UNIVERSIDADE FEDERAL DO PARANÁ

GABRIEL KOCH

SENSITIVITY OF *Phytophthora infestans* POTATO ISOLATES AND *Botrytis cinerea* STRAWBERRY ISOLATES TO FUNGICIDES

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GABRIEL KOCH

SENSITIVITY OF *Phytophthora infestans* POTATO ISOLATES AND *Botrytis cinerea* STRAWBERRY ISOLATES TO FUNGICIDES

Tese apresentada ao curso de Pós-Graduação em Produção Vegetal, Setor de Ciências Agrárias, Universidade Federal do Paraná, como requisito parcial à obtenção do título de Doutor em Produção Vegetal.

Orientador: Prof. Dr. Henrique da Silva Silveira Duarte

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O sucesso é ir de fracasso em
fracasso sem perder o entusiasmo.
- Winston Churchill

RESUMO

A requeima, provocada por *Phytophthora infestans* e o mofo cinzento, causado por *Botrytis cinerea*, são doenças que causam grandes perdas nas culturas da batateira e do morangueiro, respectivamente. O controle dessas doenças baseia-se, principalmente, no uso de fungicidas químicos, mas o uso intensivo desses produtos tem favorecido a seleção de isolados resistentes, comprometendo sua eficácia. Avaliar a sensibilidade dos patógenos aos fungicidas e monitorá-los antes da introdução de novos produtos é essencial para prolongar a vida útil dos fungicidas e adotar estratégias de manejo antirresistência. No primeiro capítulo desta tese, foi avaliada a sensibilidade de isolados de *P. infestans* coletados no Sul do Brasil aos fungicidas dimetomorphe (DM), mandipropamida (MD), cloridrato de propamocarbe (CP) e clorotalonil (CL), bem como testada a presença de resistência cruzada no isolados entre os fungicidas do grupo químico das amidas do ácido carboxílico, CAAs (dimetomorphe e mandipropamida). Para a avaliação de sensibilidade, 43 dos 68 isolados monoesporangiais coletados foram selecionados para DM, 47 para MD, 43 para CP, e 45 isolados para CL. A avaliação foi realizada por meio da concentração efetiva a 50% (CE_{50}), com avaliação do crescimento micelial. Os valores de EC_{50} variaram de 0.086 – 0.218 µg/mL para DM, 0.008 – 0.037 µg/mL para MD, 4.949 – >100.00 µg/mL para CP e 0.605 – 12.273 µg/mL para CL. Para o estudo da resistência cruzada, foi utilizada a análise de correlação de Spearman com os valores de EC_{50} de 30 isolados. O resultado demonstrou que há correlação fraca positiva (P=0.037 e r=0.38) entre a sensibilidade dos dois fungicidas estudados. No segundo capítulo, os objetivos foram desenvolver uma *baseline* para o mefentrifluconazol, um fungicida recentemente registrado para a cultura do morangueiro, para isolados de *B. cinerea* do Brasil e dos Estados Unidos; verificar resistência cruzada entre mefentrifluconazol e outros DMIs; identificar mutações no gene *CYP51*; e avaliar a eficácia comparativa do mefentrifluconazol com outros fungicidas disponíveis no mercado no controle da doença em frutos. As baselines foram desenvolvidas por meio do resultado do teste de CE_{50} de 155 isolados (70 do Brasil e 85 dos EUA). Os resultados mostraram que a CE_{50} média dos isolados do Brasil foi de 0.169 µg/ml, variando de 0.046 a 1.303 µg/ml, e a dos Estados Unidos foi de 0.084 µg/ml, variando de 0.030 a 0.384 µg/ml. Foram selecionados 20 isolados para o estudo da resistência cruzada entre DMIs com os fungicidas difenoconazol e triflumizol. A presença da resistência cruzada foi confirmada, bem como duas mutações no gene *CYP51* (G461S e R464K) nos isolados com maiores valores de CE_{50}. Nos testes com frutos destacados, foi avaliado o tratamento preventivo e curativo dos fungicidas mefentrifluconazol, fludioxonil e ciprodinil em três diferentes isolados (um sensível, um resistente ao mefentrifluconazole e um resistente ao mefentrifluconazole e ao ciprodinil). Em ambos os tratamentos, o melhor fungicida foi o fludioxonil. O mefentrifluconazol foi eficaz no controle de isolados sem mutações no gene *CYP51*, com desempenho comparável a fungicidas como fludioxonil e ciprodinil. Os resultados desses estudos destacam a importância do monitoramento contínuo da sensibilidade dos patógenos aos fungicidas, desde antes do uso do ingrediente ativo, fornecendo informações para a adoção de estratégias de manejo mais eficazes e sustentáveis, tanto na cultura da batata quanto do morangueiro, visando a mitigação do risco de resistência e a preservação da eficácia dos fungicidas disponíveis.

Palavras-chave: Controle químico; Mofo cinzento; Requeima; Sensibilidade; Resistência.

ABSTRACT

Late blight, caused by *Phytophthora infestans*, and gray mold, caused by *Botrytis cinerea*, are diseases that cause major losses in potato and strawberry crops, respectively. The control of these diseases is mainly based on the use of chemical fungicides, but the intensive use of these products has favored the selection of resistant isolates, compromising their effectiveness. Evaluating the sensitivity of pathogens to fungicides and monitoring after introducing new products is essential for extending the useful life of fungicides and adopting anti-resistance management strategies. In the first chapter of this thesis, the sensitivity of isolates of *P. infestans* collected in southern Brazil to the fungicides dimethomorph (DM), mandipropamid (MD), propamocarb hydrochloride (CP) and chlorothalonil (CL) was assessed, as well as the presence of cross-resistance in the isolates between the fungicides of the chemical group of carboxylic acid amides, CAAs (dimethomorph and mandipropamid). For the sensitivity assessment, 43 of the 68 monospore isolates collected were selected for DM, 47 for MD, 43 for CP, and 45 isolates for CL. The evaluation was carried out using the effective concentration at 50% (EC_{50}), with assessment of mycelial growth. The EC_{50} values ranged from 0.086 - 0.218 µg/mL for DM, 0.008 - 0.037 µg/mL for MD, 4.949 - >100.00 µg/mL for CP and 0.605 - 12.273 µg/mL for CL. Spearman's correlation analysis with the EC_{50} values of 30 isolates was used to study cross-resistance. Results showed a weak positive correlation (P=0.037 and r=0.38) between the sensitivity of the two fungicides studied. In the second chapter, the objectives were: i) to develop a baseline for mefentrifluconazole, a fungicide recently registered for strawberry cultivation, for isolates of *B. cinerea* from Brazil and the United States; ii) to verify cross-resistance between mefentrifluconazole and other DMIs; iii) to identify mutations in the *CYP51* gene; and iv) to evaluate the comparative efficacy of mefentrifluconazole with other fungicides available on the market in controlling the disease in fruit. The baselines were developed using the EC_{50} of 155 isolates (70 from Brazil and 85 from the USA). Results showed that the average EC_{50} of the isolates from Brazil was 0.169 µg/ml, ranging from 0.046 to 1.303 µg/ml, and that of the United States was 0.084 µg/ml, ranging from 0.030 to 0.384 µg/ml. Twenty isolates were selected for the study of cross-resistance between DMI fungicides difenoconazole and triflumizole. The presence of cross-resistance was confirmed, and two mutations in the *CYP51* gene (G461S and R464K) in the isolates with the highest EC_{50} values. In the tests with detached fruit, the preventive and curative treatment of the fungicides mefentrifluconazole, fludioxonil and cyprodinil was evaluated for three different isolates (one sensitive, one resistant to mefentrifluconazole and one resistant to mefentrifluconazole and cyprodinil). In both treatments, the best fungicide was fludioxonil. Mefentrifluconazole was effective in controlling isolates without mutations in the *CYP51* gene, with performance comparable to fungicides such as fludioxonil and cyprodinil. The results of these studies highlight the importance of continuous monitoring of the sensitivity of pathogens to fungicides, providing information for the adoption of more effective and sustainable management strategies in both potato and strawberry crops, with a view to mitigating the risk of resistance and preserving the effectiveness of available fungicides.

Keywords: Chemical control; Gray mold; Late blight; Sensitivity; Resistance.

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1 GENERAL INTRODUCTION

The potato crop (*Solanum tuberosum* L.) is one of the most important globally, serving as a basic food for millions of people and representing an important source of income for small and large producers (Food and Agriculture Organization - FAO, 2021). However, potato production is severely impacted by diseases, with late blight, caused by *Phytophthora infestans*, being one of the most important for the crop. This oomycete can devastate the entire crop within a few weeks, especially in climatic conditions favorable to its development (Haverkort et al., 2009).

Potato late blight management has focused on the use of chemical fungicides, including compounds such as phenylamides, acetamides, carbamates, carboxylic acid amides, and quinone outside inhibitors (QoIs). However, the emergence of resistance has also been reported, representing a critical challenge for the management of late blight (Gisi and Cohen, 1996; Lee et al., 1999; Corbiere et al., 2010; Miranda et al., 2010; Cooke et al., 2012). Studies to monitor the sensitivity of isolates of *P. infestans* are fundamental for understanding the dynamics of resistance and for indicating the best strategies for integrated disease management.

Similarly, the strawberry crop (*Fragaria* × *ananassa* Duch.) plays an important role in world agricultural production, standing out as one of the most valued fruits in economic and nutritional terms. Strawberries are widely appreciated for their taste and content of bioactive compounds such as vitamins, antioxidants and fiber (Giampieri et al., 2012). However, strawberry production faces major phytosanitary challenges, including the incidence of diseases caused by phytopathogens. Among these, *Botrytis cinerea*, the causal agent of gray mold, is one of the main threats, causing substantial losses both in the field and in the post-harvest period (Oliveira et al, 2017).

As with potato late blight, the control of *B. cinerea* has been based mainly on the use of chemical fungicides. However, the intensive use of these products has led to the emergence of resistant populations, reducing the effectiveness of management strategies (Hahn, 2014). Studies indicate that the resistance of isolates of *B. cinerea* to the main classes of fungicides, such as quinone outside inhibitors (QoIs), methyl benzimidazole carbamates (MBCs), dicarboxamides, succinate dehydrogenase inhibitors (SDHIs) and demethylation inhibitor (DMIs), represents a growing problem in strawberry-producing regions both in Brazil (Maia et al., 2021) and in the USA (Amiri et al., 2013), including the existence of isolates with multiple resistance to seven different fungicides (Maia et al., 2021). This problem is also found in isolates of *B. cinerea* from other crops (Bardas et al., 2010, Chen et al., 2024, Mao et al., 2024). Given this, combined with the scarcity of effective control alternatives, producers have reported the difficulty in managing the disease (Maia et al., 2023). Fungicides such as mefentrifluconazole have been developed to aid control, but to prolong the useful life of the available products and monitor the evolution of resistance over the years (Gao et al., 2018), it is essential to regularly assess the sensitivity of the isolates to the fungicides in use. Thus, establishing a baseline before introducing a new fungicide into crop management is essential to determine long-term effictive control.

The resistance of phytopathogens to fungicides compromises not only the sustainability of production systems, but also food safety. Based on this information, the objectives of this study were: i) to evaluate the sensitivity of isolates of *Phytophthora infestans* from potato plants collected in the states of Paraná, Santa Catarina and Rio Grande do Sul to the fungicides dimethomorph, mandipropamid, propamocarb hydrochloride and chlorothalonil, four fungicides that have been widely used to control late blight in southern Brazil; ii) to check for cross-resistance between fungicides from the CAA chemical group; iii) to develop a baseline for the new fungicide mefentrifluconazole for *Botrytis cinerea* isolates from Brazil and the United States; iv) to determine cross-resistance between mefentrifluconazole and other DMIs; v) to identify the presence of mutations in the *CYP51* gene; and vi) to verify practical resistance and compare the control efficiency of mefentrifluconazole compared to other fungicides available on the market.

REFERENCES

Amiri, A., Heath, S.M., and Peres, N.A. 2013. Phenotypic characterization of multifungicide resistance in Botrytis cinerea isolates from strawberry fields in Florida. **Plant Disease**. 97:393-401.

Bardas, G.A., Veloukas, T., Koutita, O., and Karaoglanidis, G.S. 2010. Multiple resistance of Botrytis cinerea from kiwifruit to SDHIs, QoIs and fungicides of other chemical groups. **Pest Manag Sci**. 66:967–973.

Chen, L., Sun, B., Zhao, Y., and Miao, Z. 2024. Molecular Mechanisms and Biological Characteristics of *Botrytis cinerea* Field Isolate Resistance to Pyrisoxazole in Liaoning Province. **Plant Disease**. 108:866-876.

Cooke, L.R., Kildea, S., Mehenni-Ciz, J., Quinn, L., Little, G., Hutton, F., Perez, F.M., Deahl, K.L., Griffin, D. 2012. Ongoing changes in the Irish potato late blight population. **13th EuroBlight Workshop**.

Corbiere, R., Magalon, H., Boulard, F., Andrivon, D. 2010. Study of invasive French populations (2006-2008) of *Phytophthora infestans*, the Oomycete causing potato late blight. **12th EuroBlight Workshop**.

Food and Ariculture Organization of the United Nations (FAO), Roma, 2025. Disponível em: http://www.fao.org/faostat/. Acesso em: 03 de janeiro de 2025.

Gao, Y., He, L., Mu, W., Li, B., Lin, J., and Liu, F. 2018. Assessment of the baseline sensitivity and resistance risk of Colletotrichum acutatum to fludioxonil. **Eur J Plant Pathol**. 150:639–651.

Giampieri, F., Tulipani, S., Alvarez-Suarez, J. M., Quiles, J. L., Mezzetti, B., Battino, M. (2012). The strawberry: Composition, nutritional quality, and impact on human health. **Nutrition**. 28(1), 9-19.

Gisi, U., Cohen, Y. 1996. RESISTANCE TO PHENYLAMIDE FUNGICIDES: A Case Study with *Phytophthora infestans* Involving Mating Type and Race Structure**. Annual Review Phytopathology**. 34:549-572.

Hahn, M. 2014. The rising threat of fungicide resistance in plant pathogenic fungi: Botrytis as a case study. **Journal of Chemical Biology**. 7(4), 133-141.

Haverkort, A.J., Struik, P.C., Visser, R.G.F., Jacobsen, E. 2009. Applied Biotechnology to Combat Late Blight in Potato Caused by *Phytophthora infestans*. **Potato Research**. 52:249–264.

Lee, T.Y., Mizubuti, E., Fry, W.E. 1999. Genetics of Metalaxyl Resistance in *Phytophthora infestans*. **Fungal Genetics and Biology**. 26:118–130.

Maia, J.N., Beger, G., Pereira, W.V., May De Mio, L.L., and Duarte, H.S.S. 2021. Gray mold in strawberries in the Paraná state of Brazil is caused by *Botrytis cinerea* and its isolates exhibit multiple-fungicide resistance. **Crop Protection**. 140:105415.

Maia, J.N., Beger, G., Peres, N.A., Nesi, C.N., May De Mio, L.L., and Duarte, H.S.S. 2023. Baseline sensitivity of *Botrytis cinerea* strawberry isolates from Brazil to fludioxonil and cyprodinil and disease control efficacy. **Tropical Plant Pathology.** 49:131–140.

Mao, Y., Meng, X., Zhang, Z., Zhang, L., Feng, Y., Wang, T., Zhang, J., Cai, Y., Wang, J., Sheng, G., Shen, Y., Zhou, M., and Duan, Y. 2024. Genetic variation of drug target CYP51 conferring resistance to ergosterol biosynthesis inhibitors in *Botrytis cinerea*, causing lily gray mould. **Industrial Crops & Products**. 208:117797.

Miranda, B.E.C., Suassuna, N.D., Reis, A. 2010. Mating type, mefenoxam sensitivity, and pathotype diversity in *Phytophthora infestans* isolates from tomato in Brazil. **Pesquisa agropecuária brasileira**. 45:671-679.

Oliveira, M.S., Amiri, A., Zuniga, A.I., and Peres, N.A. 2017. Sources of Primary Inoculum of *Botrytis cinerea* and Their Impact on Fungicide Resistance Development in Commercial Strawberry Fields. **Plant Disease.** 101:1761-1768.

