International Journal of *Plant Biology*

MDPI

Article

In Vitro Sensitivity of Four Fungi Isolated from Symptomatic White Onion (*Allium fistulosum* L.) to DMI, QoI, and MBC Fungicides

Jhon Andres Meneses-Mariño [1], Pablo Israel Alvarez-Romero [2,*], Eliana Granja-Guerra [3], Daniel Arturo Román-Robalino [2], Ana Francisca Tibúrcia Amorim Ferreira e Ferreira [4] and Eduardo Patricio Salazar-Castañeda [2]

[1] Independent Researcher, Riobamba 060155, Ecuador
[2] Escuela Superior Politécnica de Chimborazo (ESPOCH), Panamericana Sur Km 1 ½, Riobamba 060155, Ecuador
[3] Faculty of Agricultural Sciences and Natural Resources, Universidad Técnica de Cotopaxi, Barrio Salache S/N, Latacunga 050150, Ecuador
[4] Programa de Pós-Graduação em Agronomia Tropical, Federal University of Amazonas, Manaus 69067-005, Brazil
* Correspondence: pabloi.alvarez@espoch.edu.ec

Abstract

White onion (*Allium fistulosum* L.) is a major source of income for smallholders in the Ecuadorian Andes, where fungal root and foliar diseases reduce yield and commercial quality and are controlled mainly with single-site fungicides. We evaluated the in vitro sensitivity of four fungal isolates (*Fusarium* sp. SFCBDMR.01, *Penicillium* sp. SFCBDMR.24, *Alternaria* sp. SFCBDMF.04, and *Ulocladium* sp. SFCBDMR.13) obtained from symptomatic white onion in Santa Fe de Galán, Ecuador, and pathogenic on this host (Koch's postulates), to the demethylation inhibitors (DMIs) tebuconazole and mefentrifluconazole, the quinone outside inhibitors (QoIs) pyraclostrobin and azoxystrobin, and the methyl benzimidazole carbamates (MBCs) thiophanate-methyl and thiabendazole, incorporated into potato dextrose agar at 0–500 μg mL^{-1}. Sensitivity was quantified as the mycelial growth rate and the median effective concentration (EC_{50}), estimated using a three-parameter log-logistic model. Tebuconazole was highly active against *Fusarium* sp. (EC_{50} = 0.24 μg mL^{-1}) and *Penicillium* sp. (EC_{50} < 0.1 μg mL^{-1}) and moderately active against *Alternaria* sp. (7.41 μg mL^{-1}); mefentrifluconazole was highly active against *Alternaria* sp. and *Ulocladium* sp., and thiabendazole against *Ulocladium* sp. (EC_{50} < 0.1 μg mL^{-1}). The QoIs and thiophanate-methyl did not halve the mycelial growth of most isolates (EC_{50} > 500 μg mL^{-1}), except *Alternaria* sp. with pyraclostrobin (36.0 μg mL^{-1}) and *Penicillium* sp. with azoxystrobin, which showed a concentration-independent inhibition plateau of 61–64%. Responses were specific to each isolate–active ingredient combination, even within a FRAC group. Because one isolate per genus was tested and no baseline population was available, these values describe phenotypic sensitivity rather than resistance. They are, to our knowledge, the first fungicide sensitivity reference values for fungi associated with white onion in Ecuador and provide a basis for selecting active ingredients and for future resistance monitoring in this crop.

Keywords: *Allium fistulosum*; fungicide sensitivity; EC_{50}; mycelial growth; DMI; QoI; MBC

Academic Editor: Adriano Sofo

Received: 6 July 2026
Revised: 19 October 2026
Accepted: 3 November 2026
Published: 5 November 2026

Copyright: © 2026 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license.

1. Introduction

White onion (*Allium fistulosum* L.), commonly known as Welsh onion, is a vegetable species native to East Asia that is widely grown for its culinary uses [1]. In Ecuador, it is an important crop for smallholders in the Andean highlands; in Santa Fe de Galán parish (Chimborazo Province) alone, approximately 361 ha are devoted to white onion, which is one of the main sources of income for local farmers [2].

