Plant Pathology & Quarantine 14(1): 81–87 (2024) ISSN 2229-2217
www.ppqjournal.org

Article
Doi 10.5943/ppq/14/1/7

Exploring the efficacy of different fungicides in controlling *Botrytis cinerea* outbreaks in strawberry

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Monteiro FP, Mallmann G, Ogoshi C, Valmorbida J, Wamser AF 2024 – Exploring the efficacy of different fungicides in controlling *Botrytis cinerea* outbreaks in strawberry. Plant Pathology & Quarantine 14(1), 81–87, Doi 10.5943/ppq/14/1/7

Abstract

Botrytis cinerea, a widely distributed and versatile ascomycetous fungus, poses a significant threat to various agricultural crops, globally leading to substantial economic losses. Commonly known as gray mold, this phytopathogenic microorganism has been extensively noticed due to its capacity to cause diseases both pre/post–harvest, attacking over 200 plant species. This study explored the impact of registered fungicides on *B. cinerea* control in strawberry, assessing mycelial growth, halo formation, and phytotoxic effects. Two isolates from different locations were employed, and different fungicide combinations were evaluated for their efficacy in inhibiting mycelial growth and preventing strawberry fruit colonization. Notably, cyprodinil + fludioxonil, and methyl–thiophanate + fluazinam emerged as promising chemicals in controlling *B. cinerea*, with variations observed between isolates. Additionally, the study highlights the potential phytotoxic effects of adhesive spreaders in strawberry plants.

Keywords – Ascomycota – chemical control – cyprodinil – fluazinam – fludioxonil – gray mold – sclerotia – thiophanate methyl

Introduction

Botrytis cinerea, an ascomycetous fungus with broad distribution and notable versatility, poses a significant threat to a variety of agricultural crops, causing substantial economic losses and persistent challenges for farmers worldwide (Williamson et al. 2007, Romanazzi & Feliziani 2014). This phytopathogenic microorganism, popularly known as gray mold, has been the subject of intense research due to its ability to induce diseases both pre–and post–harvest, affecting more than 200 host plants species (Williamson et al. 2007).

The taxonomic placement of *B. cinerea* reveals its position in the class Leotiomycetes, order Helotiales and family *Sclerotiniaceae*. The fungal morphology is distinctive, featuring reproductive structures known as sclerotia, which contribute to its survival in adverse conditions and facilitate its spread (Groves & Drayton 1939). These sclerotia, often form on senescent plant material, and can persist in the soil for extended periods, representing a constant source of inoculum for subsequent infections.

Submitted 06 March 2024, Accepted 05 June 2024, Published 02 July 2024
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Accepting reviewer: Wei-Zhen Wang

The ecology of *B. cinerea* is intricate, demonstrating the ability to colonize a wide range of plant substrates. Its biology involves the production of a diverse array of cell wall–degrading enzymes, facilitating penetration into plant tissues and promoting effective host colonization (Nakajima & Akutsu 2014). The release of toxic secondary metabolites also plays a significant role in the pathogenicity of the fungus, inducing suppressed defense responses in the host plant (Collado & Viaud 2015).

Rosslenbroich & Stuebler (2000) provided some history background about chemical control of *B. cinerea* highlighting the molecules pyrimethanil, cyprodinil, mepanipyrim, fludioxonil and fenhexamid. Other approaches have been explored, such as biological control using *Trichoderma*, *Ulocladium*, *Bacillus subtilis*, and plant extracts along with their essential oils to manage *B. cinerea* (Abbey et al. 2019). Together with fungicides, biological and alternative pulverization, cultural, and physiological practices have also been explored over the years (Elad & Shtienberg 1995).

Given this context, the objective of this work is to examine the impact of registered fungicides for strawberry on the control of *B. cinerea*.