2 CHAPTER 1: SENSITIVITY OF ISOLATES OF *PHYTOPHTHORA INFESTANS* TO THE FUNGICIDES DIMETHOMORPH, MANDIPROPAMID, PROPAMOCARB HYDROCHLORIDE AND CHLOROTHALONIL

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ABSTRACT

The production of potato plants (*Solanum tuberosum* L.) is affected by various factors and the occurrence of diseases is one of the main ones. One of the most important diseases affecting the crop is late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary. Chemical control of the disease is the most widely used because it is the most efficient. However, there is a high number of sprays and, at the same time, difficulty in controlling it at field level with the fungicides available on the market due to the possible occurrence of reduced sensitivity of the oomycete to fungicides. The aim of this study was therefore to assess the sensitivity of isolates of *P. infestans* to the active ingredients dimethomorph (DM), mandipropamid (MD), propamocarb hydrochloride (CP) and chlorothalonil (CL); to identify the resistance factor for the fungicides CP and CL; and to verify whether there is a correlation between the EC_{50} values of the fungicides of the carboxylic acid amide group (CAAs), DM and MD. We selected 43 monospore isolates of *P. infestans* for DM, 47 for MD, 43 isolates for CP, and 45 for CL, which were collected in the main potato-growing regions in the states of Paraná, Santa Catarina and Rio Grande do Sul. Sensitivity to fungicides was determined by in vitro tests of 50% effective concentration (EC_{50}) with different doses for each fungicide, by means of mycelial growth evaluations. To study the correlation between fungicides, Spearman's analysis was used with the EC_{50} values of 30 isolates. The EC_{50} values found for dimethomorph were between 0.086 and 0.218 μg/mL, for mandipropamid between 0.008 and 0.037 μg/mL, for the fungicide propamocarb hydrochloride values were found in the range of 4.949 and >100.00 μg/mL and for chlorothalonil values between 0.605 and 12.273 μg/mL were observed. Eight isolates were phenotypically classified as highly resistant (HR) to propamocarb hydrochloride. Furthermore, despite the high sensitivity, it was possible to identify a moderate level of positive correlation in the isolates tested for the fungicides dimethomorph and mandipropamid. Finally, the results indicate that the fungicides propamocarb hydrochloride and chlorothalonil showed a wide variation in the sensitivity of the *P. infestans* isolates. These data reinforce the importance of continuous monitoring of fungicide efficacy to guide integrated management strategies and prevent the development of resistance.

Keywords: Chemical control, Late blight, Resistance, Solanum tuberosum L.

Introduction

The potato (*Solanum tuberosum* L.) originated in the Andes Mountains in South America. However, it is a crop that is widely distributed throughout the world. With a production in 2023 of 383.1 million tons on 16.8 million hectares (Food and Agriculture Organization - FAO, 2025), the potato is one of the most important food crops in the world. In 2023, the world's largest producer was China, with around 93.5 million tons and, in this scenario, Brazil appeared in 18th place, with an estimated production of 4.2 million tons (FAO, 2025). The state of Minas Gerais is the country's largest producer of the vegetable with around 1.4 million tons of production on approximately 38.8 thousand hectares planted. The southern region represents approximately 33.3% of national production, with the state of Paraná being the most representative, in second place nationally, with 791.9 thousand tons, followed by Rio Grande do Sul, with a production of 468.0 thousand tons, and Santa Catarina with 149.2 thousand tons produced (Instituto Brasileiro de Geografia e Estatística - IBGE, 2025).

This production, however, is affected by various factors and the occurrence of diseases has a prominent position. One of the most important diseases affecting the crop is late blight, caused by the oomycete *Phytophthora infestans* (Mont.) de Bary. It is a disease spread rapidly by the wind and, in conditions of high humidity and temperatures between 12 and 20ºC (Fohner et al., 1984), can devastate the crop entirely within a few weeks (Haverkort et al., 2009).

Efficiency in controlling late blight is directly linked to the adoption of a set of measures that are part of integrated disease management, such as: cultivars with a higher level of resistance, crop rotation, avoiding planting in damp and foggy places and the use of fungicides (Töfoli et al., 2013). In general, the cultivars with the highest market acceptance are susceptible or moderately susceptible to late blight, making the use of fungicides more important (Töfoli et al., 2016). Fungicides play a crucial role in the integrated control strategy for late blight (Schepers et al., 2018). Chemical control is the most widely used in the crop, as it is seen as the most efficient for the disease (Aylor et al., 2001; Kirk et al., 2005) and can be applied preventatively and curatively.

Climatic conditions favorable to the disease, together with the susceptibility of the main host cultivars, result in fungicides being sprayed 15 to 20 times per harvest (Schepers and Cooke, 2015). This high number of sprays results in selection pressure, which leads to the risk of fungicide resistance. The first reports of *P.*

infestans resistance occurred with the active ingredient metalaxyl in the Netherlands (Davidse et al., 1981) and in Ireland in 1981 (Dowley and O'Sullivan, 1981). Because it is a fungicide that is widely used and has been reported to be ineffective in the field, metalaxyl has been studied in many studies and resistance has been found in several (Gisi and Cohen, 1996; Lee et al., 1999; Corbiere et al., 2010; Miranda et al., 2010; Cooke et al., 2012).

In Brazil, the following fungicides with systemic action have been evaluated and the states in which the isolates were collected: metalaxyl with isolates from Rio Grande do Sul (RS), Santa Catarina (SC) , Paraná (PR), São Paulo (SP), Rio de Janeiro (RJ), Minas Gerais (MG), Espírito Santo (ES) (Reis et al., 2005), isolates from Goiás (GO) and Distrito Federal (DF) (Reis et al., 2006), isolates from PR, SC and RS (Santana et al., 2013), and isolates from PR, SC and RS (Casa-Coila, 2014); dimetomorph with isolates from RS, SC, PR, SP, MG, and Bahia (BA) (Oliveira, 2010), isolates from SP, PR, MG, RS and Roraima (RR) (Zanotta, 2019); cimoxanil with isolates from RS, SC, PR, SP, MG, RJ and ES (Reis et al., 2005); propamocarb hydrochloride with isolates from RS, SC, PR, SP, MG and BA (Oliveira, 2010), isolates from SP, PR, MG, RS and RR (Zanotta, 2019); mandipropamid with isolates from RS, SC, PR, SP, MG and BA (Oliveira, 2010); fenamidone with isolates from RS, SC, PR, SP, MG and BA (Oliveira, 2010); mefenoxam with isolates from DF and GO (Reis et al., 2006), isolates from SC, MG, RJ, DF, BA, ES and GO (Miranda et al., 2010); and cyazofamid with isolates from RS, PR, SP, MG and RR (Zanotta, 2019). To date, only five studies have examined the sensitivity of Phytophthora infestans to the multi-site fungicide chlorothalonil in Brazil, using isolates from the states of RS, SC, PR, SP, MG, BA, ES and RR (Reis et al., 2005; Oliveira, 2010; Casa-Coila, 2014; Zanotta, 2019). Considering the historical and current relevance of this pathogen, the number of studies carried out on its sensitivity to fungicides is still limited.

Cross-resistance occurs when pathogen populations that develop resistance to one fungicide also automatically and simultaneously become resistant to other fungicides that share the same mechanism of action and are affected by the same genetic mutation (Brent and Hollomon, 2007). This phenomenon has been widely reported among fungicides from the chemical group of carboxylic acid amides (CAAs) for various oomycetes (Lu et al., 2010; Wang et al., 2010, FRAC, 2025). However, as far as we know, there are still no records of cross-resistance involving *P. infestans*.

Most studies on the sensitivity of Phytophthora infestans to fungicides were carried out more than 10 years ago, the most recent being by Zanotta (2019), who evaluated only seven isolates collected in southern Brazil. Despite the lack of recent studies, four fungicides have been widely used to control late blight in the South: dimethomorph and mandipropamid, belonging to the chemical group of carboxylic acid amides (subgroup H5 of the Fungicide Resistance Action Committee - FRAC); propamocarb hydrochloride, from the carbamate group (subgroup F4 of FRAC); and chlorothalonil, a non-systemic multisite fungicide classified in group M of FRAC. Therefore, there is a need to carry out studies on the sensitivity of isolates to these fungicides currently used, with a focus on collecting a greater number of isolates in the southern region of Brazil. In addition, there is a need to verify the correlation between isolates to fungicides from the CAA group, assessing the risks of cross-resistance. In view of this, the aim of this study was i) to assess the sensitivity of isolates collected in the states of Paraná, Santa Catarina and Rio Grande do Sul to the fungicides dimethomorph, mandipropamid, propamocarb hydrochloride and chlorothalonil; and ii) to check for correlation between fungicides from the CAAs chemical group.

MATERIAL AND METHODS

Isolate collection

Samples from potato production fields were collected in 16 different cities in the three southern Brazilian states of Paraná, Santa Catarina and Rio Grande do Sul during the years 2021, 2022 and 2023. A total of 68 isolates of *P. infestans* were obtained from 22 fields in the region (Figure 1). Monospore isolates were obtained from all the isolates collected to reduce variability.

Sensitivity of isolates to fungicides

A preliminary literature search was carried out to identify the main studies, with the location of the study and the range of EC_{50} values found for the fungicides also studied in this work: dimethomorph, mandipropamid, propamocarb hydrochloride and chlorothalonil (Supplementary Table 2). The sensitivity of the isolates was assessed by evaluating mycelial growth to the four fungicides. We used 43 isolates for the fungicide dimethomorph (Forum®), 47 isolates for mandipropamid (Revus®), 43 isolates for propamocarb hydrochloride (Proplant®) and 45 isolates for the protective fungicide chlorothalonil (Absoluto®). The four fungicides were diluted in autoclaved distilled water following the concentrations for each fungicide: 0.0, 0.03, 0.1, 0.3, 0.5, 1.0 and 3.0 µg/mL for dimethomorph, 0.0, 0.01, 0.02, 0. 03, 0.04, 0.05 and 0.1 µg/mL for mandipropamid, 0.0, 1.0, 5.0, 10.0, 50.0, 100.0 and 1000.0 µg/mL for propamocarb hydrochloride and 0.0, 0.5, 1.0, 5.0, 10.0, 25.0 and 100.0 µg/mL for chlorothalonil. A plug of culture medium with mycelium (5 mm in diameter) seven days old was transferred to V8 medium (100 mL of V8, 1 g CaCO3 and 15 g of agar and topped up with distilled water to 1 L of medium), along with the concentrations of each fungicide mentioned above in 90 mm Petri dishes. All the plates were incubated at 17ºC for 7 to 14 days in the dark. This variation was based on the growth rate of each isolate. After this period, the diameter of the colonies was measured using a digital caliper and the diameter was used to calculate the sensitivity of the isolates. The EC_{50} was evaluated using regression analysis with the logarithm of the percentage of mycelial inhibition in relation to the control. The percentage of inhibition was calculated using the formula: %IMG = (Mc - Mt) / Mc x 100, where IMG = inhibition of mycelial growth, Mc = average mycelial growth of the control and Mt = average mycelial growth of the treatment analyzed. All experiments were carried out in duplicate.

The resistance factor (RF) was determined for the fungicides propamocarb hydrochloride and chlorothalonil. The calculation was based on the ratio between the EC_{50} value of each isolate and the lowest EC_{50} observed among the isolates tested, as described by Gouot (1994) in Rekanovic et al. (2011). The level of resistance was classified according to the following scale (Gouot, 1994 in Rekanovic et al., 2011): RF < 3 - Sensitive isolate (S); 3 ≤ RF ≤ 20 - moderately resistant isolate (MR); and RF > 20 - highly resistant isolates (HR).

Correlation between dimethomorph and mandipropamid

Thirty isolates with EC_{50} values for the fungicides dimethomorph and mandipropamid, both belonging to the chemical group of carboxylic acid amides (CAAs), were selected for correlation analysis. Spearman's analysis was carried out with the log-EC_{50} values to test the correlation between the fungicides. Spearman's correlation analysis was carried out using R software version 4.2.2 (R Core Team, 2024). The EC_{50} values of dimethomorph from 30 isolates were plotted against the EC_{50} values of the same isolates for mandipropamid.

RESULTS

Sensitivity of isolates to fungicides

The EC_{50} values for the fungicide dimethomorph (DM) ranged from 0.086 µg/mL in an isolate from Mangueirinha, Paraná (MAPR3) to 0.218 µg/mL in an isolate from Castro, Paraná (CAPR3). For mandipropamid, the EC_{50} values ranged from 0.008 µg/mL from an isolate collected in Canoinhas, Santa Catarina (CASC3) to 0.037 µg/mL from an isolate from Guarapuava, Paraná (GUPR6). For the fungicide propamocarb hydrochloride, the EC_{50} values found ranged from 4,949 µg/mL from an isolate from Mangueirinha, Paraná (MAPR2) to isolates with EC_{50}s above 100.00 µg/mL from eight different isolates, all from the state of Paraná. As for the fungicide chlorothalonil, the values observed ranged from 0.605 µg/mL from an isolate from Água Doce, Santa Catarina (ADSC3) to 12.273 µg/mL observed in an isolate collected in Antônio Olinto, Paraná (AOPR6) (Table 1 and Supplementary table 1).

For the fungicide dimethomorph, 4.6% of the isolates had EC_{50} values below 0.105 µg/mL and 11.6% (5 out of 43 isolates) had values above 0.2 µg/mL. Most isolates (81.4%) had EC_{50} values between 0.105 and 0.2 µg/mL. The results for mandipropamid showed that only 4.3% of the isolates had EC_{50} values below 0.0121 µg/mL, 6.4% of the isolates had EC_{50} values above 0.033 µg/mL and most of the isolates (89.4%) had EC_{50} values between 0.0121 and 0.0329 µg/mL. Around 18.60% of the isolates tested had an EC_{50} value above 100.00 µg/mL for propamocarb hydrochloride. For this fungicide, 37.2% of the isolates had EC_{50} values below 20,791 µg/mL, 23.3% of the isolates had EC_{50} values between 20,791 and 36,633 µg/mL and the remaining isolates (20.9%) had EC_{50} values between 36,634 and 84,159 µg/mL. In chlorothalonil, only one isolate (2.2%) showed an EC_{50} above 10,607 µg/mL, most isolates (55.6%) showed EC_{50} values between 2,272 and 3,939 μg/mL, while 15.6% of the isolates tested showed values below 2,727 μg/mL (Figure 2 and Supplementary figure 1).

The resistance factor analysis revealed that ten isolates were classified as sensitive to propamocarb hydrochloride. Most isolates (58%) were considered moderately resistant, while eight isolates showed high resistance to this fungicide. For chlorothalonil, three isolates were classified as sensitive. However, the vast majority (91%) were phenotypically classified as moderately resistant. Only one isolate, from Antônio Olinto/PR, was identified as highly resistant (Table 2).

Correlation between dimethomorph and mandipropamide

When comparing the EC_{50} values of 30 isolates with the fungicides dimethomorph and mandipropamid, there was a positive correlation (r=0.38) between the resistance of *P. infestans* to dimethomorph and mandipropamid using the Spearman test ($p<0.05$) with the log-EC_{50} values (Figure 3).

DISCUSSION

The assessment of the sensitivity of *P. infestans* to the fungicides used in this study is little explored in the literature, despite the importance of the pathogen and the limited availability of fungicides on the market. Seven studies were identified on the sensitivity of *P. infestans* to the fungicide dimetomorph, from the CAA group, two of which were carried out in Brazil. For mandipropamid, another fungicide from the same group, five relevant studies were found, only one of which was conducted in Brazil (Oliveira, 2010). Regarding to propamocarb hydrochloride, which belongs to the carbamate chemical group, five studies were identified, two of which were carried out in Brazil. Finally, seven studies were carried out on the multisite fungicide chlorothalonil, four of which focused on Brazilian isolates (Supplementary table 2). One possible explanation for this lack of studies is the complexity involved in laboratory experiments, from the need for traps to avoid contamination during isolation to the absence of effective storage methods. Even so, the available studies indicate that the *P. infestans* population in this study has proved sensitive to fungicides from the CAAs group, mandipropamid and dimethomorph. On the other hand, the high EC_{50} values observed for propamocarb hydrochloride draw attention, suggesting a possible reduction in sensitivity to the fungicide. The values obtained for chlorothalonil reinforce the need for continuous monitoring, as they were higher than the average reported in previous studies.

The EC_{50} values for dimethomorph (0.086 to 0.218 µg/mL) suggest that there was no reduction in sensitivity in the pathogen population, since in Oliveira, 2010 EC_{50} values ranging from 0.03 to 1.46 µg/mL were observed, in Stein and Kirk (2004) the values found were between 0.11 and 0.84 µg/mL, in addition to corroborating what was observed in Mazakova et al. (2011 and 2018) where all the isolates tested were sensitive to dimethomorph. However, the valeus found in this work differ from those observed for some isolates where it has already been possible to observe resistance of the pathogen to the fungicide at field level in the states of São Paulo and Rio Grande do Sul (Zanotta, 2019) and also induced under UV light in the laboratory (Rubin et al., 2008). In this study, the highest EC_{50} values for dimethomorph occurred in cities which, according to conversations with producers, have a high number of sprays per cycle (Guarapuava and Castro), helping to select fewer sensitive isolates. These facts show that there is a need for periodic studies to monitor this reduction in sensitivity.