Production is constrained by fungal diseases that affect roots, foliage, and harvested tissues. In Allium crops, *Fusarium* spp. cause basal and root rot, characterized by leaf yellowing that progresses from the tips, wilting, and brown to reddish-violet decay of the roots and basal plate [3]; in Welsh onion, *Fusarium* isolates also cause wilt and stunting of seedlings [4]. *Alternaria* spp. cause foliar blights such as purple blotch (*A. porri*), which begins as small, whitish to water-soaked spots that enlarge into elliptical, sunken, purple lesions with concentric zones and a yellowish border and, when they coalesce, cause leaf-tip dieback [5,6]. *Penicillium* spp. are mainly associated with blue mold, a soft, watery, pale yellow to light brown decay of bulbs, pseudostems, and stored tissues covered by blue-green sporulation, which usually starts at wounds [7,8]. *Ulocladium* spp., now regarded as a section of *Alternaria* [9], are mostly saprophytic fungi that colonize necrotic onion leaf tissue [10], although some species can cause leaf blight with small, dark lesions that turn black and coalesce into irregular necrotic patches [11]. These fungi can reduce yield and commercial quality and increase production costs through repeated fungicide applications [12].

Chemical control remains an important component of disease management. However, the repeated use of fungicides with a single site of action imposes selection pressure on fungal populations and may favor resistant phenotypes [13,14]. Reduced sensitivity or resistance to the three groups evaluated in this study—demethylation inhibitors (DMIs; FRAC group 3), quinone outside inhibitors (QoIs; FRAC group 11), and methyl benzimidazole carbamates (MBCs; FRAC group 1)—has been documented in the same genera in other crops and regions: reduced sensitivity of *Fusarium graminearum* to DMI and QoI fungicides [15], benzimidazole and multiple resistance in *Penicillium* spp. [16], and QoI resistance in *Alternaria alternata* [17]. Fungi causing postharvest deterioration of garlic (*Allium sativum*) in Mexico also differed in their sensitivity to fungicides [18]. In contrast, no information is available on the fungicide sensitivity of fungi associated with white onion in Ecuador.

In vitro sensitivity assays can detect differences in the response of fungi to active ingredients before reduced efficacy becomes evident in the field. Sensitivity is commonly quantified by exposing the fungus to a range of concentrations and estimating, from the dose–response curve, the median effective concentration (EC_{50}), i.e., the concentration that reduces growth by 50% relative to the untreated control [19]. EC_{50} values allow the comparison of isolates and active ingredients and, when obtained from representative collections of isolates, are the basis for establishing baseline sensitivity distributions and detecting shifts associated with resistance [13].

Sensitivity can vary considerably among species and among isolates of the same species, depending on genetic background, previous exposure to fungicides, and resistance mechanisms such as target-site alterations or increased efflux activity [12,20]. Evaluating fungi against several active ingredients from different chemical groups is therefore useful to identify differences in sensitivity and to support more rational fungicide use.

Santa Fe de Galán was selected because it is one of the main white onion-producing parishes of Chimborazo Province [2], where growers rely on chemical control and where no information exists on the fungicide sensitivity of the fungi associated with the crop. The six fungicides were selected because they are commercial products available in the local agro-input market and represent the three single-site FRAC groups (DMI, QoI, and MBC) most commonly used in vegetable production, with two active ingredients per

https://doi.org/10.3390/ijpb17110121

group to examine within-group variation. Accordingly, the objective of this study was to evaluate the in vitro sensitivity of isolates of *Fusarium* sp., *Penicillium* sp., *Alternaria* sp., and *Ulocladium* sp. obtained from symptomatic white onion plants in Santa Fe de Galán to DMI, QoI, and MBC fungicides, using the mycelial growth rate and EC_{50} values as response variables.

2. Materials and Methods

2.1. Sample Collection

Samples were collected from established white onion crops in the Barrio Norte sector of Santa Fe de Galán parish (Guano canton, Chimborazo Province, Ecuador; 1°29′49.2″ S, 78°32′15.7″ W; 3546 m a.s.l.). Two sampling events were carried out at different times, and a total of 50 samples of root and foliar tissues were collected from plants showing disease symptoms (leaf yellowing and dieback, foliar lesions, and root discoloration and decay; Figure 1a). The samples were placed individually in sterile plastic bags, transported under refrigeration, and stored at 4–8 °C until processing at the Plant Pathology Laboratory, Faculty of Natural Resources, Escuela Superior Politécnica de Chimborazo (ESPOCH), Riobamba, Ecuador.