Materials & Methods

Mycelial growth influenced by fungicides

Two *B. cinerea* isolates from different locations were employed in this study. They were obtained in Caçador (26°49'03.1"S, 50°59'25.0"W) and Celso Ramos (27°37'03.3"S, 51°19'48.6"W, approximate location). The experiment was conducted in Petri dishes of 9 cm. After pouring the culture media malt extract (malt extract 20 g/L and agar 20 g/L). Fungicides were mixed in malt culture medium at the label dose (Table 1) to assess their effect on the mycelial growth. A disk measuring 0.5 cm containing fungal mycelium was deposited at the center of each Petri dish. The radial growth of the mycelium was measured after seven days until the fungicide–free treatment covered the entire growth medium.

Table 1 Fungicides and doses employed expressed as the amount of active ingredient/100 L of water.

Fungicides	Dose - Amout of active ingredient/100L
Boscalid	40 g
Iprodione	75 mL
Methyl-thiophanate	49 g
Pyrimetanil	60 mL
Procymidone	50 g
Difenoconazole	10 mL
Benzalkonium chloride	25 mL
Fluazinam	39.8 mL
Tebuconazole + Trifloxystrobin	15 + 7.5 mL
Pyraclostrobin + Fluxapyroxad	33.33 + 16.7 mL
Azoxystrobin	8 g
Boscalid + Cresoxim-methyl	40 + 20 mL
Tebuconazole	15.98 mL
Metconazole	9 mL
Cyprodinil + Fludioxonil	46.88 + 31.25 mL
Methyl-thiophanate + Fluazinam	48.75 + 48.75 mL

Halo formation due the presence of fungicide

The experiment was conducted in Petri dishes of 9 cm. After pouring the culture media, 100 µL of a spore suspension at 10^5 spores per mL was spread over the culture media surface using a Drigalski handle. In the center of the Petri dish, it was placed 10 µL of the fungicide solution at the label dose (Table 1). The inhibition halo was measured after seven days of incubation at 25 ºC and a photoperiod of 12 h.

Strawberry fruits colonization influenced by the fungicides

Strawberry fruits were previously immersed in a *B. cinerea* suspension at 10^5 spores per mL using both isolates. After 24h the strawberry fruits were re–immersed in the fungicide solution according to the treatment. The fungicides cyprodinil + fludioxonil, cyprodinil + fludioxonil + methyl–thiophanate + fluazinam, methyl–thiophanate + fluazinam, fluazinam, tebuconazole, difenoconazole and tebuconazole + trifloxystrobin were selected for this experiment because they were more promising to control *B. cinerea* based on the previous experiments. They were incubated in a plastic box closed with plastic at 20 ºC and 12 h of photoperiod for more seven days. They were assessed for the presence or absence of *B. cinerea* growing on the fruit.

Possible fitotoxicity effect of the fungicide on strawberry plants

The fungicides cyprodinil + fludioxonil, methyl–thiophanate + fluazinam, cyprodinil + fludioxonil + methyl–thiophanate + fluazinam, with and without an adhesive spreader, were applied to strawberry plants to ensure the safety of their application because they were the most promising in *B. cinerea* control. Fungicides were applied to the strawberry plants at the recommended label dose (Table 1), and after a three–day period, the foliage was examined to detect any potential phytotoxic effects.

Results

Considering the two isolates, the fungicides that stood out in the control of *B. cinerea* were cyprodinil + fludioxonil, fluazinam, methyl–thiophanate + fluazinam, difenoconazole, tebuconazole, and tebuconazole + trifloxystrobin (Fig. 1). There were differences between the isolates regarding the efficacy of the fungicides (Fig. 1).