The EC_{50} values obtained for mandipropamid (0.008 to 0.037 µg/mL) were lower than those observed for dimethomorph. Although none of the isolates showed a reduction in sensitivity, a positive correlation was identified between the EC_{50}

values of the two fungicides, suggesting a possible relationship between them within the CAA group. This finding is relevant, as it indicates a potential risk of cross-resistance should resistance to one of the active ingredients emerge - a phenomenon already reported for CAAs in other oomycetes (Lu et al., 2010; Wang, 2010). The low EC_{50} values observed in this study corroborate the results of previous research with mandipropamid (Cohen et al., 2007; Oliveira, 2010; Kildea et al., 2014; Saville, 2015), in which highly sensitive isolates were also reported.

The high sensitivity of the isolates to fungicides from the CAA group observed may be linked to a number of factors. The first is partly due to good fungicide management strategies on farms, where there is a rotation of active ingredients and chemical groups and consequently less use of these products. In addition, most of the commercial products used are already developed in combinations of specific fungicides with multi-sites (Ojiambo et al., 2010), which generates a low selection pressure in the pathogen population. Another factor to consider is the fact that the artificially generated mutations (Blum et al., 2010) found in the genes where these fungicides act are not persistent, i.e. resistance is unstable (Rubin et al., 2008). This is probably because the inheritance of resistance in oomycetes is more complicated than in true fungi, due to their diploid nature. Most studies show that resistance to fungicides from the CAA group depends on recessive alleles (Cai et al., 2021). For all these reasons, the risk of resistance of *P. infestans* to CAA fungicides is considered low (Gisi and Sierotzki, 2008).

The EC_{50} values found for the systemic fungicide propamocarb hydrochloride were the highest observed in this study (4,494 to >100.00 µg/mL), with eight isolates classified as highly resistant. However, these results corroborate the values in other studies in which the isolates were not characterized as resistant (Grünwald et al., 2006; Lehtinen et al., 2007), even though there is evidence of a significant reduction in sensitivity (Lehtinen et al., 2007). In this study, all the isolates tested showed mycelial growth at a concentration of 1000 µg.mL^{-1}, but only isolates with growth at concentrations above 10000 µg.mL^{-1} were considered resistant (Zanotta, 2019). On the other hand, other studies have shown that the *P. infestans* population studied was already more sensitive to propamocarb hydrochloride, with EC_{50} values below 3.0 µg/mL in Colombia (Garcia et al., 2008) and also did not exceed 34.1 µg/mL in isolates collected in different regions of Brazil (Oliveira, 2010).

The multi-site fungicide chlorothalonil is an alternative widely used by producers to control late blight, mainly because it is considered a low-risk product for developing resistance. In addition, these fungicides are widely used on the crop, as they are mainly present in mixtures with difenoconazole, cymoxanil and azoxystrobin (Agrofit, 2025). The EC_{50} values observed in this study (0.605 to 12.273 µg/mL) show that some isolates were classified as sensitive and only one was phenotypically classified as highly resistant to chlorothalonil, and therefore demand attention, since the values found are, on average, above those found in other studies both in Brazil (Reis et al., 2005; Oliveira, 2010; Casa-Coila, 2014) and in other locations around the world (Sujkowski et al., 1995; Kato et al., 1997). On the other hand, there is a more recent report of isolates found in Brazil that were resistant to chlorothalonil using the microtiter technique (Zanotta, 2019). This resistance to multisite fungicides, as well as the difference in sensitivity found in this work in the different isolates, may be linked to the way in which resistance to multisite fungicides occurs, which would be the overproduction of ABC transporters (Stergiopoulos et al., 2003) or by the excess production of thiols that end up detoxifying the fungicides (Leroux et al., 2002). The AOPR6 isolate with the highest EC50 value (12.273 µg/mL) comes from an area in the city of Antônio Olinto-PR, where the number of applications per crop cycle is low (12 to 15 sprays) compared to some areas in Guarapuava, for example (20 to 25 sprays), but there is no rotation of active ingredients, due to the low range of products, generating greater selection pressure for the fungicides used there.

Experiments were conducted on detached leaflets using other active ingredients with different modes of action. However, due to the difficulty in setting up the experiment, especially in relation to the viability of the isolates of *P. infestans*, and the lack of repeatability between trials, it was decided not to include this part in the thesis.

The importance of potato growing in Brazil and around the world is great and, given the aggressiveness of late blight and the dependence on fungicides to control the disease, despite the low EC_{50} values found in this study, there is a need for periodic studies into the sensitivity of the products. Research with a larger number of isolates and a wider range of fungicides is essential. This is all to help the producer manage spraying, increasing the useful life of the product in the field, so as to maintain its efficiency in controlling the disease.

REFERENCES

Agrofit (2025) Sistema de agrotóxicos fitossanitários. Available at: http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons. Accessed on January, 2025.

Aylor DE, Fry WE, Mayton H, Andrade-Piedra JL (2001) Quantifying the rate of release and escape of *Phytophthora infestans* sporangia from a potato canopy. Phytopathology 91:1189-1196.

Blum M, Boehler M, Randall E, Young V, Csukai M, Kraus S, Moulin F, Scalliet G, Avrova AO, Whisson SC, Fonne-Pfister R (2010) Mandipropamid targets the cellulose synthase-like PiCesA3 to inhibit cell wall biosynthesis in the oomycete plant pathogen, *Phytophthora infestans*. Molecular Plant Pathology 11(2):227–243.

Brent KJ, Hollomon DW (2007) Fungicide resistance in crop pathogens: How can it be managed? FRAC Monograph No. 1, 2 nd Ed. CropLife International, Brussels, Belgium.

Cai M, Li T, Lu X, Chen L, Wang Q, Liu X (2021) Multiple mutations in the predicted transmembrane domains of the cellulose synthase 3 (CesA3) of *Phytophthora capsici* can confer semi-dominant resistance to carboxylic acid amide fungicides. International Journal of Biological Macromolecules 193:2343-2351.

Casa-Coila VH (2014) Caracterização de isolados de *Phytophthora infestans* (Mont.) De Bary provenientes de batata do Sul do Brasil associada ao estudo da resistência em Solanum spp. Tese (Doutorado em Agronomia) 126f.

Cohen Y, Rubin E, Hadad T, Gotlieb D, Sierotzki H, Gisi U (2007) Sensitivity of *Phytophthora infestans* to mandipropamid and the effect of enforced selection pressure in the field. Plant Pathology 56:836–842.

Cooke LR, Kildea S, Mehenni-Ciz J, Quinn L, Little G, Hutton F, Perez FM, Deahl KL, Griffin D (2012) Ongoing changes in the Irish potato late blight population. 13th EuroBlight Workshop.

Corbiere R, Magalon H, Boulard F, Andrivon D (2010) Study of invasive French populations (2006-2008) of *Phytophthora infestans*, the Oomycete causing potato late blight. 12th EuroBlight Workshop.

Davidse LC, Looijen D, Turkensteen LJ, Van der Wal, D (1981) Occurrence of metalaxyl-resistant strains of *Phytophthora infestans* in Dutch potato fields. Netherlands Journal of Plant Pathology 87:65-68.

Dowley LJ, O'Sullivan E (1981) Metalaxyl-resistant strains of *Phytophthora infestans* (Mont.) de Bary in Ireland. Potato Research 24:417-421.

Fohner GR, Fry WE, White GB (1984) Computer simulation raises question about timing protectant fungicide application frequency according to a potato late blight forecast. Phytopathology. Vol. 74. No. 10.

Fungicide Resistance Action Committee – FRAC. 2025. Information on CAA fungicides. Disponível em: https://www.frac.info/frac-teams/working-groups/caa-fungicides/information. Acesso em: 10 jan. 2025.

Food and Ariculture Organization of the United Nations (FAO), Roma, 2025. Disponível em: http://www.fao.org/faostat/. Acesso em: 03 de janeiro de 2025.

García HG, Marín M, Jaramillo S, Cotes JM (2008) Sensibilidad de aislamientos colombianos de *Phytophthora infestans* a cuatro fungicidas sistémicos. Agronomía Colombiana 26:47-57.

Gisi U, Cohen Y (1996) RESISTANCE TO PHENYLAMIDE FUNGICIDES: A Case Study with *Phytophthora infestans* Involving Mating Type and Race Structure. Annual Review Phytopathology 34:549-572.

Gisi U, Sierotzki H (2015) Oomycete Fungicides: Phenylamides, Quinone Outside Inhibitors, and Carboxylic Acid Amides. In: Ishii H, Hollomon DW. Fungicide Resistance in Plant Pathogens. Springer 145-174.

Gouot, JM (1994) Characteristics and population dynamics of *Botrytis cinerea* and other pathogens resistant to dicarboximides. In: Fungicide Resistance in North America. (Delp C.J., ed.), The American Phytopatological Society, St. Paul, Minnesota, USA, pp. 53-55.

Grünwald NJ, Sturbaum AK, Romero Montes G, Garay Serrano, E, Lozoya-Saldaña H, Fry WE (2006) Selection for fungicide resistance within a growing season in field populations of *Phytophthora infestans* at the center of origin. Phytopathology 96:1397-1403.

Haverkort AJ, Struik PC, Visser RGF, Jacobsen E (2009) Applied Biotechnology to Combat Late Blight in Potato Caused by *Phytophthora infestans*. Potato Research 52:249–264.

Instituto Brasileiro De Geografia e Estatística – IBGE. 2025. Produção de Batata. Disponível em: https://www.ibge.gov.br/explica/producao-agropecuaria/batata/br. Acesso em: 09 jan. 2025.

Kato M, Mizubuti ES, Goodwin SB, Fry WE (1997) Sensitivity to protectant fungicides and pathogenic fitness of clonal lineages of *Phytophthora infestans* in the United States. Phytopathology 87:973-978.

Kildea S, Mehenni-Ciz J, Griffin D, Cooke L (2014) Sensitivity of Irish *Phytophthora infestans* to the CAA fungicide mandipropamid. Fourteenth Euroblight Workshop 16:221–222.

Kirk WW, Abu-El Samen FM, Muhinyuza JB, Hammerschmidt R, Dousches DS; Thill CA, Groza H, Thompson AL (2005) Evaluation of potato late blight management utilizing host plant resistance and reduced rates and frequencies of fungicide applications. Crop Protection 24:961-970.

Lee TY, Mizubuti E, Fry WE (1999) Genetics of Metalaxyl Resistance in *Phytophthora infestans*. Fungal Genetics and Biology 26:118–130.

Lehtinen A, Hannukkala A, Rantanen T, Jauhiainen L (2007) Phenotypic and genetic variation in Finnish potato-late blight populations, 1997–2000. Plant Pathology 56:480–491.

Leroux P, Fritz R, Debieu D, Albertini C, Lanen C, Bach J, Gredt M, Chapeland F (2002) Mechanisms of resistance to fungicides in field strains of *Botrytis cinerea*. Pest Management Science 58:876-888.

Lu, XH, Zhu, SS, Bi, Y, Liu, XL, Hao, JJ (2010) Baseline sensitivity and resistance-risk assessment of *Phytophthora capsici* to iprovalicarb. Phytopathology 100:1162-1168.

Mazáková J, Zouhar M, Ryšánek P, Táborský V, Hausvater E, Doležal P (2011) Sensitivity to fungicides in the isolates of *Phytophthora infestans* (Mont.) de Bary in the Czech Republic from 2003 to 2008. Plant Protection Science 47:5–12.

Mazáková J, Zouhar M, Sedlák P, Zusková E, Ryšánek P, Hausvater E (2018) Sensitivity to Fungicides and Essential Oils in Czech Isolates of *Phytophthora infestans*. Scientia agriculturae bohemica, 49:69–77.

Miranda BEC, Suassuna ND, Reis A (2010) Mating type, mefenoxam sensitivity, and pathotype diversity in *Phytophthora infestans* isolates from tomato in Brazil. Pesquisa agropecuária brasileira 45:671-679.

Ojiambo PS, Paul PA, Holmes GJ (2010) A quantitative review of fungicide efficacy for managing downy mildew in cucurbits. Phytopathology 100:1066-1076.

Oliveira SAS (2010) Genetic structure, aggressiveness and fungicide sensitivity of *Phytophthora infestans* populations from the South and southeast regions of Brazil. Tese (Doctor Scientiae) 96f.

Reis A, Ribeiro FHS, Maffia LA, Mizubuti ESG (2005) Sensitivity of Brazilian isolates of *Phytophthora infestans* to commonly used fungicides in tomato and potato crops. Plant Disease 89:1279-1284.

Reis A, Ribeiro FHS, Mizubuti ESG (2006) Caracterização de isolados de *Phytophthora infestans* do Distrito Federal e de Goiás. Fitopatologia Brasileira 31:270-276.

Rekanović E, Potočnik I, Milijašević-Marčić S, Stepanović M, Todorović B, Mihajlović M (2011) Sensitivity of *Phytophthora infestans* (Mont.) de Bary Isolates to Fluazinam, Fosetyl-Al and Propamocarb-hydrochloride. Pestic. Phytomed. (Belgrade), 26(2), 111–116.

Rubin AE, Gotlieb D, Gisi U, Cohen Y (2008) Mutagenesis of *Phytophthora infestans* for resistance against carboxylic acid amide and phenylamide fungicides. Plant Disease 92:675-683.

Santana FM, Gomes CB, Rombaldi C, Bianchi VJ, Reis A (2013) Characterization of *Phytophthora infestans* populations of southern Brazil in 2004 and 2005. Phytoparasitica 41:557–568.

Saville A, Graham K, Grünwald NJ, Myers K, Fry WE, Ristaino JB (2015) Fungicide sensitivity of U.S. genotypes of *Phytophthora infestans* to six oomycete-targeted compounds. Plant Disease 99:659-666.

Schepers HTAM, Cooke LR (2015) Potato Pathogens in Northern and Western Europe. In: Ishii H, Hollomon DW. Fungicide Resistance in Plant Pathogens. Springer 355-378.

Schepers HTAM, Kessel GJT, Lucca F, Förch MG, Van den Bosch GBM, Topper CG, Evenhuis A (2018) Reduced efficacy of fluazinam against *Phytophthora infestans* in the Netherlands. European Journal of Plant Pathology 151:947–960.

Stein JM, Kirk WW (2004) The generation and quantification of resistance to dimethomorph in *Phytophthora infestans*. Plant Disease 88:930-934.

Stergiopoulos I, van Nistelrooy JGM, Kema GHJ, De Waard MA (2003) Multiple mechanisms account for variation in base-line sensitivity to azole fungicides in field isolates of *Mycosphaerella graminicola*. Pest Management Science 59:1333-1343.

Sujkowski LS, Fry BA, Power RJ, Coodwin SB, Peever TL, Hamlen RA, Fry WE (1995) Sensitivities of Mexican isolates of *Phytophthora infestans* to chlorothalonil, cymoxanil, and metalaxyl. Plant Disease 79:1117-1120.

Töfoli JG, Melo PCT, Domingues RJ, Ferrari JT (2013) Requeima e pinta preta na cultura da batata: importância, características e manejo sustentável. O Biológico, 75:33-40.

Töfoli JG, Melo PCTD, Domingues RJ, Ferrari JT (2016) Controle da requeima e pinta preta da batata por fungicidas e seu reflexo sobre a produtividade e a qualidade de tubérculos. Arquivos do Instituto Biológico 83.

Wang, HC, Sun, HY, Stammler, G, Ma, JX, Zhou, MG (2010) Generation and characterization of isolates of *Peronophythora litchi* resistant to carboxylic acid amide fungicides. Phytopathology 100:522-527.

Zanotta S (2019) Caracterização da população de *Phytophthora infestans* (Mont) de Bary em regiões produtoras de batata (*Solanum tuberosum L.*) no Brasil. Tese (Doutorado em Segurança Alimentar e Sanidade no Agroecossistema) 124f.

Tables

Table 1. Locality and EC_{50} values for dimethomorph, mandipropamid, propamocarb hydrochloride and chlorothalonil of the isolates.