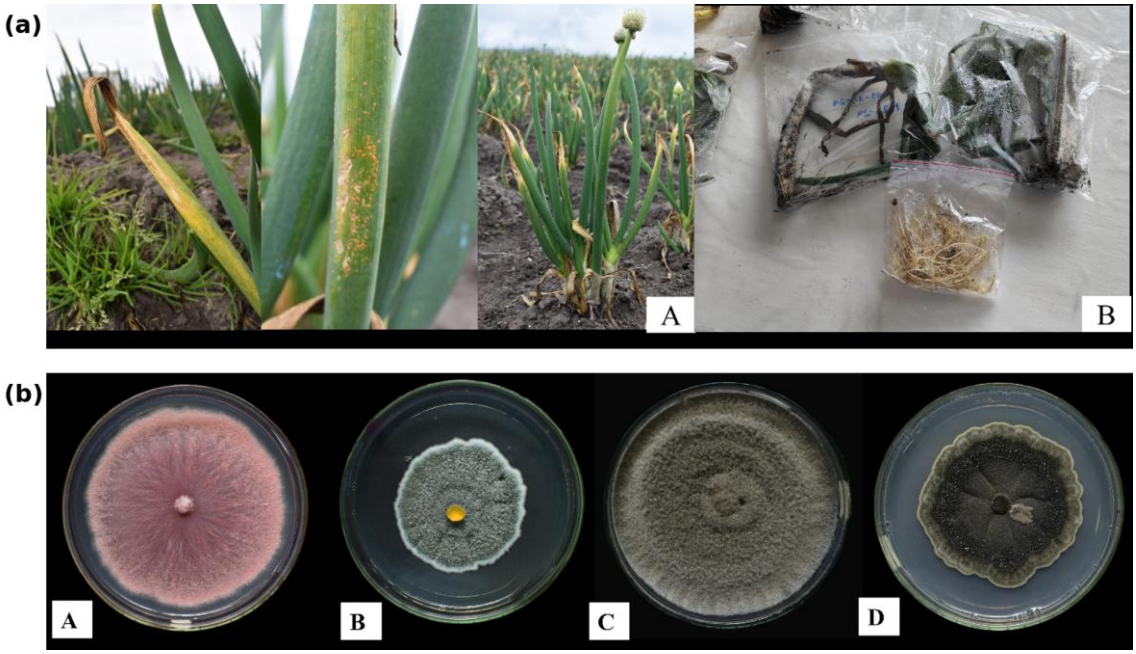


Figure 1. (**a**) Symptomatic white onion plants in Santa Fe de Galán (A) and root and foliar samples (B). (**b**) Colonies on PDA of the four isolates evaluated: (A) *Fusarium* sp. SFCBDMR.01, (B) *Penicillium* sp. SFCBDMR.24, (C) *Alternaria* sp. SFCBDMF.04, and (D) *Ulocladium* sp. SFCBDMR.13.

2.2. Isolation and Identification of Fungi

Root and foliar tissues were processed by direct and indirect isolation [6]. Tissue fragments (approximately 4 mm) were excised from the margin between healthy and symptomatic tissue with a sterile scalpel. For direct isolation, fragments were rinsed in sterile distilled water and plated without chemical disinfection. For indirect isolation, fragments were surface-disinfected by immersion in 70% ethanol for 30 s and 2% sodium hypochlorite for 2 min and rinsed in sterile distilled water for 2 min. Fragments were placed in Petri dishes containing potato dextrose agar (PDA) and Rose Bengal agar supplemented with ampicillin and a benzylpenicillin formulation (Biconcilina®, Life, Quito, Ecuador), sealed with Parafilm, and incubated at 25 ± 2 °C for 10 days.