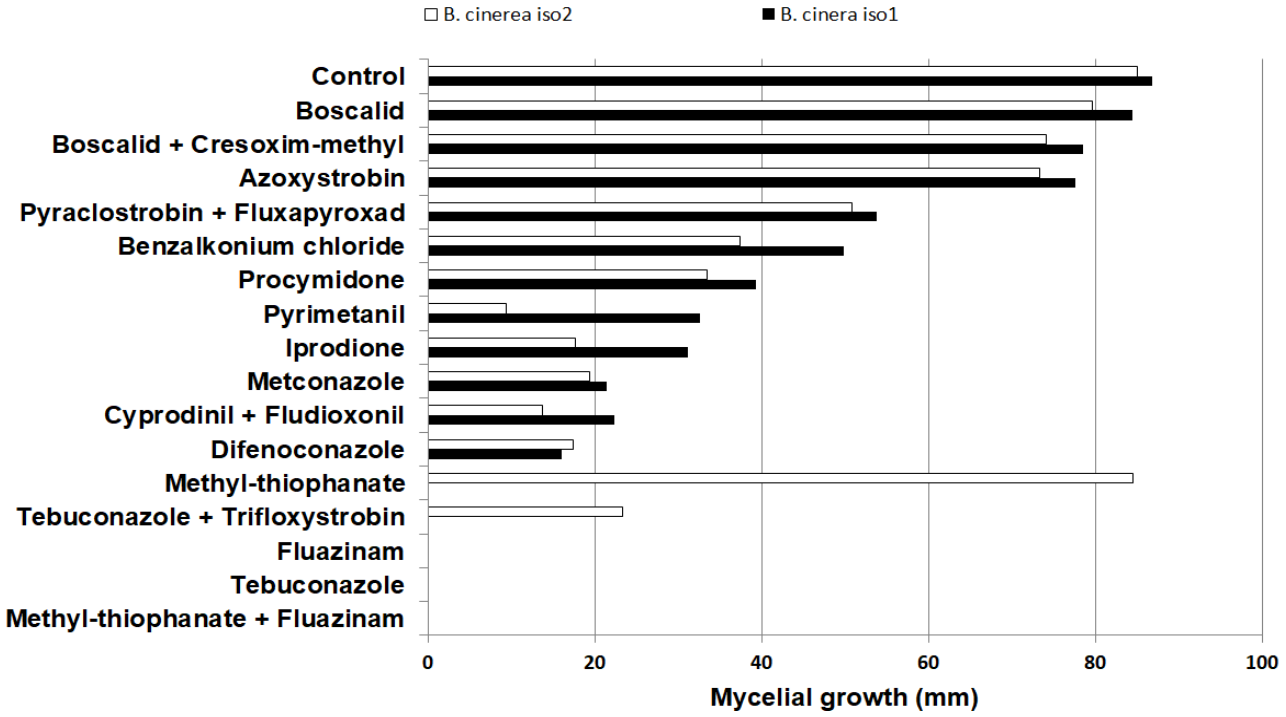

Fig. 1 – Effect of fungicides on the control of the mycelial growth of *Botrytis cinerea*.

In terms of inhibition zone formation, only fluazinam (Fig. 2), cyprodinil + fludioxonil, and methyl–thiophanate + fluazinam demonstrated the inhibition halo for both isolates (Table 2).

Table 2 Inhibition halo promoted by the fungicide.

Fungicide	Caçador Isolate	Celso Ramos isolate
Boscalid	0.00	0.00
Iprodione	0.00	0.00
Methyl-thiophanate	0.00	0.00
Pyrimetanil	0.00	0.00
Procymidone	0.00	0.00
Difenoconazole	0.00	0.00
Benzalkonium chloride	0.00	0.00
Fluazinam	16.99 ± 0.28	19.63 ± 0.59
Tebuconazole + Trifloxystrobin	0.00	0.00
Pyraclostrobin + Fluxapyroxad	0.00	0.00
Azoxystrobin	0.00	0.00
Boscalid + Cresoxim-methyl	0.00	0.00
Tebuconazole	0.00	0.00
Metconazole	15.68 ± 2.13	0.00
Cyprodinil + Fludioxonil	16.43 + 0.26	24.07 ± 3.90
Methyl-thiophanate + Fluazinam	19.14 ± 1.03	22.09 ± 0.04
Control	0.00	0.00