City	DM*		MD*		CP*		CL*	
	No. of isolates	EC_{50} (µg/mL)	No. of isolates	EC_{50} (µg/mL)	No. of isolates	EC_{50} (µg/mL)	No. of isolates	EC_{50} (µg/mL)
Antônio Olinto/PR	5	0.105 – 0.150	3	0.016 -0.021	2	17.541 – 44.091	3	2.131 – 12.273
Contenda/PR	3	0.136 – 0.160	1	0.026	3	11.063 - 20.696	3	2.053 – 3.994
Guarapuava/PR	7	0.147 – 0.206	4	0.023 – 0.037	4	7.093 – 30.138	5	1.320 – 3.697
Castro/PR	5	0.127 – 0.218	7	0.019 – 0.033	6	6.465 – 77.079	6	2.782 – 7.273
Lapa/PR	6	0.093 – 0.167	5	0.012 – 0.030	3	5.462 - 61.838	4	2.999 - 6.532
Palmeira/PR	2	0.150 - 0.205	3	0.022 – 0.027	3	17.201 - 32.414	4	2.867 – 9.704
Mangueirinha/PR	4	0.086 - 0.188	4	0.019 – 0.025	4	5.344 - >100	3	2.321 – 4.953
São Mateus do Sul/PR	3	0.105 - 0.158	3	0.018 - 0.020	2	4.494 - >100	2	3.663 – 4.134
Araucária/PR	-	-	2	0.021 – 0.022	1	>100	2	2.634 – 3.089
Palmas/PR	-	-	1	0.025	1	>100	1	3.116
Witmarsum/PR	-	-	2	0.022 – 0.023	2	34.063 - >100	2	2.328 - 2.638
Água Doce/SC	2	0.136 – 0.176	6	0.022 - 0.032	4	12.139 – 39.191	3	0.605 - 5.890
Canoinhas/SC	2	0.119 – 0.171	2	0.008 – 0.023	3	11.707 – 70.309	2	1.346 - 3.490
Major Vieira/SC	-	-	-	-	2	43.477 - >100	-	-
Bom Jesus/RS	2	0.113 - 0.125	1	0.021	2	44.705 – 69.527	2	3.207 - 3.974
Cambará do Sul/RS	2	0.159 – 0.167	2	0.017 – 0.021	1	21.191	3	2.481 – 7.020

- no EC_{50} test was carried out for the fungicide.
* DM - Dimetomorph, MD - Mandipropamid, CP - Propamocarb hydrochloride and CL - chlorothalonil.

Table 2. Sensitivity of isolates of *Phytophthora infestans* to propamocarb-hydrochloride and chlorothalonil, from different cities in South Brazil and their respective resistance factor (RF) and phenotype.

Isolate	City	CP*			CL*		
		EC_{50} (µg/mL)	FR	Phenotype	EC_{50} (µg/mL)	FR	Phenotype
AOPR1	Antônio Olinto	17.541	3.54	MR	2.131	3.52	MR
AOPR5	Antônio Olinto	44.091	8.91	MR	2.369	3.91	MR
AOPR6	Antônio Olinto	-	-	-	12.273	20.28	HR
COPR1	Contenda	20.696	4.18	MR	2.053	3.39	MR
COPR2	Contenda	11.063	2.23	S	2.694	4.45	MR
COPR3	Contenda	15.646	3.16	MR	3.994	6.60	MR
GUPR1	Guarapuava	18.808	3.80	MR	2.075	3.43	MR
GUPR2	Guarapuava	7.093	1.43	S	3.697	6.11	MR
GUPR5	Guarapuava	-	-	-	1.320	2.18	S
GUPR6	Guarapuava	30.138	6.09	MR	2.559	4.23	MR
GUPR7	Guarapuava	22.534	4.55	MR	2.808	4.64	MR
CAPR1	Castro	18.259	3.69	MR	3.473	5.74	MR
CAPR3	Castro	77.079	15.58	MR	3.806	6.29	MR
CAPR4	Castro	12.732	2.57	S	3.630	6.00	MR
CAPR5	Castro	34.845	7.04	MR	7.273	12.02	MR
CAPR7	Castro	6.465	1.31	S	2.782	4.60	MR
CAPR8	Castro	32.879	6.64	MR	2.812	4.65	MR
LAPR1	Lapa	61.838	12.50	MR	6.532	10.79	MR
LAPR2	Lapa	5.462	1.10	S	5.194	5.58	MR
LAPR3	Lapa	11.907	2.41	S	4.061	6.71	MR
LAPR5	Lapa	-	-	-	2.999	4.96	MR
MAPR1	Mangueirinha	>100	38.08	HR	2.321	3.84	MR
MAPR2	Mangueirinha	5.344	1.08	S	3.945	6.52	MR
MAPR3	Mangueirinha	>100	>100	HR	-	-	-
MAPR4	Mangueirinha	>100	95.45	HR	4.953	8.18	MR
SMPR3	São Mateus	>100	>100	HR	3.663	6.05	MR

	do Sul						
SMPR4	São Mateus do Sul	4.494	1	S	4.134	6.83	MR
ARPR1	Araucária	>100	25.00	HR	2.634	4.35	MR
ARPR2	Araucária	-	-	-	3.089	5.10	MR
PAPR1	Palmeira	32.414	6.55	MR	2.933	4.85	MR
PAPR2	Palmeira	29.933	6.05	MR	3.694	6.10	MR
PAPR3	Palmeira	17.201	3.48	MR	2.867	4.74	MR
PAPR5	Palmeira	-	-	-	9.704	16.03	MR
PSPR1	Palmas	-	-	-	3.116	5.15	MR
PSPR4	Palmas	>100	>100	HR	-	-	-
WIPR1	Witmarsum	>100	25.23	HR	2.638	4.36	MR
WIPR2	Witmarsum	34.063	6.88	MR	2.328	3.84	MR
ADSC1	Água Doce	12.139	2.45	S	5.890	9.73	MR
ADSC3	Água Doce	38.042	7.69	MR	0.605	1	S
ADSC4	Água Doce	31.509	6.37	MR	1.934	3.20	MR
ADSC6	Água Doce	39.191	7.91	MR	-	-	-
CASC1	Canoinhas	21.586	4.36	MR	3.490	5.77	MR
CASC3	Canoinhas	11.701	2.36	S	-	-	-
CASC5	Canoinhas	70.309	14.21	MR	1.346	2.22	S
MVSC1	Major Vieira	>100	93.32	HR	-	-	-
MVSC2	Major Vieira	43.477	8.79	MR	-	-	-
BJRS1	Bom Jesus	44.705	9.03	MR	3.974	6.57	MR
BJRS2	Bom Jesus	69.527	14.05	MR	3.207	5.30	MR
TARS1	Cambará do Sul	-	-	-	2.481	4.10	MR
TARS2	Cambará do Sul	21.191	4.28	MR	7.020	11.60	MR
TARS3	Cambará do Sul	-	-	-	2.740	4.53	MR

- no EC_{50} test was carried out for the isolate.

* CP - Propamocarb hydrochloride and CL - chlorothalonil.

Supplementary table 1. Isolate code, city, state and EC50 values for dimethomorph, mandipropamid, propamocarb hydrochloride and chlorothalonil.

Isolate	City	State	DM*	MD*	CP*	CL*
			EC_{50} (µg/mL)			
AOPR1	Antônio Olinto	Paraná	0.105	0.020	17.541	2.131
AOPR3	Antônio Olinto	Paraná	0.128	-	-	-
AOPR4	Antônio Olinto	Paraná	0.150	0.021	-	-
AOPR5	Antônio Olinto	Paraná	0.140	0.016	44.091	2.369
AOPR6	Antônio Olinto	Paraná	0.134	-	-	12.273
COPR1	Contenda	Paraná	0.145	-	20.696	2.053
COPR2	Contenda	Paraná	0.136	-	11.063	2.694
COPR3	Contenda	Paraná	0.160	0.026	15.646	3.994
GUPR1	Guarapuava	Paraná	0.192	-	18.808	2.075
GUPR2	Guarapuava	Paraná	0.147	0.030	7.093	3.697
GUPR3	Guarapuava	Paraná	0.206	0.023	-	-
GUPR4	Guarapuava	Paraná	0.181	-	-	-
GUPR5	Guarapuava	Paraná	0.198	-	-	1.320
GUPR6	Guarapuava	Paraná	0.189	0.037	30.138	2.559
GUPR7	Guarapuava	Paraná	0.200	0.035	22.534	2.808
CAPR1	Castro	Paraná	0.127	0.023	18.259	3.473
CAPR2	Castro	Paraná	-	0.025	-	-
CAPR3	Castro	Paraná	0.218	0.030	77.079	3.806
CAPR4	Castro	Paraná	-	0.026	12.732	3.630
CAPR5	Castro	Paraná	0.165	0.033	34.845	7.273
CAPR7	Castro	Paraná	0.156	0.019	6.465	2.782
CAPR8	Castro	Paraná	0.206	0.028	32.879	2.812
LAPR1	Lapa	Paraná	0.137	0.015	61.838	6.532
LAPR2	Lapa	Paraná	0.133	0.030	5.462	5.194
LAPR3	Lapa	Paraná	0.151	-	11.907	4.061
LAPR4	Lapa	Paraná	0.093	0.015	-	-
LAPR5	Lapa	Paraná	0.152	0.022	-	2.999
LAPR6	Lapa	Paraná	0.167	0.012	-	-
MAPR1	Mangueirinha	Paraná	0.188	0.019	>100	2.321
MAPR2	Mangueirinha	Paraná	0.145	0.025	5.344	3.945
MAPR3	Mangueirinha	Paraná	0.086	0.025	>100	-
MAPR4	Mangueirinha	Paraná	0.105	0.023	>100	4.953

SMPR1	São Mateus do Sul	Paraná	0.158	0.020	-	-
SMPR3	São Mateus do Sul	Paraná	0.112	0.018	>100	3.663
SMPR4	São Mateus do Sul	Paraná	0.105	0.021	4.494	4.134
ARPR1	Araucária	Paraná	-	-	>100	2.634
ARPR2	Araucária	Paraná	-	0.022	-	3.089
ARPR4	Araucária	Paraná	-	0.021	-	-
PAPR1	Palmeira	Paraná	-	0.022	32.414	2.933
PAPR2	Palmeira	Paraná	-	0.024	29.933	3.694
PAPR3	Palmeira	Paraná	-	-	17.201	2.867
PAPR4	Palmeira	Paraná	-	0.027	-	-
PAPR5	Palmeira	Paraná	-	-	-	9.704
PSPR1	Palmas	Paraná	-	-	-	3.116
PSPR4	Palmas	Paraná	-	0.025	>100	-
WIPR1	Witmarsum	Paraná	-	0.022	>100	2.638
WIPR2	Witmarsum	Paraná	-	0.023	34.063	2.328
ADSC1	Água Doce	Santa Catarina	-	0.031	12.139	5.890
ADSC3	Água Doce	Santa Catarina	0.176	0.024	38.042	0.605
ADSC4	Água Doce	Santa Catarina	0.136	0.032	31.509	1.934
ADSC5	Água Doce	Santa Catarina	-	0.022	-	-
ADSC6	Água Doce	Santa Catarina	-	-	39.191	-
ADSC7	Água Doce	Santa Catarina	-	0.025	-	-
ADSC8	Água Doce	Santa Catarina	-	0.023	-	-
CASC1	Canoinhas	Santa Catarina	-	-	21.586	3.490
CASC3	Canoinhas	Santa Catarina	0.119	0.008	11.701	-
CASC5	Canoinhas	Santa Catarina	0.171	0.023	70.309	1.346
MVSC1	Major Vieira	Santa Catarina	-	-	>100	-
MVSC2	Major Vieira	Santa Catarina	-	-	43.477	-
BJRS1	Bom Jesus	Rio Grande do Sul	0.125	0.021	44.705	3.974
BJRS2	Bom Jesus	Rio Grande do Sul	-	-	69.527	3.207
BJRS3	Bom Jesus	Rio Grande do Sul	0.113	-	-	-
TARS1	Cambará do Sul	Rio Grande do Sul	-	-	-	2.481
TARS2	Cambará do Sul	Rio Grande do Sul	0.159	-	21.191	7.020
TARS3	Cambará do Sul	Rio Grande do Sul	0.167	0.017	-	2.740
TARS4	Cambará do Sul	Rio Grande do Sul	-	0.021	-	-

- no EC_{50} test was carried out for the fungicide.
* DM - Dimetomorph, MD - Mandipropamid, CP - Propamocarb hydrochloride and CL - chlorothalonil.

Supplementary table 2. Survey of studies carried out on the sensitivity of *Phytophthora infestans* to the fungicides dimethomorph, mandipropamid, propamocarb hydrochloride and chlorothalonil.

Fungicide	Country	Range of EC_{50} µg/mL	Reference
Dimetomorph	USA	0.11 – 0.84	Stein e Kirk, 2004
	Netherlands	0.5*	Ziogas, 2006
	Israel	Total inhibition at 0.5	Rubin, 2008
	Brazil	0.03 – 1.46	Oliveira, 2010
	Czech Republic	All sensitive	Mazakova, 2011
	Czech Republic	All sensitive	Mazakova, 2017
	Brazil	$1.27.10^{-9}$ - >10,000	Zanotta, 2019
	Brazil	0.086 – 0.218	**This work**
Mandipropamid	09 European countries + Israel	0.02 – 2.98	Cohen, 2007
	Israel	Total inhibition at 0.0005 – 0.005	Rubin, 2008
	Brazil	0.05 – 1.4	Oliveira, 2010
	Ireland	All sensitive	Kildea, 2014
	USA	0.01 – 0.03	Saville, 2015
	Brazil	0.008 - 0.037	**This work**
Propamocarb hydrochloride	Finland	Isolates growing >1000	Lehtinen, 2007
	Colombia	0.71	Garcia, 2008
	Brazil	0.1 – 34.1	Oliveira, 2010
	Serbia	12.1 – 31.1	Rekanovic, 2011
	Brazil	0.008 - >10000	Zanotta, 2019
	Brazil	4.949 - >100	**This work**
Chlorothalonil	Mexico	3.1, with three isolates >10	Sujkowski, 1995
	USA	0.21 – 1.47	Kato, 1997
	Brazil	Majority of isolates (38 of 50) <1.0 µg/ml.	Reis, 2005
	Netherlands	0.075*	Ziogas, 2006
	Brazil	0.2 – 8.2	Oliveira, 2010
	Brazil	0.01 – 4.67	Casa-Coila, 2014
	Brazil	$1.4.10^{-11}$ - >10000	Zanotta, 2019
	Brazil	0.605 - 12.273	**This work**

Figures

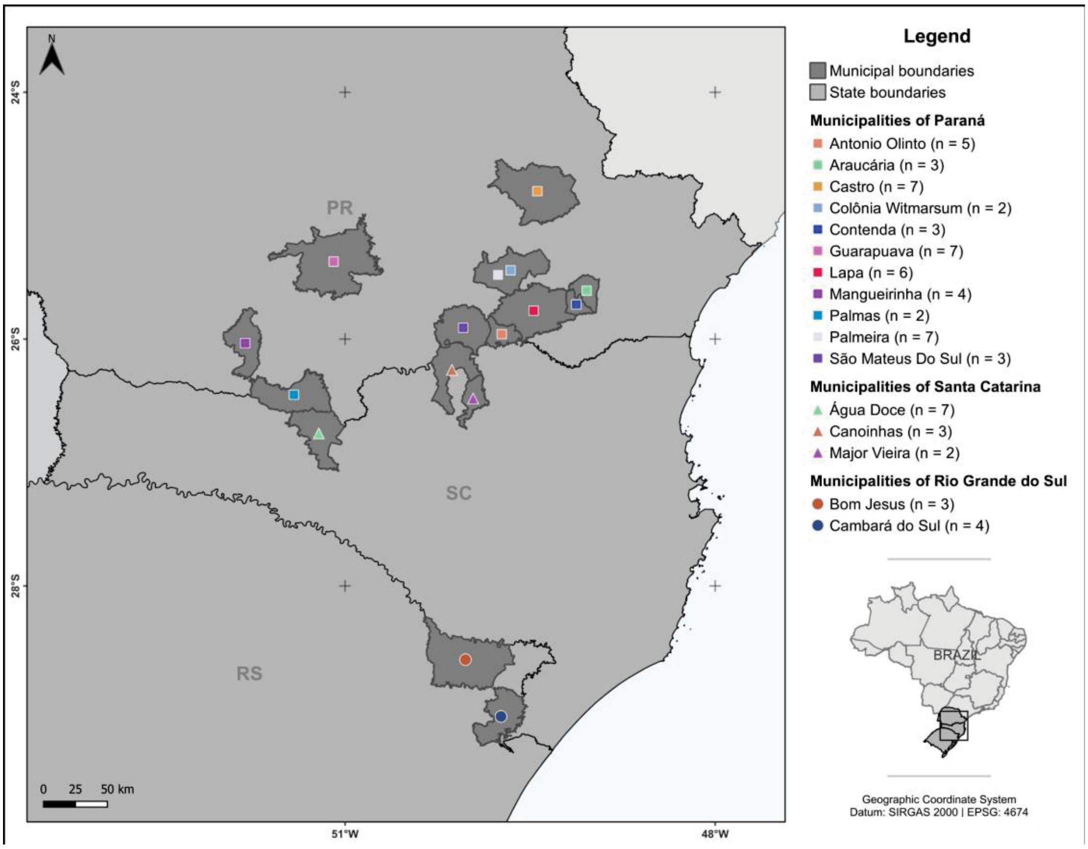


Figure 1. Number of fields and isolates collected between 2021, 2022 and 2023 in the states of Paraná, Santa Catarina and Rio Grande do Sul obtained and tested for sensitivity to fungicides.