Emerging colonies were subcultured by transferring actively growing mycelial fragments to PDA supplemented with 100 mg L^{-1} chloramphenicol and incubated at 25 ± 2 °C in the dark for 10 days until pure cultures were obtained. Isolates were identified to genus level by morphology, i.e., on the basis of colony characteristics on PDA (color, texture, pigmentation, and growth) and of the microscopic characters diagnostic for each genus (conidiophores and conidia observed in lactoglycerol mounts under a light microscope), following the descriptions of Leslie and Summerell for *Fusarium* [21], Visagie et al. for *Penicillium* [22], and Woudenberg et al. for *Alternaria* and *Ulocladium* [9]. No biochemical or molecular methods were used; therefore, isolates are reported at genus level only. One representative isolate of each genus was selected for the pathogenicity tests (Section 2.3) and the sensitivity assays: *Fusarium* sp. SFCBDMR.01, *Penicillium* sp. SFCBDMR.24, and *Ulocladium* sp. SFCBDMR.13 from roots, and *Alternaria* sp. SFCBDMF.04 from foliar tissue (Table 1; Figure 1b). Isolate codes are laboratory accession codes composed of the locality (SF, Santa Fe de Galán), the host (CB, *cebolla blanca*, white onion), the collector's initials (DM), the tissue of origin (R, root; F, foliar), and the isolate number. Because a single isolate per genus was used, the genus name (e.g., *Fusarium* sp.) refers throughout the text to the corresponding coded isolate in Table 1.

Table 1. Fungal isolates recovered from symptomatic white onion in Santa Fe de Galán (Chimborazo, Ecuador) and representative isolates used in the pathogenicity tests and sensitivity assays (total, 30 isolates).

Representative Isolate (Code) [a]	Isolates Recovered, *n* (%)	Crop	Tissue of Origin [b]
(A) *Fusarium* sp. SFCBDMR.01	10 (33.3)	White onion	Root
(B) *Penicillium* sp. SFCBDMR.24	8 (26.7)	White onion	Root
(C) *Alternaria* sp. SFCBDMF.04	7 (23.3)	White onion	Foliar
(D) *Ulocladium* sp. SFCBDMR.13	5 (16.7)	White onion	Root

[a] SF, Santa Fe de Galán; CB, *cebolla blanca* (white onion); DM, collector's initials; R, root; F, foliar; final digits, isolate number. [b] Tissue from which the representative isolate was obtained.

2.3. Pathogenicity Tests (Koch's Postulates)

The pathogenicity of the four representative isolates later used in the sensitivity assays (Table 1) was tested in a greenhouse on healthy, 45-day-old white onion (*A. fistulosum*) plants; ten plants were inoculated per isolate. *Alternaria* sp. was inoculated by placing mycelial discs, cut from the margin of actively growing PDA cultures, on the leaves. *Fusarium* sp., *Penicillium* sp., and *Ulocladium* sp. were inoculated with conidial suspensions of 1×10^7 conidia mL^{-1} prepared from PDA cultures, applied to the roots (*Fusarium* sp. and *Penicillium* sp.) or to the leaves (*Ulocladium* sp.). Control plants were treated with sterile distilled water. Plants were kept in the greenhouse at a mean temperature of 18 °C and a relative humidity above 60% and were evaluated for symptoms 10 days (*Penicillium* sp.), 12 days (*Fusarium* sp. and *Ulocladium* sp.), and 15 days (*Alternaria* sp.) after inoculation. To complete Koch's postulates, fragments from the margin of the symptomatic tissue were plated on PDA as described in Section 2.2, and the re-isolated fungi were identified by comparing their colony and microscopic characters with those of the inoculated isolates.

2.4. Fungicides and Preparation of Amended Media

Six commercial fungicides from three FRAC groups [14] were evaluated (Table 2): the DMIs (FRAC group 3; target site code G1) tebuconazole and mefentrifluconazole, the QoIs (group 11; code C3) pyraclostrobin and azoxystrobin, and the MBCs (group 1; code B1) thiophanate-methyl and thiabendazole. For each product, a stock solution of 100,000 μg a.i.

mL^{-1} was prepared in sterile distilled water on an active-ingredient basis, sterilized by filtration, and diluted to a 10,000 μg a.i. mL^{-1} working solution. Appropriate volumes of the working solution were added to 240 mL batches of molten PDA supplemented with chloramphenicol (100 mg L^{-1}) to obtain final concentrations of 0 (untreated control), 0.1, 1, 10, 100, and 500 μg a.i. mL^{-1}, and the amended media were dispensed into 90 mm Petri dishes.

Table 2. Fungicides evaluated and active ingredient content of the commercial products used to prepare the stock solutions.

