vapours is AITC (allyl isothiocyanate), which is extracted from the seeds of cruciferous plants (Brassicaceae family). In their study, Ugolini et al. (2014) found that a four-hour treatment of the atmosphere (in a closed room) with AITC vapours was effective in controlling *B. cinerea* on stored strawberries. The incidence of fruit rot was 47 to 91% lower than in the control treatment. AITC residues on the fruit were less than 1 mg/kg.

Some wild strawberry varieties are naturally more tolerant to *B. cinerea*, which may reduce the need for protective measures to prevent infection. Currently, there are no varieties available that are resistant to the infection (Bestfleisch et al., 2015; Petrasch et al., 2022). Some varieties, such as 'Florence', are significantly less susceptible to damage compared to others, such as 'Senga Sengana' (Bestfleisch et al., 2013). In most cases, strawberry varieties that are more resistant to grey mould infection have better morphological characteristics and a higher content of antimicrobial compounds (e.g. phenols, flavonoids, acids, etc.) that make them more tolerant to infection (Zhao et al., 2022).

Researchers Vagelas et al. (2009) tested the antifungal activity of wastewater from an olive oil mill on the growth of *B. cinerea* mycelium directly on strawberry fruits and under *in vitro* conditions. Sterilised wastewater from the oil mill was added to the culture medium and to the surface of potato dextrose agar plates and inoculated with *B. cinerea* mycelium discs. For inoculation, the fruits were soaked in a suspension of *B. cinerea* spores. After drying, they were treated with a mixture of oil mill effluent and tallow powder (1:6 v/v). The results showed that the inhibition of mycelia growth on agar plates was significantly stronger when a few drops of the sterilised and filtered wastewater were added to the surface (as opposed to addition to the medium alone). On fruits, the most effective inhibition of *B. cinerea* growth was observed with the combination of wastewater from the oil mill and tallow powder. It is assumed that the growth inhibitory effect on *B. cinerea* is due to the presence of phenolic compounds that remain in the wastewater after sterilisation.

An innovative and not widely used method to control *B. cinerea* is the use of preen (uropygial) oil extracted from bird feathers. Hassan et al. (2021) found that oil isolated from chicken feathers has a growth-inhibiting effect on the fungus *B. cinerea*. They tested the efficacy of different oil concentrations mixed in potato dextrose agar. Under *in vitro* conditions, a concentration of 25 µL of oil/mL agar reduced the growth of the fungus by 41%,

and the germination of spores that could form colonies by 28%. On strawberry fruits, the oil reduced the level of fungal infection by 87%. They therefore concluded that the minimum concentration of uropygial oil required to inhibit the growth of *B. cinerea* on fruits by at least 41% was 25 µL/mL. They concluded that the effectiveness of the oil is due to its waxy composition and the content of 17 different compounds.

CONCLUSIONS

To sum up, the pathogenic fungus *B. cinerea* causes considerable damage to cultivated strawberry plants and a reduction in marketable yield. The fungus has various strategies to survive and infect plant parts. Due to the development of tolerance and resistance to some fungicides and the negative impact of chemical agents on the environment, the use of appropriate agronomic techniques and products to control pathogens based on biological agents is of great importance. There are currently no strawberry varieties on the market that are resistant to infection with the fungus *B. cinerea*. Several biological control products based on fungi, bacteria and their metabolites with an antagonistic activity are authorised and commercially available. The use of these products is associated with more complex instructions for use and requires compliance with the recommended instructions, as the efficacy of the control of pathogenic organisms may otherwise be inconsistent and poor. More and more research is being conducted in the search for new effective microorganisms, as well as their metabolites and naturally derived compounds, which may be successful in controlling *B. cinerea*. It has been shown that certain combinations of biological agents are more effective in controlling *B. cinerea* on strawberry plants than the use of single agents. The biological control of *B. cinerea* using antagonistic microorganisms, such as filamentous fungi from the genus *Trichoderma*, yeast fungi from the genus *Aureobasidium* and bacteria from the genera *Bacillus* and *Staphylococcus*, is generally considered more effective and reliable than other non-chemical methods, such as the utilisation of essential oils. While non-chemical control options for *B. cinerea*

are not yet a complete substitute for chemical fungicides, they represent effective and environmentally friendly alternatives that can significantly reduce fungicide use. Non-chemical methods to control *B. cinerea* have been researched for decades and will certainly continue to be investigated, as the discovery of new strategies or combinations of different compounds and biological agents could reduce the post-harvest incidence of *B. cinerea* on strawberry plants and fruits.

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