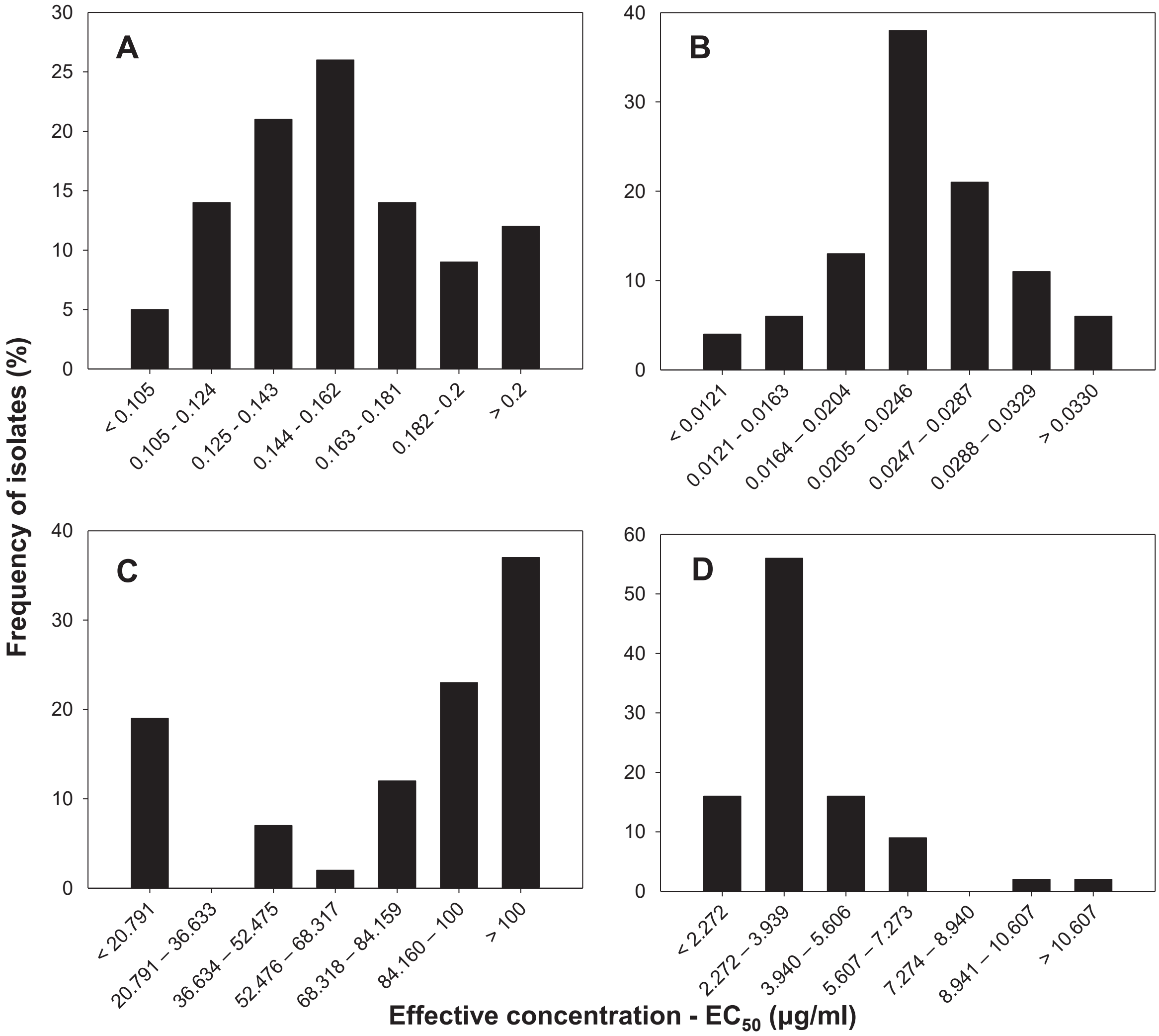


Figure 2. Frequency of *Phytophthora infestans* isolates in different classes of estimated EC_{50} values for the fungicides: **A**, Dimethomorph; **B**, Mandipropamid; **C**, Propamocarb hydrochloride; **D**, Chlorothalonil.

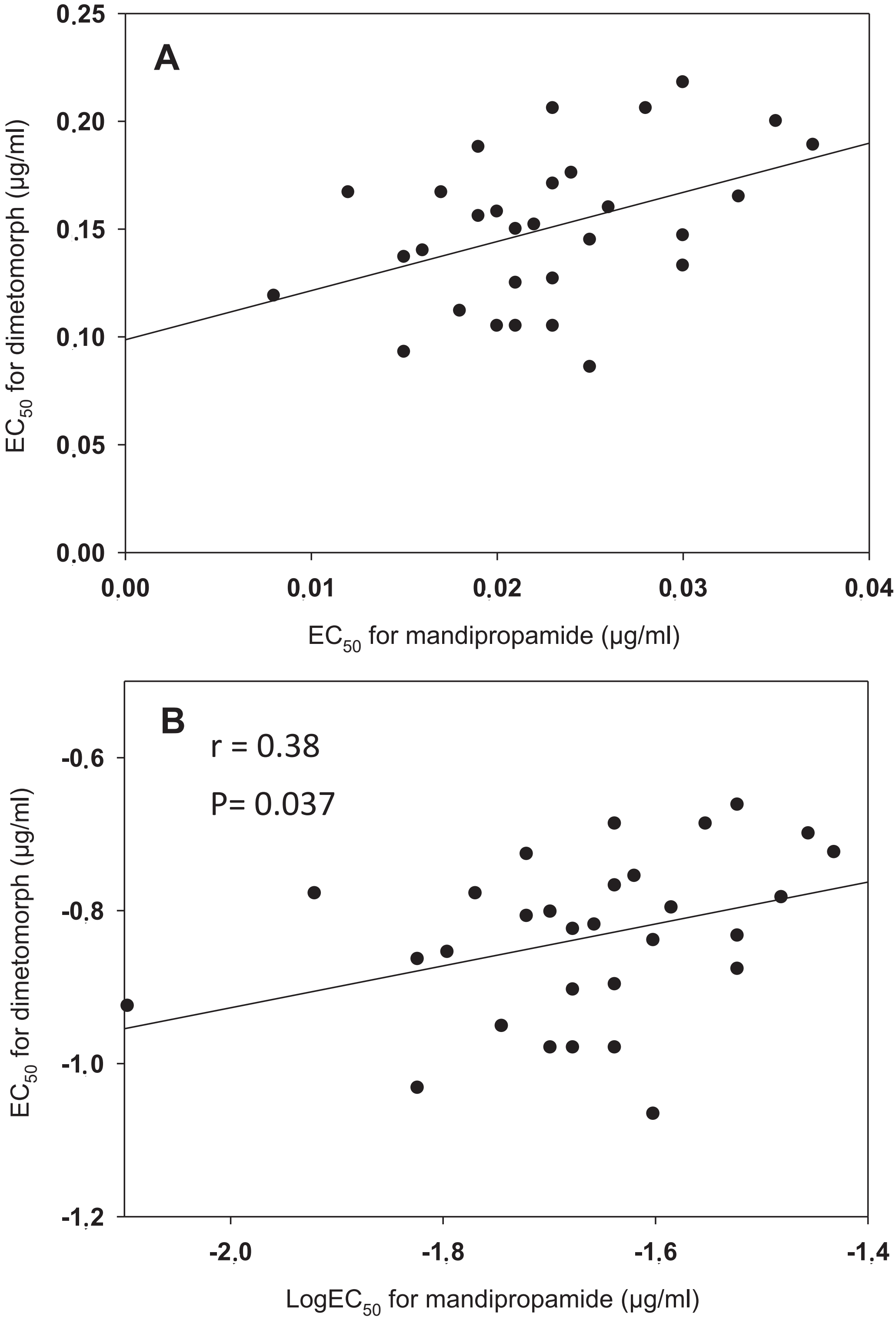
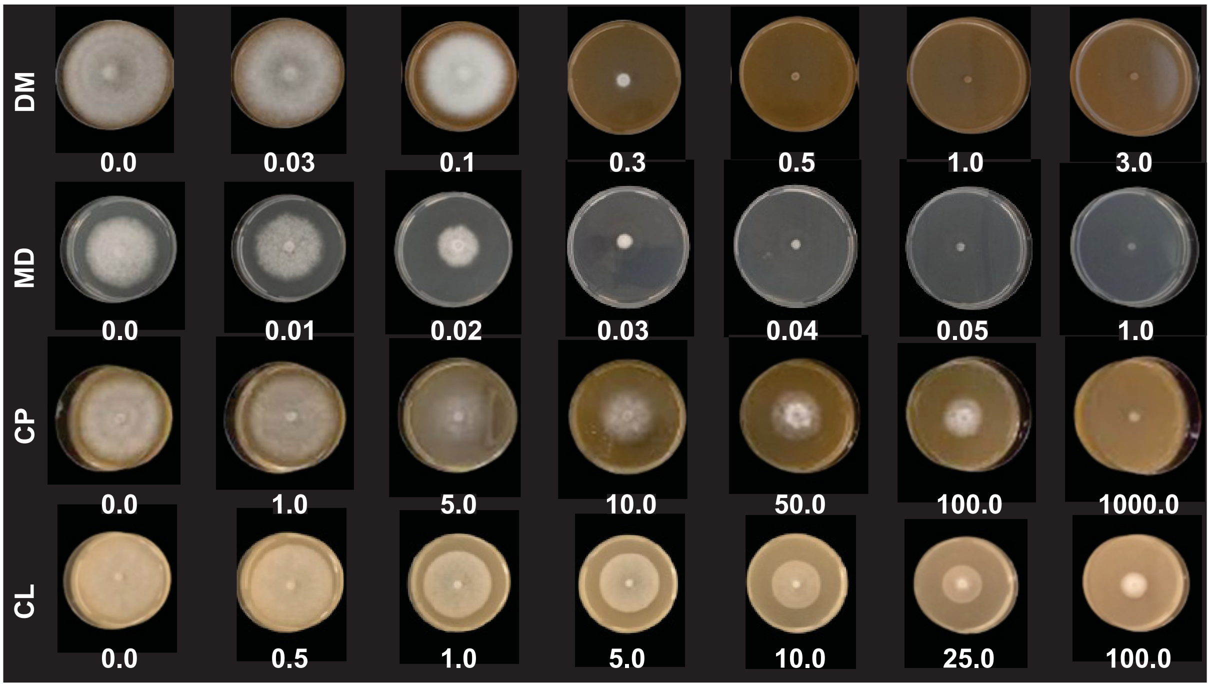


Figure 3. Correlation analysis between dimetomorph and mandipropamid (**A** - EC_{50} values and **B** - EC_{50} values in log) in *Phytophthora infestans* isolates from potato.



Supplementary figure 1. Illustration of the experiment with the fungicides **DM** - dimethomorph, **MD** - mandipropamid, **CP** - propamocarb hydrochloride and **CL** – chlorothalonil and the respective doses used. All values are in µg/mL.

3 CHAPTER 2: Baseline sensitivity of *Botrytis cinerea* strawberry isolates to mefentrifluconazole and cross-resistance with other DMIs

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ABSTRACT

Gray mold, caused by the fungus *Botrytis cinerea* Pers., is one of the main diseases affecting the strawberry crop, representing a problem during cultivation and post-harvest. The intensive use of chemical fungicides has favored the selection of isolates that are resistant to the products currently available on the market. In this study, we established a baseline for mefentrifluconazole for populations of *B. cinerea* from Florida (USA) and Parana (Brazil) not yet exposed to this new fungicide. In addition, this study verified the occurrence of cross-resistance between mefentrifluconazole and other fungicides from the DMI group (difenoconazole and triflumizole); identified potential mutations associated with the loss of sensitivity of the isolates; evaluated the practical resistance of the isolates; and compared the efficacy of this new product with fungicides already available on the market. To build the baseline, the EC_{50} of 155 isolates (70 isolates from Brazil and 85 from the United States) was determined. The results showed that isolates from Parana were less sensitive than those from Florida, with two isolates presenting EC_{50} values above 1.00 µg/ml. Average EC_{50} of isolates from Parana was 0.169 µg/ml, ranging from

0.046 to 1.303 μg/ml, and average of isolates from Florida was 0.084 μg/ml, ranging from 0.030 to 0.384 μg/ml. Twenty of these 155 isolates were selected for the cross-resistance study, and their EC_{50} was determined for difenoconazole and triflumizole. Spearman's correlation analysis confirmed the existence of cross-resistance between the fungicides. DNA was extracted for three isolates with the highest EC_{50} and three with the lowest EC_{50} for mefentrifluconazole, followed by PCR and sequencing of the *CYP51* gene. The two isolates with the highest EC_{50} values had the G461S mutation, whereas the third isolate with a high EC_{50} showed the R464K mutation. Isolates with low EC_{50} did not show any mutations. The efficacy of mefentrifluconazole was compared with cyprodinil and fludioxonil on detached fruit assays using three isolates (one sensitive, one with the G461S mutation and one with R464K). Mefentrifluconazole was effective for isolates with no resistance, with similar performance to fludioxonil and cyprodinil.

Keywords: Gray mold, fungicide, resistance.

INTRODUCTION

The United States and Brazil stand out as major strawberry producers, occupying second and tenth place in the ranking of the largest producers of the fruit in 2022, respectively (Faostat, 2024). There are, however, several adversities that affect the crop, reducing productivity. One of the most important pre- and post-harvest diseases is gray mold, caused by the fungus *Botrytis cinerea* Pers. (Oliveira et al, 2017).

Botrytis cinerea is capable of infecting more than 200 plant species (Dean et al., 2012). Management of gray mold is generally carried out through a combination of cultural practices, the use of less susceptible cultivars and, above all, chemical control (Mertely et al., 2000; Baggio et al., 2018). Fungicides with various mechanisms of action are available on the market to control this disease, including succinate dehydrogenase inhibitors - SDHIs (boscalid, penthiopyrad), quinone outside inhibitors - QoIs (trifloxystrobin, azoxystrobin), benzimidazoles (thiophanate methyl), dicarboxamides (iprodione, procymidone), anilinopyrimidines (pyrimethanil, cyprodinil), phenylpyrrole (fludioxonil) and demethylation inhibitors - DMIs (difenoconazole, triflumizole). The high genetic variability, short life cycle and prolific production of spores make the fungus evolve very quickly under the high pressure of fungicides (Bardas et al., 2010).

Resistant populations of *B. cinerea* to several active ingredients have already been found in several countries such as Brazil (Maia et al., 2021), the United States (Amiri et al., 2013), Greece (Bardas et al., 2010), China (Chen et al., 2024; Mao et al., 2024), among others. Because of this, along with the few alternatives for effective control of the disease, growers are reporting difficulties in management (Maia et al., 2023). New active ingredients, such as mefentrifluconazole, have been developed for plant disease control. Mefentrifluconazole is a systemic fungicide belonging to the group of demethylation inhibitors (DMI's), inhibiting cytochrome P450 sterol 14α-demethylase (Zhang et al., 2019). This fungicide does not inhibit the germination of conidia, but rather the elongation of the germ tube and mycelial growth (Leroux, 2007). Although it is not yet registered for the control of *B. cinerea* on strawberries in the United States, in Brazil it was recently registered for the crop, in a mixture with the active ingredient fluxapiroxade, and has shown potential against this pathogen, acting on mycelial growth and germ tube elongation (Li et al, 2021), as well as for other pathosystems (Ishii et al., 2022, He et al., 2023, Rocha et al., 2024).