FRAC Group (Target Site Code)	Chemical Group	Active Ingredient	Trade Name	Active Ingredient Concentration
DMI, 3 (G1)	Triazoles	Tebuconazole	Stratego® SC	250 g L^{-1}
		Mefentrifluconazole	Belanty®	400 g L^{-1}
QoI, 11 (C3)	Strobilurins	Pyraclostrobin	Comet® 25 CE	250 g L^{-1}
		Azoxystrobin	Amistar 50 WG	500 g kg^{-1}
MBC, 1 (B1)	Benzimidazoles	Thiophanate-methyl	Novak® 500 SC	500 g L^{-1}
		Thiabendazole	Mertect 500 SC	500 g L^{-1}

2.5. Mycelial Growth Assay

Mycelial discs (approximately 5.13 mm in diameter) were cut with sterile straws from the margin of actively growing colonies on PDA and placed on the fungicide-amended medium. Untreated control plates received a single disc at the center, whereas each amended plate received two discs placed equidistantly on opposite sides of the center; in amended plates, the two colonies were measured separately and their mean was used, so that the plate was the experimental unit. Each isolate–fungicide combination was set up as an independent experiment with six concentrations and three replicate plates per concentration, arranged in a randomized complete block design in which each block (replicate) contained one plate of every concentration. Plates were labeled, sealed with plastic film, and incubated in the dark at 25 ± 2 °C for 8–10 days (fixed for each experiment; Table S3). The final reading was taken when the control colonies of the fast-growing isolates (*Fusarium* sp. and *Alternaria* sp.) approached the edge of the plate; because the incubation time was set by these isolates, the control colonies of the slower-growing *Penicillium* sp. and *Ulocladium* sp. did not reach the plate edge (Figure 2). This does not bias the comparison, because MGR is expressed per day and inhibition is calculated relative to the control of the same experiment. Colony diameter was measured in two perpendicular directions with a digital caliper. In three assays—tebuconazole with *Penicillium* sp. and the untreated controls of the azoxystrobin assays with *Penicillium* sp. and *Ulocladium* sp.—colony diameters were measured in the same two perpendicular directions on calibrated digital photographs of the plates taken at the final reading (one plate per concentration), using the outer diameter of the 90 mm Petri dish as the scale (Table S1). The mycelial growth rate of each plate was calculated from the final reading as MGR (mm day^{-1}) = $(D - d_0)/t$, where D is the mean colony diameter (mm), d_0 is the disc diameter (5.13 mm), and t is the incubation time (days). Percentage inhibition was calculated as PI (%) = $[(MGRc - MGRt)/MGRc] \times 100$, where MGRc is the mean MGR of the control plates and MGRt is the MGR of a treated plate. For descriptive purposes only, inhibition was categorized as low (0–25%), moderate (>25–50%), or high (>50%) (Table 3); these classes are not a standardized guideline and were used only to summarize the data, whereas sensitivity was compared on the basis of EC_{50} values (Section 2.6).



Figure 2. Mycelial growth of (A) *Fusarium* sp. SFCBDMR.01, (B) *Penicillium* sp. SFCBDMR.24, (C) *Alternaria* sp. SFCBDMF.04, and (D) *Ulocladium* sp. SFCBDMR.13 on PDA amended with tebuconazole, mefentrifluconazole, pyraclostrobin, azoxystrobin, thiophanate-methyl, and thiabendazole at 0 (untreated control), 0.1, 1, 10, 100, and 500 μg mL^{-1} (left to right), photographed at the final reading. Control plates contained one colony and amended plates two. Because the incubation time (8–10 days) was set by the fast-growing isolates, the control colonies of the slower-growing *Penicillium* sp. and *Ulocladium* sp. did not reach the plate edge.

Table 3. Descriptive classes of mycelial growth inhibition used to summarize the data.

Category	Range
Low inhibition	0–25%
Moderate inhibition	>25–50%
High inhibition	>50%