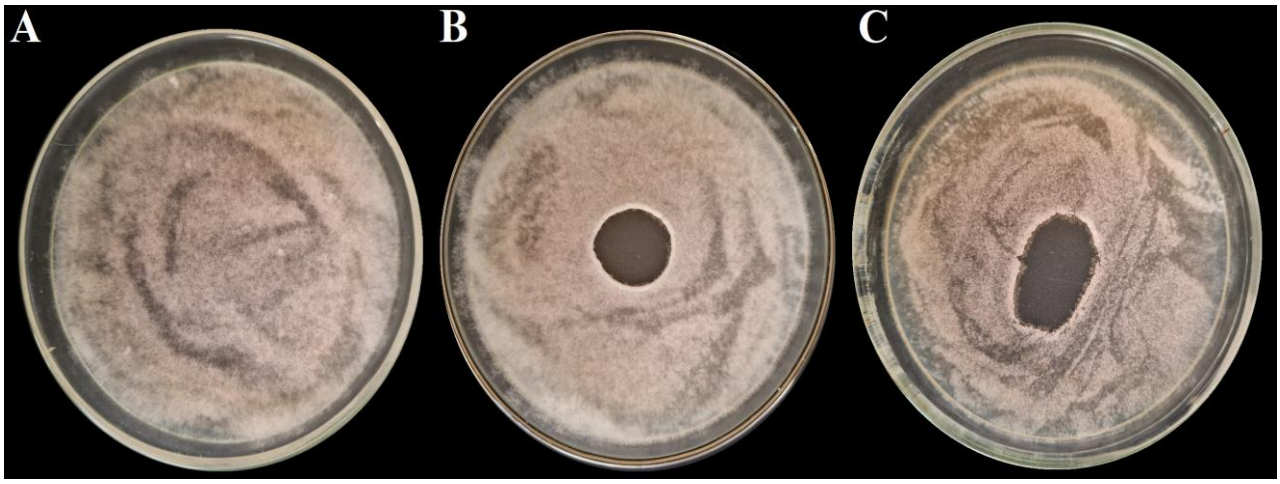

Fig. 2 – Effect of fluazinam on the control of *B. cinerea*. A treatment without fungicide. B halo promoted by fluazinam in *B. cinerea* isolate Caçador. C halo promoted by fluazinam in *B. cinerea* isolate Celso Ramos.

The fungicides cyprodinil + fludioxonil and methyl–thiophanate + fluazinam, as well as the combined application of these four fungicides, proved to be the most effective in controlling mycelial growth (Fig. 3).