DMIs are classified as fungicides with a medium risk of resistance and, because they act on the same target site, they are subject to cross-resistance between the active ingredients in the group (FRAC, 2024). Cross-resistance between mefentrifluconazole and other DMIs has already been reported in *Monilinia fructicola*, *Colletotrichum* spp., *Alternaria* spp., *Cercospora beticola* and *Podosphaera xanthii* (Ishii et al., 2021) and in *Botrytis cinerea* in other crops (Zhang et al., 2020, Chen et al., 2024). However, this occurrence has not yet been observed in *B. cinerea* on strawberries (Li et al., 2021). In general three mechanisms of resistance to DMIs are reported: i) point mutations in the sequence of the CYP51 gene, which encodes ergosterol 14α-demethylase (Leroux, 2007); ii) overexpression of the CYP51 enzyme (Carter et al., 2014) and iii) increased efflux of the fungicide, mediated by the overexpression of efflux pumps, such as ABC (ATP-binding cassette) transporters and MFS (major facilitator superfamily) transporters (Kretschmer et al., 2009, Carter et al., 2014). Although the mechanisms of resistance are known, there is no knowledge about the time it takes for resistance to develop to a new active ingredient. This highlights the need for anti-resistance management, starting with monitoring the pathogen before starting to include a new active ingredient in crop management. For this reason, studying the baseline of isolates is essential to follow the development of fungicide resistance in pathogen populations over the years (Gao et al, 2018). In addition, it is important to compare the control efficacy of a new fungicide with products already in use, so that it can be incorporated into disease management strategies.

Given this context, the objectives of this work were: i) to develop a baseline for mefentrifluconazole for *Botrytis cinerea* isolates from Brazil and the United States; ii) to verify the occurrence of cross-resistance between mefentrifluconazole and other DMIs; iii) to identify the presence of mutations in the CYP51 gene; and iv) to check for practical resistance and compare the control efficiency of mefentrifluconazole on fruit compared to other fungicides available on the market.

MATERIALS AND METHODS

Isolate collection

A total of 155 isolates of *B. cinerea* were collected between the years 2010 and 2023 from different production fields in Florida (USA) (n = 85) and Parana (Brazil) (n = 70). The collected samples showed typical disease symptoms, and fields that had never been sprayed with mefentrifluconazole were selected. Subsequently, single-spore isolation was performed for all isolates. In order not to lose their characteristics after successive transfers, all isolates were stored using two different methods: in silica-gel using filter paper disks, stored at -20ºC (Fong et al., 2000). For the experiments, the isolates were recovered from storage.

Baseline sensitivity of *B. cinerea* to mefentrifluconazole

A baseline was carried out for the population of 70 Brazilian isolates and another for the population of 85 American isolates. All 155 isolates were evaluated for sensitivity to mefentrifluconazole (Cyvia®, BASF) using the effective concentration at 50% (EC_{50}) test of mycelial growth. Aliquots of the stock solution were added to the PDA medium and transferred to Petri dishes (60 mm) to obtain final concentrations of mefentrifluconazole of 0 (control), 0.01, 0.05, 0.1, 0.5, 1.0, and 5.0 µg/mL. Plugs of 5 mm from each isolate were transferred to the Petri dishes containing the different concentrations. The evaluation was performed by measuring the diameter of the fungal colonies 2 days after transferring the plugs. For each concentration, three plates were prepared, and two measurements were taken in perpendicular directions. The experiment was performed in duplicate. EC_{50} was evaluated using regression analysis with the logarithm of the percentage of mycelial inhibition relative to the control. The percentage of inhibition was calculated using the formula: %MCI = (Mc – Mt) / Mc x 100, where MCI = mycelial growth inhibition, Mc = mean mycelial growth of the control, and Mt = mean mycelial growth of the treatment analyzed. Sturges Law was used to identify the number of classes within the baseline graph, considering the number of isolates used (n=155).

Cross-resistance

Twenty isolates (ten from each country) were selected based on their sensitivity to mefentrifluconazole for evaluation of sensitivity to difenoconazole (Inspire®, Syngenta Crop Protection) and triflumizole (Procure® 480SC, brand). The isolates from each country were subdivided into three EC_{50} groups using Scott-Knott analysis. Three isolates with the highest EC_{50}, three with an intermediate EC_{50} and 4 isolates with the lowest EC_{50} were selected for each country. Difenoconazole was chosen due to the number of commercial products released with this active ingredient for use in strawberry cultivation in Brazil, while triflumizole represents the application of the DMI chemical group in the USA. The procedure followed the same conditions described for mefentrifluconazole. The final concentrations of the difenoconazole was 0 (control), 0.01, 0.05, 0.1, 0.5, 1.0, and 5.0 µg/mL and for triflumizole was 0 (control), 0.05, 0.1, 0.5, 1.0, 2.0, and 5.0 µg/mL. Each experiment used three plates per concentration and was performed in duplicate. Spearman's analysis was carried out with the log-EC_{50} values to test the cross-resistance relationship between the fungicides mefentrifluconazole, difenoconazole and triflumizole. Spearman correlation analyses were conducted using the R software version 4.2.2 (R Core Team, 2024). The EC_{50} values of mefentrifluconazole from 20 isolates were plotted against the EC_{50} values of the same isolates for difenoconazole and triflumizole.

Analysis of the *CYP51* gene sequence

Genomic DNA was extracted from 06 isolates using FastDNAKit (MP Biomedicals, LCC), according to the manufacturer's protocol. The isolates were chosen according to their sensitivity to mefentrifluconazole. The three isolates with the highest EC_{50} and the three with the lowest EC_{50} were chosen. The *CYP51* gene was amplified by polymerase chain reaction (PCR) using the primer pairs CYP51-F (5'- ACCACCTTTTCGTGTATT -3') and CYP51-R (5'- TCATTGTAACCTATCCTCC - 3') (Mao et al, 2024). The PCR conditions were as follows: initial denaturation for 2 minutes at 95ºC; followed by 35 cycles of 1 minute at 94ºC denaturation, annealing at 55ºC for 1 minute and elongation at 72ºC for 2 minutes; followed by elongation at 72ºC for 10 minutes. The PCR product was sequenced along with other primers that were designed for sequencing (Table 1).

Table 1. List of primers used for sequencing.

Primer	Seq (5' to 3')	Reference
CYP51-F	ACCACCTTTTCGTGTATT	Mao et al., 2024
CYP51-R	TCATTGTAACCTATCCTCC	Mao et al., 2024
CYP51-S1	CATTGGCTCAGGAAATTTCGCAGCGGTC	This work
CYP51-S2	CTCAGGAAATTTCGCAGCGGTCAACTGG	This work
CYP51-S3	CTTCTTCGATTGTCGCGCAAAGGTATGT	This work

CYP51-S4	ACTGTTTTGACAACCCCCGTATTTGGCA	This work
CYP51-S5	TTGACAACCCCCGTGTTTGGCAAAGATG	This work
CYP51-S6	GTTCTTCGGCGTTCAAAGGTCCAAAGGG	This work
CYP51-S7	ATCATGCCCAGCGAACTGTAGCCAAAAC	This work
CYP51-S8	TGCTCTTCTCATGGCCGGACAACATTCT	This work
CYP51-S9	CGCCTCCACACCCCAATCCATTCTATCA	This work
CYP51-S10	GCAAAGGCGCTTCCAGTCCTTAC	This work

Efficacy of preventive and curative applications of mefentrifluconazole, cyprodinil and fludioxonil in the control of gray mold using sensitive and resistant isolates

To evaluate the efficacy of mefentrifluconazole, two experiments were conducted in a double factorial design (4 x 3), completely randomized, with seven replications. The factors evaluated were fungicides and isolates. One experiment was carried out to evaluate the preventive action and the other to evaluate the curative action of the fungicides. The fungicides evaluated were mefentrifluconazole (Belanty®, BASF) at a concentration of 75 mg/L a.i.; fludioxonil (Maxim®, Syngenta) at a concentration of 250 mg/L a.i.; cyprodinil (Unix®, Syngenta) at a concentration of 375 mg/L a.i.; and the control treatment (water application). The fungicides were diluted in distilled water to obtain spray solutions at the concentrations recommended by the manufacturers. In the case of the fungicide fludioxonil, which is not registered on its own with the Ministério da Agricultura, Pecuária e Abastecimento (MAPA) for strawberries, 250 mg/L a.i. was used, following the same a.i. concentration in the mixture of fludioxonil and cyprodinil, which is registered with MAPA for the crop (AGROFIT, 2025). Three isolates were used in these two experiments: isolate SJ374 with the G461S mutation (resistant to mefentrifluconazole - EC_{50} of 1.303 μg/ml) and sensitive to cyprodinil (EC_{50} of 0.07 μg/ml); isolate RA081 with the R464K mutation (resistant to mefentrifluconazole - EC_{50} of 0.465 μg/ml) and resistant to cyprodinil

(EC_{50} of 12.46 µg/ml) and isolate SJ463 which has no mutations (sensitive to mefentrifluconazole - EC_{50} of 0.046 µg/ml) and sensitive to cyprodinil (EC_{50} of 0.036 µg/ml). The preventive application of the fungicides was carried out 24 hours before inoculation and the curative application was carried out 24 hours after inoculation. Each repetition consisted of five fruits in a plastic pot 15 cm in diameter and 4.5 cm high with filter paper moistened with distilled water. The strawberry fruit used were of the Albion cultivar at the green to red stage of ripeness, with no previous fungicide application. The fruit was disinfected with 1.0% sodium hypochlorite for 2 min, followed by three washes in distilled water and dried at room temperature. Immediately afterwards, the fruit for the preventive control were sprayed with the adjusted concentration of the fungicides. After 24 hours, all the fruit for the preventive and curative control were inoculated without wounding with 30 µl of a suspension containing 10^5 spores/ml and placed in BOD incubators at 22ºC with a 12-hour photoperiod. The fruit used for the curative control were sprayed with the fungicides 24 hours after inoculation. The incidence (% of fruit with signs of the pathogen) was monitored five days after inoculation. The experiments were carried out twice.

RESULTS

Baseline sensitivity of *B. cinerea* to mefentrifluconazole

Based on mycelial growth, the EC_{50} values ranged from 0.030 to 1.303 µg/ml, with an average of 0.123 ± 0.156 µg/ml. The difference between the highest and lowest EC_{50} values found was 1.273. The baseline developed showed eight equal intervals with values of 0.16 µg/ml. Most isolates, 88%, had EC_{50} values below 0.19 µg/ml. Only two isolates, 1.3% of the total, had EC_{50} values above 1.14 µg/ml (fig.1A). The average EC_{50} of the isolates from Brazil was 0.169 µg/ml, ranging from 0.046 to 1.303 µg/ml, with two isolates (3%) having EC_{50} values above 1.14 µg/ml (fig.1B) and from the United States was 0.084 µg/ml, ranging from 0.030 to 0.384 µg/ml (fig.1C).

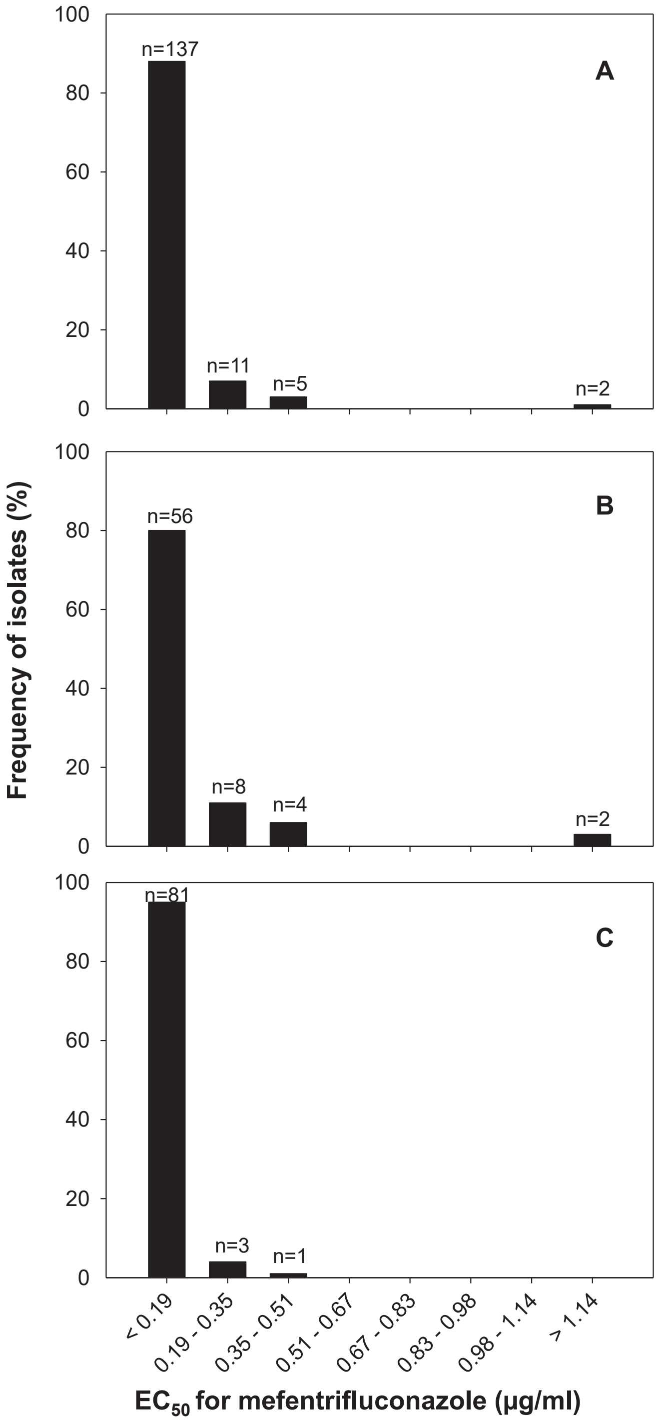

Fig. 1 Frequency distribution of EC_{50} (concentration to inhibit 50% of the mycelial growth) values for mefentrifluconazole. **A.** From 155 *Botrytis cinerea* isolates collected in Brazil and United States. **B.** From 70 *B. cinerea* isolates collected in

Brazil. **C.** From 85 *B. cinerea* isolates collected in United States. Individual EC_{50} values were grouped into class intervals of 0.16 µg/ml.

Cross-resistance

When comparing the EC_{50} values of 20 isolates to the fungicides mefentrifluconazole, difenoconazole and triflumizole, there was a correlation between the resistance of *B. cinerea* to mefentrifluconazole and that of the other two fungicides using the Spearman test ($p<0.05$) with the log-EC_{50} values (Fig. 2).

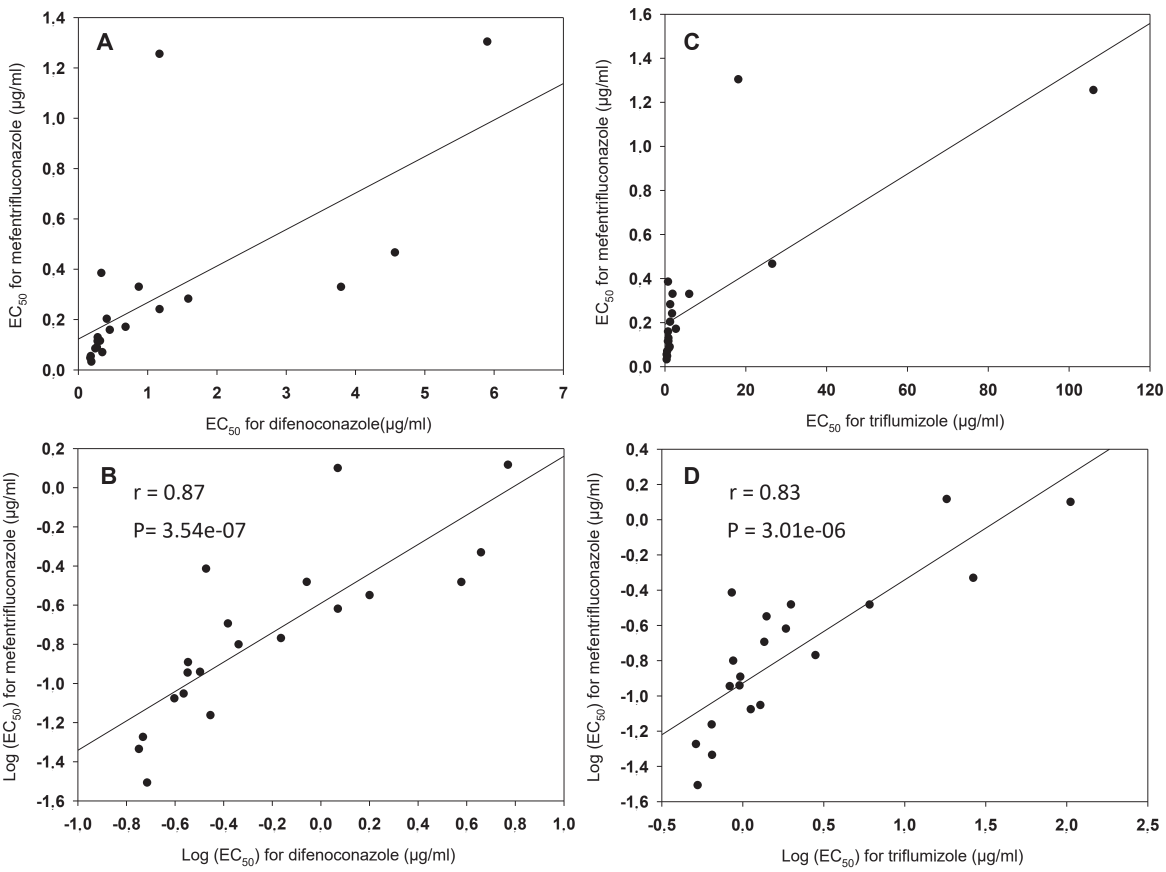

Fig. 2 Cross-resistance correlation analysis between mefentrifluconazole and difenoconazole (**A** - EC_{50} values and **B** - EC_{50} values in log) and between mefentrifluconazole and triflumizole (**C** - EC_{50} values and **D** - EC_{50} values in log) in *Botrytis cinerea* isolates from strawberry.