2.6. Estimation of the Median Effective Concentration (EC_{50})

For each isolate–fungicide combination, the EC_{50} was estimated by nonlinear least-squares regression of plate-level MGR on fungicide concentration, using the three-parameter log-logistic model with the lower limit fixed at zero, $f(x) = d/\{1 + \exp[b(\ln x - \ln EC_{50})]\}$, where d is the upper limit (the MGR of the control) and b is the slope. This model is equivalent to the LL.3 model of the drc package [19], which is used by the ec50estimator package [23]; because the lower limit is fixed at zero, the EC_{50} corresponds to the concentration that halves the control MGR. Models were fitted in Python 3.13 with SciPy 1.18 (least_squares, trust-region reflective algorithm) [24] and NumPy 2.5 [25]; the EC_{50} was estimated on the logarithmic scale, and 95% confidence intervals were computed from its standard error and back-transformed. Goodness of fit was assessed with the residual standard error and a lack-of-fit F-test against a one-way ANOVA model, and the three-parameter log-logistic model was compared with the four-parameter log-logistic and two three-parameter Weibull models using Akaike's information criterion (Table S1). EC_{50} values were not extrapolated beyond the tested range: when mean inhibition at 500 μg mL^{-1} was below 50%, the EC_{50} is reported as >500 μg mL^{-1}, and when the estimate was lower than the lowest concentration tested, it is reported as <0.1 μg mL^{-1}.

2.7. Sensitivity Categories

To facilitate comparison among combinations, EC_{50} values were assigned to five descriptive sensitivity categories (Table 4), adapted from the EC_{50} ranges used by Gudmestad et al. [26] for *Alternaria solani* and consistent with the general criterion of Edgington et al. [27], in which EC_{50} values <1 μg mL^{-1} indicate high fungitoxicity. The original ranges were developed for a single pathogen–fungicide group and are not validated thresholds of resistance for the genera studied here; in addition, the category for 100–500 μg mL^{-1} was made explicit to avoid gaps between classes. Therefore, the categories describe the relative phenotypic sensitivity of each isolate and were not used to infer resistance, which would require a baseline sensitivity distribution of the population and, ideally, the identification of resistance mechanisms [13,14].

Table 4. Descriptive sensitivity categories based on EC_{50} values (adapted from [26,27]).

Category	Range
Highly sensitive (HS)	<1 μg mL^{-1}
Sensitive (S)	1–5 μg mL^{-1}
Moderately sensitive (MS)	>5–20 μg mL^{-1}
Low sensitivity (LS)	>20–100 μg mL^{-1}
Very low sensitivity (VLS)	>100 μg mL^{-1}

2.8. Statistical Analyses

Each isolate–fungicide combination was analyzed as an independent experiment. In the 21 combinations with three replicate plates per concentration, MGR data were subjected to analysis of variance for a randomized complete block design, with concentration as a fixed factor and replicate as block (error df = 10). Normality of residuals and homogeneity of variances were checked with the Shapiro–Wilk and Levene (median-centered)

tests, respectively (Table S2); data were analyzed untransformed. Means were compared with Tukey's honestly significant difference (HSD) test ($p \leq 0.05$); letters therefore compare concentrations within one isolate–fungicide combination only. Analyses were performed in Python 3.13 with SciPy [24].

3. Results

3.1. Fungal Isolates and Pathogenicity

Fungi belonging to four genera, *Fusarium*, *Penicillium*, *Alternaria*, and *Ulocladium*, were recovered from the 50 symptomatic samples and identified morphologically (Figure 1b). In total, 30 isolates were obtained: 10 of *Fusarium* sp., 8 of *Penicillium* sp., 7 of *Alternaria* sp., and 5 of *Ulocladium* sp., corresponding to isolation frequencies of 33.3%, 26.7%, 23.3%, and 16.7%, respectively (Table 1). One isolate per genus (*Fusarium* sp. SFCBDMR.01, *Penicillium* sp. SFCBDMR.24, *Alternaria* sp. SFCBDMF.04, and *Ulocladium* sp. SFCBDMR.13; Table 1) was used in the pathogenicity tests and sensitivity assays, and all results below therefore refer to these four individual isolates.

Pathogenicity was confirmed for the four representative isolates. *Fusarium* sp. caused reddish discoloration of the roots 12 days after inoculation, and *Penicillium* sp. caused root rot after 10 days; *Alternaria* sp. caused necrotic leaf spots after 15 days, and *Ulocladium* sp. necrotic leaf spots after 12 days. For each isolate, more than 70% of the inoculated plants (≥7 of 10) developed symptoms, whereas control plants remained asymptomatic. Each fungus was re-isolated from the symptomatic tissue and re-identified morphologically as the inoculated genus, thus fulfilling Koch's postulates.