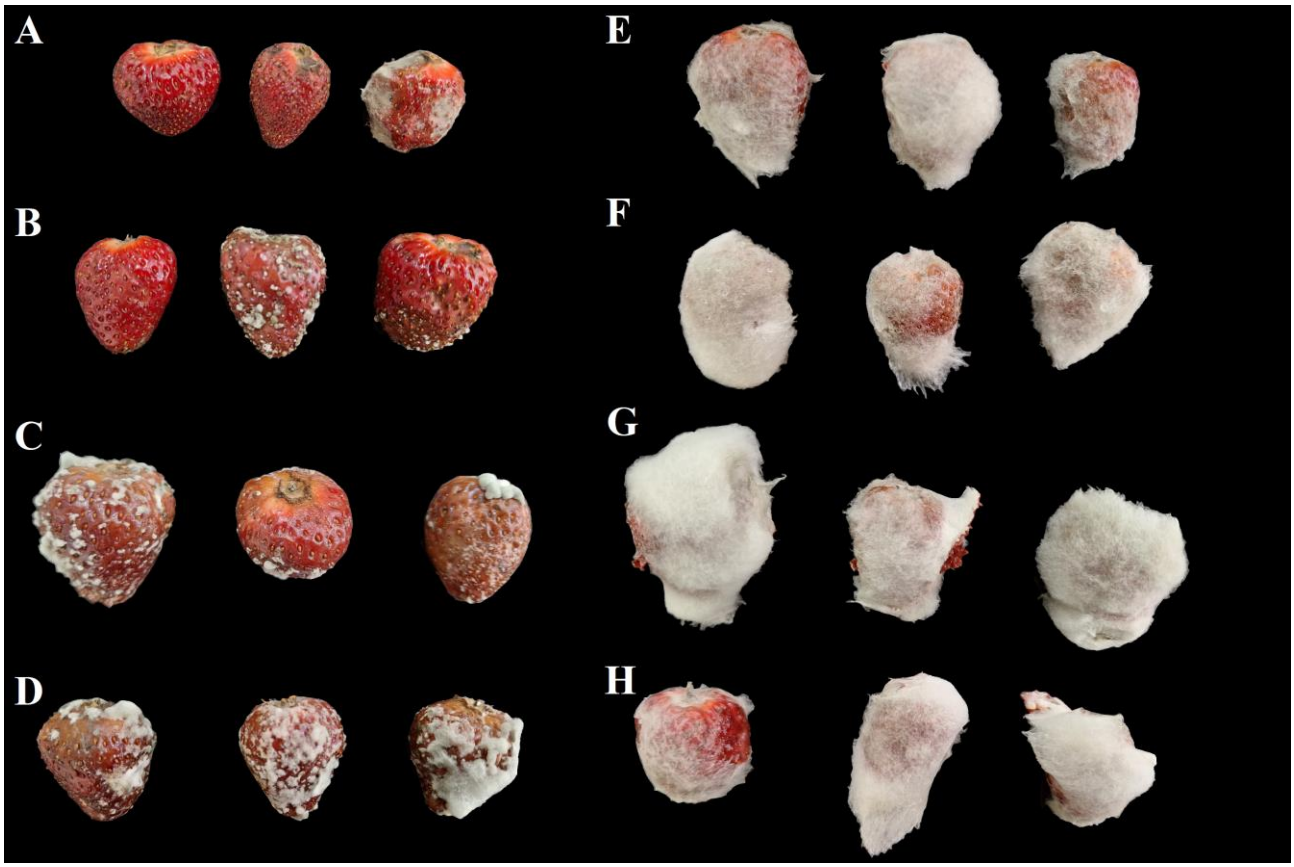

Fig. 3 – Effect of fungicides on the control of *B. cinerea* in strawberry fruits. A Cyprodinil + Fludioxonil. B Cyprodinil + Fludioxonil + Methyl–thiophanate + Fluazinam. C Methyl–thiophanate + Fluazinam. D Fluazinam. E Tebuconazole. F Difenoconazole. G Tebuconazole + Trifloxystrobin. H Control.

Phytotoxicity was only observed when the fungicides were used with the adhesive spreader (Fig. 4). The younger leaves exhibited not only necrosis but also some deformation. Pulverization without the adhesive spreader did not cause any phytotoxic symptoms.