Mutation analysis

The amino acid sequence translated from the molecular characterization of the CYP51 gene of the isolates used revealed a substitution of glycine for serine at codon 461 in two isolates (SJ374 and JP041, from São José dos Pinhais-PR and Japira-PR, respectively) whose EC50 was the highest (1,303 and 1,254 μg/ml, respectively). In addition, a substitution of the amino acid arginine for lysine at codon 464 was observed in one isolate (RA081, from the city of Rio Azul-PR) which obtained the third highest EC50 among the isolates tested. The other isolates submitted to mutation analysis showed no mutations (Table 2).

Table 2. Identification of mutations in the CYP51 protein in isolates of *Botrytis cinerea*.

Isolates	Country	Translated protein sequences[a]		Mutation	EC_{50}
		461	464		μg/ml
SJ374	Brazil	LPF**S**AGR	SAG**R**HRC	G461S	1.303
JP041	Brazil	LPF**S**AGR	SAG**R**HRC	G461S	1.254
RA081	Brazil	LPF**G**AGK	GAG**K**HRC	R464K	0.465
20-431	USA	LPF**G**AGR	GAG**R**HRC	-	0.053
SJ463	Brazil	LPF**G**AGR	GAG**R**HRC	-	0.046
22-186	USA	LPF**G**AGR	GAG**R**HRC	-	0.031

[a] Fragments of the translated protein sequences after alignment showing replacement of glicine (G) by serine (S) and arginine (R) by lysine (K) at codons 461 and 464, respectively.

Efficacy of the fungicides in the control of gray mold in fruit inoculated with *B. cinerea* sensitive and resistant isolates

When the preventive effect of the fungicides was evaluated, for the sensitive isolate (SJ463), the fungicides mefentrifluconazole, cyprodinil and fludioxonil showed superior control efficacy when applied compared to the control treatment, with incidences of less than 7.14%. For the isolate characterized with the G461S mutation (SJ374), the incidence of the disease was 48.6% when the fungicide mefentrifluconazole was applied, showing it to be more effective in controlling the disease compared to the control, but with lower efficacy when compared to the fungicides cyprodinil and fludioxonil, which showed incidences of 2.9 and 0.0%, respectively. The RA081 isolate, whose R464K mutation was found and phenotypically characterized as resistant to cyprodinil, did not show control efficacy when applied with either the fungicide mefentrifluconazole or cyprodinil. For this isolate, the only fungicide able to effectively control the disease was fludioxonil (2.86% disease incidence) (Table 3).

When disease control efficacy was evaluated for the different resistance phenotypes to the fungicide mefentrifluconazole, disease control efficacy with this fungicide was higher for the sensitive isolate (SJ463) compared to the two resistant isolates (Fig. 3). For the fungicide cyprodinil, the isolate phenotypically resistant to mefentrifluconazole and cyprodinil (RA081) showed lower control efficacy. For the fungicide fludioxonil and the control treatment, there were no differences in control efficacy between the isolates (Table 3).

Table 3. Incidence of gray mold on strawberry fruit after preventive treatment with the fungicides mefentrifluconazole, cyprodinil and fludioxonil and inoculation of a sensitive isolate (SJ463), without the presence of a mutation; one resistant (SJ374) to mefentrifluconazole, with the G461S mutation and sensitive to cyprodinil and one resistant (RA081) to mefentrifluconazole, with the R464K mutation and resistant to cyprodinil.

Treatment	**Disease incidence (%)**		
	Sensitive (SJ463)	**Resistant (SJ374)**	**Resistant (RA081)**
	No mutation	**G461S**	**R464K + Cyprodinil**

			resistance
Control	70.0 Aa*	91.4 Aa	80.0 Aa
Mefentrifluconazole	7.1 Bb**	48.6 Ab	40.0 Ab
Cyprodinil	1.4 Bb	2.9 Bc	51.4 Aab
Fludioxonil	0.0 Ab	0.0 Ac	2.9 Ac

[*] Means followed by the same letters, lowercase in the column and uppercase in the row do not differ, respectively, by the ANOVA and Tukey tests at 5% probability.

[**] Data were transformed into log(x+3) for analysis but are presented in the original scale.

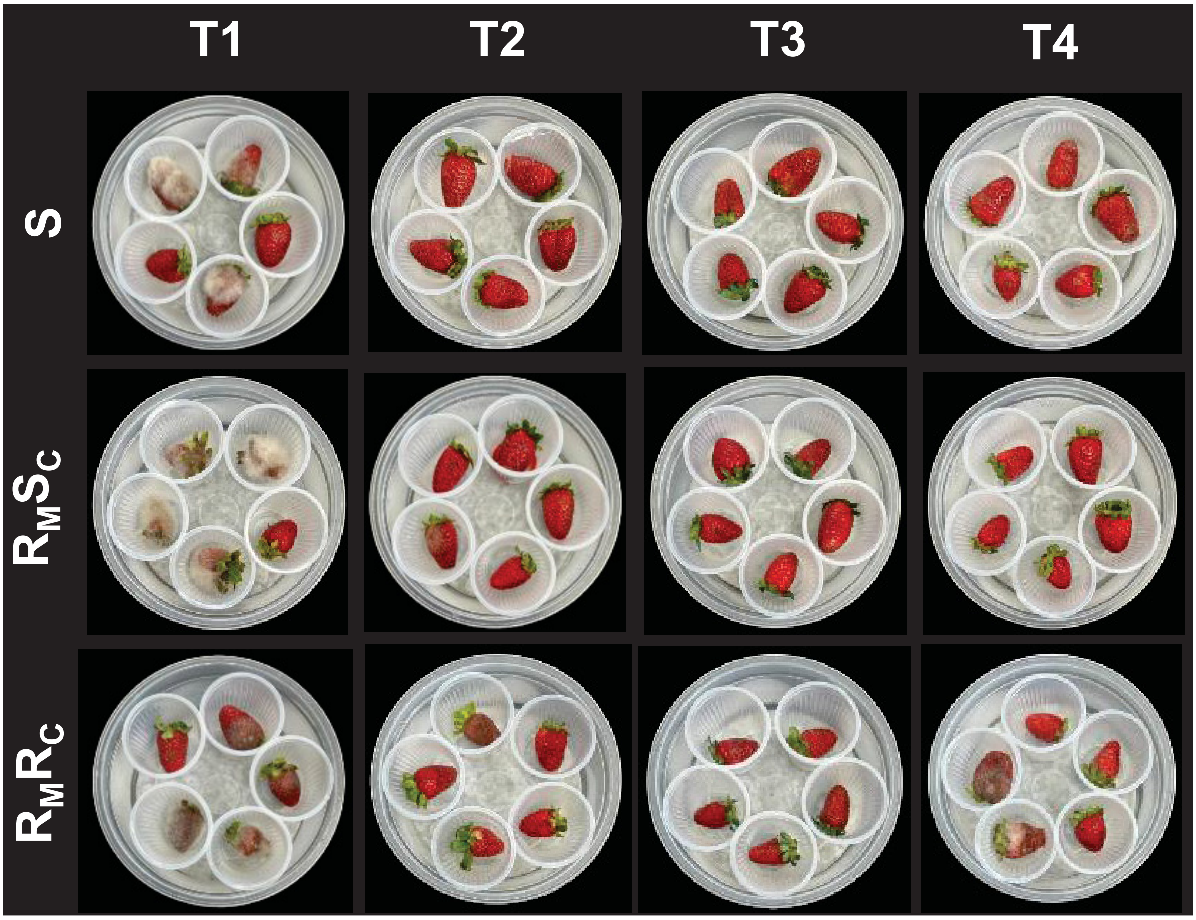

Fig. 3 Experiment testing the efficacy of the preventive control of the fungicides mefentrifluconazole (T2), fludioxonil (T3) and cyprodinil (T4) in comparison with the control, without fungicide application (T1) on three different isolates: S - sensitive isolate; RmSc - isolate resistant to mefentrifluconazole and sensitive to cyprodinil; and RmRc - isolate resistant to mefentrifluconazole and cyprodinil.

When the curative effect of the fungicides was evaluated, for the mefentrifluconazole-sensitive isolate (SJ463), the control efficacy of the mefentrifluconazole fungicide (44.3% incidence) was higher than the control treatment, but it showed lower control efficacy when compared to the cyprodinil and fludioxonil fungicides, which showed 1.4 and 2.9% incidence, respectively. For both DMI-resistant mutant isolates, treatment with mefentrifluconazole did not differ from the control, while treatment with the fungicide fludioxonil was the most effective control. The fungicide cyprodinil differed from the control only for the isolate that was resistant to the fungicide mefentrifluconazole (SJ374), and was not effective for the isolate (RA081) that was resistant to the fungicides mefentrifluconazole and cyprodinil (Table 4).

When the disease control efficacy was evaluated for the different resistance phenotypes to the fungicide mefentrifluconazole, the disease control efficacy with the fungicide mefentrifluconazole was higher for the sensitive isolate (SJ463) compared to the two resistant isolates. For the fungicide cyprodinil and fludioxonil, the isolate phenotypically resistant to mefentrifluconazole and cyprodinil (RA081) showed lower control efficacy (Fig. 4). For the control treatment, there were no differences in control efficacy between the isolates (Table 4).

Table 4. Incidence of gray mold on strawberry fruit after curative treatment with the fungicides mefentrifluconazole, cyprodinil and fludioxonil and inoculation of a sensitive isolate (SJ463), without the presence of a mutation; one resistant (SJ374) to mefentrifluconazole, with the G461S mutation and sensitive to cyprodinil and one resistant (RA081) to mefentrifluconazole, with the R464K mutation and resistant to cyprodinil.

Treatment	**Disease incidence (%)**		
	Sensitive (SJ463)	**Resistant (SJ374)**	**Resistant (RA081)**
	No mutation	**G461S**	**R464K + Cyprodinil resistance**
Control	70.0 Aa*	91.4 Aa	80.0 Aa
Mefentrifluconazole	44.3 Bb**	74.3 Aa	58.6 Aa
Cyprodinil	11.3 Cc	27.1 Bb	60.0 Aa
Fludioxonil	2.9 Bc	1.4 Bc	25.7 Ab

* Means followed by the same letters, lowercase in the column and uppercase in the row do not differ, respectively, by the ANOVA and Tukey tests at 5% probability.

** Data were transformed into √(x+1) for analysis but are presented in the original scale.

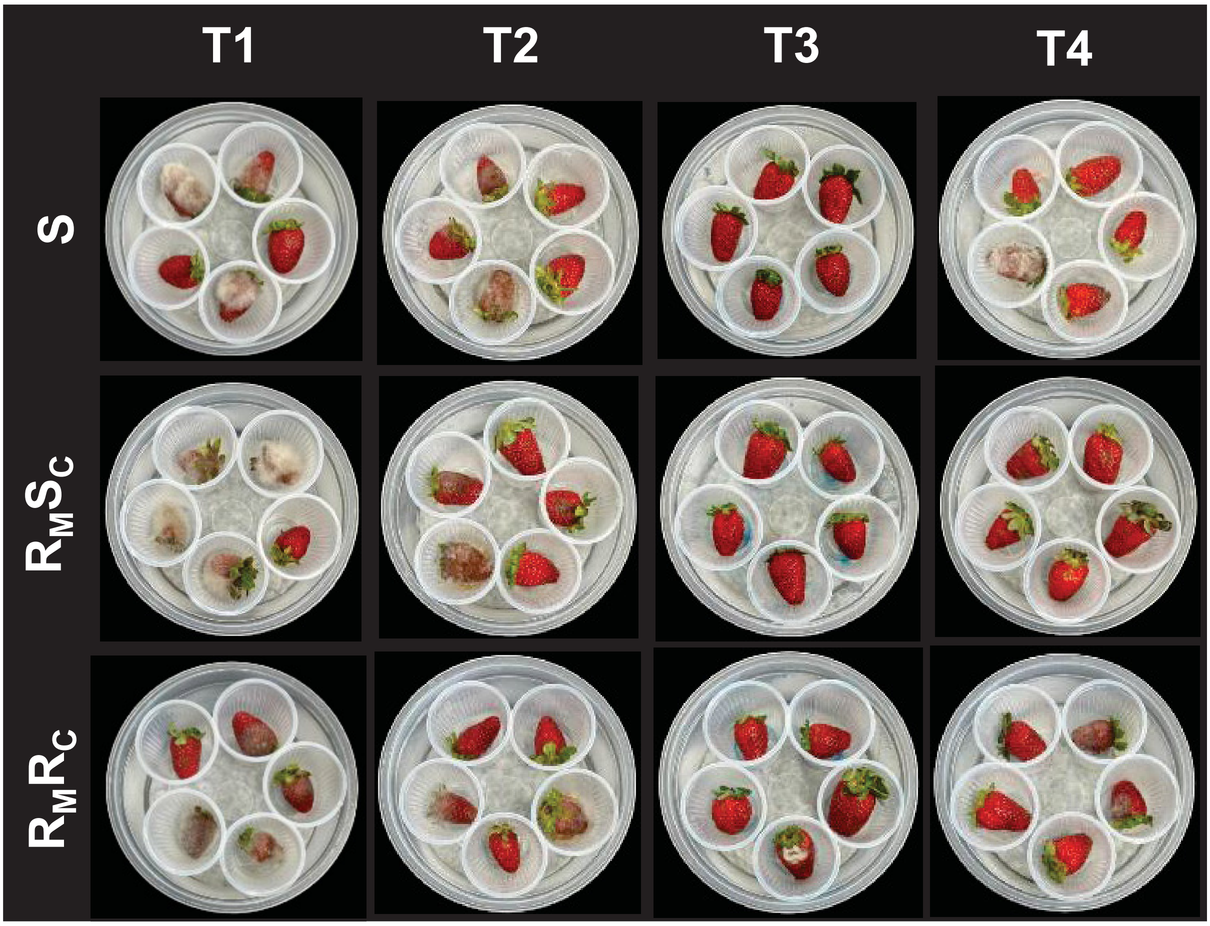

Fig. 4 Experiment testing the efficacy of the curative control of the fungicides mefentrifluconazole (T2), fludioxonil (T3) and cyprodinil (T4) in comparison with the control, without fungicide application (T1) on three different isolates: S - sensitive isolate; RmSc - isolate resistant to mefentrifluconazole and sensitive to cyprodinil; and RmRc - isolate resistant to mefentrifluconazole and cyprodinil.

DISCUSSION

The report of *Botrytis cinerea* resistance to mefentrifluconazole in other crops (Mao et al., 2024; Chen et al., 2024) underlines the importance of establishing a baseline before exposing the pathogen to the new active ingredient in strawberry cultivation, even with other DMIs already registered for the crop. This study identified cross-resistance between DMI group fungicides and detected two mutations: G461S in two isolates and R464K in one isolate. The resistance observed in the laboratory was confirmed in detached fruit. Despite this, mefentrifluconazole can be integrated into disease management in areas without resistant isolates, where its efficacy was similar to that of established fungicides such as fludioxonil and cyprodinil. This information is essential for appropriate management in the field, considering the potential of mefentrifluconazole against *B. cinerea*.

Most of the isolates were sensitive to mefentrifluconazole (the average EC_{50} of the isolates from Brazil was 0.169 µg/ml, ranging from 0.046 to 1.303 µg/ml and from the United States was 0.084 µg/ml, ranging from 0.030 to 0.384 µg/ml)), forming a unimodal curve with a right tail at the baseline of both countries. This behavior corroborates with isolates tested with mefentrifluconazole (Li et al., 2021), other DMIs (Fan et al., 2016) and other fungicide groups on *B. cinerea* (Maia et al., 2023). However, some EC_{50} values found suggest the presence of resistant isolates. The highest EC_{50} values in strawberry *B. cinerea* reported by Li et al. (2021) for mefentrifluconazole were 0.313 µg/ml, four times lower than those observed in the present study.

In general, some isolates collected in Brazil (mean EC50: 0.169 µg/ml) proved to be less sensitive than the American isolates (mean EC50: 0.084 µg/ml). The cross-resistance between active ingredients of the DMI chemical group, proven in this study, explains this fact, since the use of this group of fungicides is more intense in Brazil, especially with the active ingredient difenoconazole. In the USA, the use of other active ingredients from the DMI group in strawberries is mainly for powdery mildew control, with low efficacy against *B. cinerea* (personal communication). Cross-resistance between DMIs has already been proven and observed in other pathosystems (Fonseka and Gudmestad, 2016, Dutra et al., 2020, Wei et al., 2020, Ishii et al., 2021), including in *B. cinerea* in tomato isolates (Zhang et al., 2020, Chen et al., 2024), but there were no reports in strawberry (Li et al., 2021). Knowledge of the existence of cross-resistance in this pathosystem in strawberry cultivation is indispensable and will serve as a guide in fungicide rotation, to delay the selection of resistant isolates (Fan et al., 2016).

Although there are three different mechanisms of resistance to DMIs, point mutations in the *CYP51* gene are the most reported in field isolates (Price et al., 2015). Changes in the amino acid sequence, resulting from mutations in the *CYP51* gene, associated with phenotypic changes in sensitivity to DMIs have already been reported, such as: Y136F, V136A, D134G, S524T (Cools et al., 2013). The two mutations found in this work, G461S and R464K, have also been reported to cause loss of sensitivity to fungicides in *Botrytis cinerea* isolates in China, increasing EC_{50} values for fungicides from the DMI group by up to 10 times (Mao et al., 2024). G461S and R464K were also associated with the resistance of *B. cinerea*, collected from tomato plants, to pyrisoxazole (Chen et al., 2024). The G464S and R467K mutations (corresponding to G461S and R464K in *B.cinerea*) have also been associated with resistance of *Candida albicans* to fluconazole (Morschhäuser, 2002). The same mutation, G461S, was identified in *Monilinia fructicola*, leading to resistance to tebuconazole (Lichtemberg et al., 2017). The G459S mutation (corresponding to G461S in *B. cinerea*), together with F506I, are involved in resistance to the fungicide prochloraz in *Penicilium digitatum* (Wang et al., 2014). These reports show that the mutations found in the present work are strictly related to the loss of sensitivity of *B. cinerea* to mefentrifluconazole, as well as to difenoconazole and triflumizole, other DMIs tested for cross-resistance.

The most effective fungicide for controlling gray mold was fludioxonil, given the low incidence values of the disease on fruit, both in preventive and curative treatments. This shows that fludioxonil is the best option for controlling the disease (Amiri et al., 2013, Maia et al., 2023, Maia et al., 2024). Despite this, the product must be applied at the right time for its activity to reach its potential (Amiri et al., 2013). Care must be taken when using this fungicide, with anti-resistance management in mind, such as use limits and even mixing with fungicides with different modes of action (Ren et al., 2016). In Brazil, the commercial product has already been registered in a mixture with cyprodinil, which could help with anti-resistance management.

The presence of the isolate previously characterized as resistant to cyprodinil showed that there was a difference in the incidence of the disease between sensitive and resistant isolates. Therefore, an isolate with reduced sensitivity will not have completely inhibited the biosynthesis of methionine and other amino acids, where this fungicide acts (Masner et al., 1994).

Although practical resistance to the fungicide mefentrifluconazole was observed in detached fruit in both the preventive treatment (7.1% incidence in the susceptible isolate, and 48.6% and 40.0% in resistant isolates) and the curative treatment (44.3% incidence in the susceptible isolate, and 74.3% and 58.8% in resistant isolates), the use of this fungicide is an excellent alternative for managing the disease when there is no reported resistance, equivalent to other fungicides available on the market. The efficacy of mefentrifluconazole has already been reported both for the *Botrytis cinerea* pathosystem (Li et al., 2021) and in other pathosystems (Ishii et al., 2022).

A comparison between the absolute values of the two experiments reveals a higher incidence of the disease in the fruit treated with the curative treatment. For the fungicide mefentrifluconazole, the incidence in the preventive treatment was 7.1%, 48.6% and 40.0%, while in the curative treatment the same isolates showed rates of 44.3%, 74.3% and 58.6%, respectively. This same trend was observed for the other two fungicides evaluated (tables 3 and 4). This difference corroborates other studies that have shown the better preventive effect of fungicides in controlling gray mold (Li et al., 2021, Maia et al., 2023). Therefore, mefentrifluconazole should be sprayed in advance or at an early stage of gray mold spread to improve disease control (Li et al., 2021).

The number of effective fungicides is decreasing worldwide, mainly due to the selection of resistant pathogens. Given reports of cross-resistance among DMIs, the resistance observed in this study is likely associated with the use of other DMIs in the crop. In Brazil, fungicides such as difenoconazole, tebuconazole, and metconazole are widely used and registered (ADAPAR, 2025), while in the United States, DMI-based products are also authorized, mainly for the control of powdery mildew in strawberries (personal communication). In this context, mefentrifluconazole emerges as a promising alternative, as its potential, observed in the fruits evaluated in this study, has already been demonstrated in field tests with commercial products. The combination of mefentrifluconazole and fluxapyroxad resulted in a 30.8% incidence in fruits, outperforming both the untreated control (48.4%) and the treatment with fluxapyroxad alone (38.4%) (Zuniga et al., 2024). Furthermore, a key aspect is that the product recently approved in Brazil contains mefentrifluconazole in combination with the carboxamide fluxapyroxad, a formulation that helps reduce the risk of fungal resistance to the fungicide, making it a more sustainable strategy for crop disease management.

Although mefentrifluconazole is considered a fungicide with a low to medium risk of resistance (FRAC), *B. cinerea* is a pathogen listed as a “high risk” for resistance. This is due to its wide host range, various positions where the fungus attacks, high genetic variability and prolific reproduction capacity (FRAC, Chen et al., 2024). In conclusion, our results indicate the importance of a baseline study, as well as the efficaccy of the product, even before it is made available to the crop and for the control of a pathogen with a high resistance potential. The baseline developed could serve as a comparison for the periodic sensitivity studies that are needed. The presence of mutations alerts us to appropriate management strategies, using mixtures with fungicides with a low risk of resistance and rotating active ingredients in the crop.

REFERENCES

ADAPAR – AGÊNCIA DE DEFESA AGROPECUÁRIA DO PARANÁ. *Agrotóxicos no Paraná*. Available at: https://celepar07web.pr.gov.br/agrotoxicos/pesquisar.asp. Accessed on: January, 2025.

AGROFIT. 2025. Sistema de agrotóxicos fitossanitários. Available at: http://agrofit.agricultura.gov.br/agrofit_cons/principal_agrofit_cons. Accessed on January, 2025.

Amiri, A., Heath, S.M., and Peres, N.A. 2013. Phenotypic characterization of multifungicide resistance in *Botrytis cinerea* isolates from strawberry fields in Florida. Plant Disease. 97:393-401.

Baggio, J.S., Peres, N.A., and Amorim, L. 2018. Sensitivity of *Botrytis cinerea* Isolates from Conventional and Organic Strawberry Fields in Brazil to Azoxystrobin, Iprodione, Pyrimethanil, and Thiophanate-Methyl. Plant Disease. 120:1803-1810.

Bardas, G.A., Veloukas, T., Koutita, O., and Karaoglanidis, G.S. 2010. Multiple resistance of *Botrytis cinerea* from kiwifruit to SDHIs, QoIs and fungicides of other chemical groups. Pest Manag Sci. 66:967–973.

Carter, H.E., Fraaije, B.A., West, J.S., Kelly, S.L., Mehl, A., Shaw, M.W., and Cools, H.J. 2014. Alterations in the predicted regulatory and coding regions of the sterol 14α-demethylase gene (CYP51) confer decreased azole sensitivity in the oilseed rape pathogen *Pyrenopeziza brassicae*. Molecular Plant Pathology. 15(5), 513–522.

Chen, L., Sun, B., Zhao, Y., and Miao, Z. 2024. Molecular Mechanisms and Biological Characteristics of *Botrytis cinerea* Field Isolate Resistance to Pyrisoxazole in Liaoning Province. Plant Disease. 108:866-876.

Cools, H.J., Hawkins, N.J., and Fraaije, B.A. 2013. Constraints on the evolution of azole resistance in plant pathogenic fungi. Plant Pathology. 62:36–42.

Dean, R., Van Kan, J.A.L., Pretorius, Z.A., Hammond-Kosack, K.E., Di Pietro, A., Spanu, P.D., Rudd, J.J., Dickman, M., Kahmann, R., Ellis, J., and Foster, G.D. 2012. The Top 10 fungal pathogens in molecular plant pathology. Molecular Plant Pathology. 13(4), 414–430.

Dutra, P.S.S., Lichtemberg, P.S.F., Martinez, M.B., Michailides, T.J., May De Mio, L.L. 2020. Cross-Resistance Among Demethylation Inhibitor Fungicides With Brazilian *Monilinia fructicola* Isolates as a Foundation to Discuss Brown Rot Control on Stone Fruit. Plant Disease. 104:2843-2850.

Fan, K., Wang, J., Fu, L., Li, X., Zhang, Y., Zhang, X., Zhai, H., and Qu, J. 2016. Sensitivity of *Botryosphaeria dothidea* from apple to tebuconazole in China. Crop Protection. 87:1-5.

Fong, Y.K., Anuar, S., Lim, H.P., Tham, F.Y., and Sanderson, F.R. 2000. A modified filter paper technique for long-term preservation of some fungal cultures. Mycologist 14:127–130.

Fonseka, D.L., and Gudmestad, N.C. 2016. Spatial and Temporal Sensitivity of *Alternaria* Species Associated With Potato Foliar Diseases to Demethylation Inhibiting and Anilo-Pyrimidine Fungicides. Plant Disease. 100:1848-1857.

Gao, Y., He, L., Mu, W., Li, B., Lin, J., and Liu, F. 2018. Assessment of the baseline sensitivity and resistance risk of *Colletotrichum acutatum* to fludioxonil. Eur J Plant Pathol. 150:639–651.

He, D., Shi, J., Qiu, J., Hou, Y., Du, Y., Gao, T., Huang, W., Wu, J., Lee, Y.W., Mohamed, S.R., Liu, X., and Xu, J. 2023. Antifungal activities of a novel triazole fungicide, mefentrifluconazole, against the major maize pathogen *Fusarium verticillioides*. Pesticide Biochemistry and Physiology. 192:105398.

Ishii, H., Bryson, P.K., Kayamori, M., Miyamoto, T., Yamaoka, Y., and Schnabel, G. 2021. Cross-resistance to the new fungicide mefentrifluconazole in DMI-resistant fungal pathogens. Pesticide Biochemistry and Physiology. 171:104737.

Ishii, H., Watanabe, H., Yamaoka, Y., and Schnabel, G. 2022. Sensitivity to fungicides in isolates of *Colletotrichum gloeosporioides* and *C. acutatum* species complexes and efficacy against anthracnose diseases. Pesticide Biochemistry and Physiology. 182:105049.

Kretschmer, M., Leroch, M., Mosbach, A., Walker, A.S., Fillinger, S., Mernke, D., Schoonbeek, H.J., Pradier, J.M., Leroux, P., De Waard, M.A., and Hahn, M. 2009. Fungicide-Driven Evolution and Molecular Basis of Multidrug Resistance in Field Populations of the Grey Mould Fungus *Botrytis cinerea*. PLoS Pathogens. 5:12.

Li, T., Li, H., Liu, T., Zhu, J., Zhang, L., Mu, W., and Liu, F. 2021. Evaluation of the antifungal and biochemical activities of mefentrifluconazole against *Botrytis cinerea*. Pesticide Biochemistry and Physiology. 173:104784.

Lichtemberg, P.S.F., Luo, Y., Morales, R.G., Muehlmann-Fischer, J.M., Michailides, T.J., and May De Mio, L.L. 2017. The point mutation G461S in the MfCYP51 gene is associated with tebuconazole resistance in *Monilinia fructicola* populations in Brazil. Phytopathology 107:1507-1514.

Maia, J.N., Beger, G., Pereira, W.V., May De Mio, L.L., and Duarte, H.S.S. 2021. Gray mold in strawberries in the Paraná state of Brazil is caused by *Botrytis cinerea* and its isolates exhibit multiple-fungicide resistance. Crop Protection. 140:105415.

Maia, J.N., Beger, G., Peres, N.A., Nesi, C.N., May De Mio, L.L., and Duarte, H.S.S. 2023. Baseline sensitivity of *Botrytis cinerea* strawberry isolates from Brazil to fludioxonil and cyprodinil and disease control efficacy. Tropical Plant Pathology. 49:131–140.

Maia, J.N., Beger, G., Boldrini, I.B., Peres, N.A., May De Mio, L.L., and Duarte, H.S.S. 2024. Sensitivity of Brazilian *Botrytis cinerea* isolates from strawberry to pyrimethanil and its control efficacy. 49:141–146.

Masner, E., Muster, E., and Schmid, J. 1994. Possible methionine biosynthesis inhibition by pyrimidinamine fungicides. Pesticide Science. 42:163–166.

Mao, Y., Meng, X., Zhang, Z., Zhang, L., Feng, Y., Wang, T., Zhang, J., Cai, Y., Wang, J., Sheng, G., Shen, Y., Zhou, M., and Duan, Y. 2024. Genetic variation of drug target CYP51 conferring resistance to ergosterol biosynthesis inhibitors in *Botrytis cinerea*, causing lily gray mould. Industrial Crops & Products. 208:117797.

Mertely, J.C., Chandler, C.K., Xiao, C.L., and Legard, D.E. 2000. Comparison of sanitation and fungicides for management of Botrytis fruit rot of strawberry. Plant Disease. 84:1197-1202.

Morschhäuser, J. 2002. The genetic basis of fluconazole resistance development in *Candida albicans*. Biochimica et Biophysica Acta. 1587:240–248.

Oliveira, M.S., Amiri, A., Zuniga, A.I., and Peres, N.A. 2017. Sources of Primary Inoculum of *Botrytis cinerea* and Their Impact on Fungicide Resistance Development in Commercial Strawberry Fields. Plant Disease. 101:1761-1768.

Price, C.L., Parker, J.E., Warrilow, A.G.S., Kelly, D.E., and Kelly, S.L. 2015. Azole fungicides – understanding resistance mechanisms in agricultural fungal pathogens. Pest Manag Sci. 71:1054–1058.

R Core Team. 2024. R: A language and environment for statistical computing. R Foundation for Statistical Computing. https:// www. R-project. org/

Ren, W., Shao, W., Han, X., and Chen, C. 2016. Molecular and biochemical characterization of laboratory and field mutants of *Botrytis cinerea* resistant to fludioxonil. Plant Disease. 100:1414-1423.

Rocha, M.G.C., Custódio, A.A.P., Fantin, L.H., Oliveira, K.B., Campos, H.D., Canteri, M.G., and Duarte, H.S.S. 2024. Development and validation of a standard area diagram set to assess corn grey leaf spot severity and foliar fungicide control efficacy. Journal of Phytopathology. 172:e13311.

Wang, J., Yu, J., Liu, J., Yuan, Y., Li, N., He, M., Qi, T., Hui, G., Xiong, L., and Liu, D. 2014. Novel Mutations in CYP51B from *Penicillium digitatum* Involved in Prochloraz Resistance. Journal of Microbiology. 52:762–770.

Wei, L.L., Chen, W.C., Zhao, W.C., Wang, J., Wang, B.R., Li, F.J., Wei, M.D., Guo, J., Chen, C.J., Zheng, J.Q., and Wang, K. 2020. Mutations and Overexpression of CYP51 Associated with DMI-Resistance in *Colletotrichum gloesporioides* from Chili. Plant Disease. 104:668-676.

Zhang, Y., Wu, X., Li, X., Duan, T., Xu, J., Dong, F., Liu, X., Guo, L., and Zheng, Y. 2019. A fast and sensitive ultra-highperformance liquid chromatography-tandem mass spectrometry method for determining mefentrifluconazole in plant- and animal-derived foods. Food Additives & Contaminants: Part A.

Zhang, C., Li, T., Xiao, L., Zhou, S., and Xili Liu, X. 2020. Characterization of tebuconazole resistance in *Botrytis cinerea* from tomato plants in China. Phytopathology Research. 2:25.

Zuniga, A.I., Rebello, C.S., Peres, N.A. 2024. Evaluation of products for the control of Botrytis fruit rot in annual strawberry, 2023-24. Plant Disease Management Reports. 18:PF046.