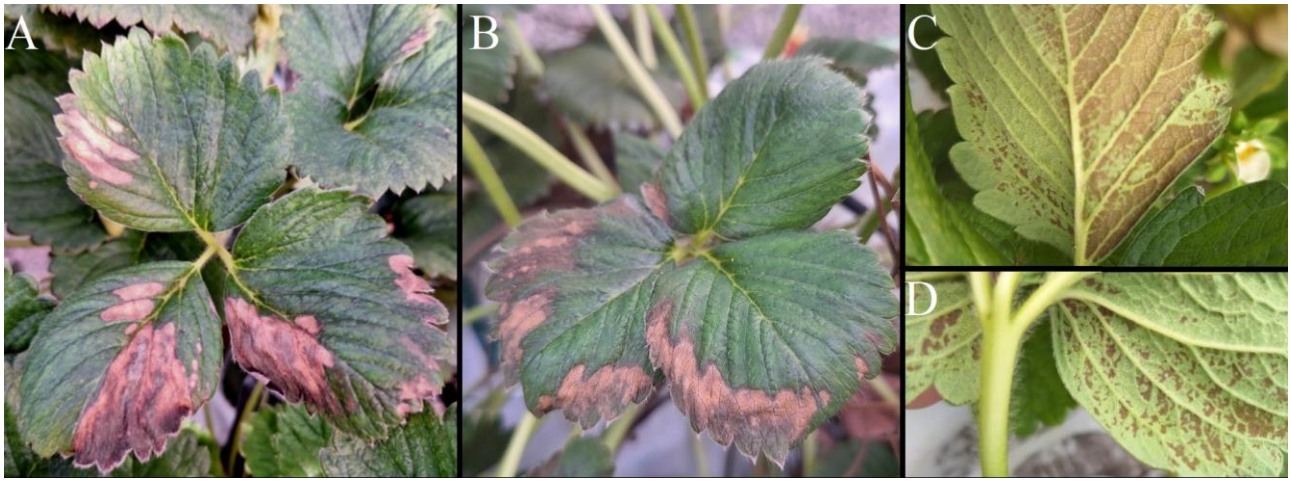

Fig. 4 – Fitotoxicity caused by methyl–thiophanate + fluazinam on strawberries when applied with adhesive spreader. A Strawberry leaves when applied methyl–thiophanate + fluazinam with adhesive spreader. B Strawberry leaves when applied methyl–thiophanate + fluazinam + Cyprodinil + Fludioxonil with adhesive spreader. C–D Strawberry leaves when applied with adhesive spreader only.

Discussion

The results of this study provided valuable insights into the effectiveness of various fungicides in controlling *B. cinerea* outbreaks in strawberry crops. The performance of selected fungicides was assessed in terms of their impact on *B. cinerea* control, mycelial growth inhibition, inhibition zone formation, and potential phytotoxic effects.

Among the tested fungicides, cyprodinil + fludioxonil, fluazinam, methyl–thiophanate + fluazinam, difenoconazole, tebuconazole, and tebuconazole + trifloxystrobin emerged as notable candidates effectively for *B. cinerea* control (Fig. 1). These fungicides have demonstrated promising results, showcasing their potential in addressing the challenges posed by *B. cinerea* in strawberry crops. However, it was crucial to acknowledge that there was variability in the efficacy of these fungicides between the two isolates, emphasizing the importance of considering regional differences and adaptability in disease management strategies (Fig. 1).

In terms of inhibition zone formation, fluazinam, cyprodinil + fludioxonil, and methyl–thiophanate + fluazinam exhibited consistent inhibition halos for both isolates, indicating their potential to impede the growth of *B. cinerea* (Table 2). This aligns with recent studies highlighting the role of these fungicides in creating effective barriers against the pathogen. Notably, fludioxonil and cyprodinil have been previously identified as excellent fungicides for controlling *B. cinerea* (Rosslenbroich & Stuebler 2000), similarly, fluazinam also demonstrated efficacy in controlling *B. cinerea* (Maia et al. 2021). These findings underscore the importance of considering synergistic effects when developing fungicide strategies against *B. cinerea*.

However, it is essential to note the observed phytotoxicity when fungicides were used in conjunction with the adhesive spreader (Fig. 4). Recent research indicated that the interaction between fungicides and adhesive spreaders may lead to adverse effects on plant health (Cowgill et al. 2013), as evidenced by the necrosis and deformation of younger leaves observed in this study. It seemed that when the spreader was applied together with the fungicide methyl–thiophanate + fluazinam, the phytotoxic effect was potentiated. Pulverization without the adhesive spreader, on the other hand, did not induce any phytotoxic symptoms, suggesting a need for careful consideration of application techniques to mitigate adverse effects on plant health.

This study emphasized the significance of selecting appropriate fungicides tailored to regional variations, understanding their inhibitory mechanisms, and considering the potential phytotoxic effects associated with specific application methods. The identified effective fungicides, along with insights into their application nuances, contributed valuable knowledge for enhancing strategies to combat *B. cinerea* in strawberry crops. Future research could explore further nuances in fungicide application, considering additional factors such as environmental conditions and crop varieties for a more comprehensive disease management approach.

Acknowledgements

The authors are thankful to the Agricultural Research and Rural Extension Enterprise of Santa Catarina (EPAGRI) and the Foundation for Research and Innovation Support of the State of Santa Catarina (FAPESC) for financing and encouraging the execution of the project that gave rise to this publication. In addition, we would like to thank the research assistant Daniela Alves Cardoso for her help during the execution of the experiments, and Edivânio Rodrigues de Araújo for donating the fungicide cyprodinil + fludioxonil to conduct this study.

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