3.2. Mycelial Growth Rate

Colony growth of the four isolates on PDA amended with each of the six fungicides, including the untreated control of every experiment, is shown in Figure 2. Fungicide concentration significantly reduced MGR in 19 of the 21 replicated isolate–fungicide combinations ($p < 0.05$; Table S2); the exceptions were *Ulocladium* sp. with pyraclostrobin ($p = 0.23$) and *Penicillium* sp. with thiophanate-methyl ($p = 0.24$). The shape of the response, however, differed markedly: some combinations showed a progressive decline that approached complete inhibition, whereas others showed a sharp reduction at 0.1 µg mL^{-1} followed by a plateau of partial inhibition up to 500 µg mL^{-1} (Figures 3–8; Table S3).

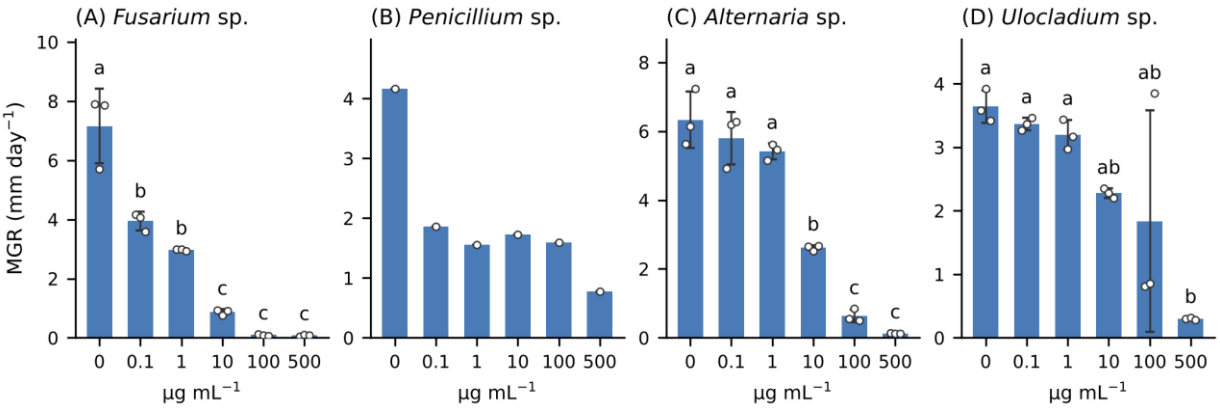


Figure 3. Mycelial growth rate (MGR) of (**A**) *Fusarium* sp., (**B**) *Penicillium* sp., (**C**) *Alternaria* sp., and (**D**) *Ulocladium* sp. on PDA amended with tebuconazole. Bars are means ± standard deviation (n = 3 replicate plates) and points are individual plates; MGR = (D − 5.13 mm)/t, calculated from the final colony diameter. Within each panel, different letters indicate significant differences among concentrations (randomized complete block ANOVA followed by Tukey's HSD test, $p \leq 0.05$).

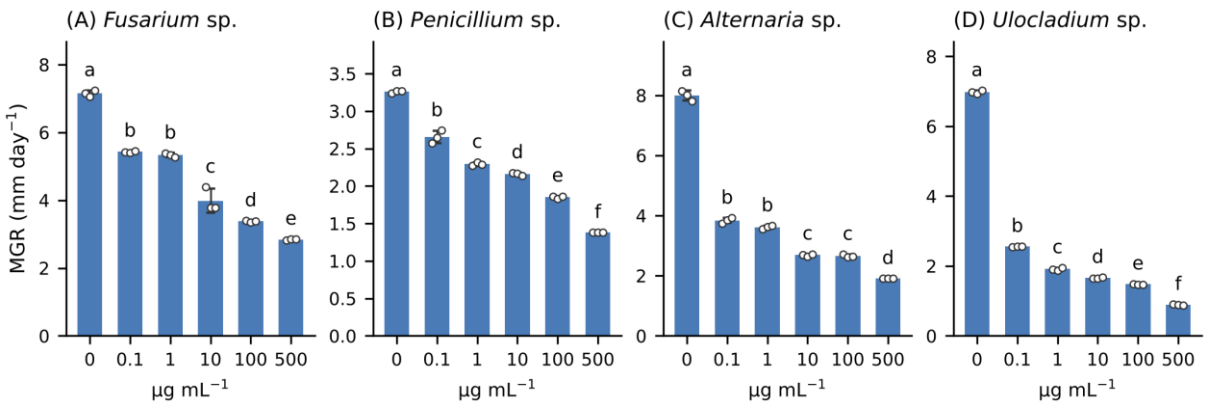


Figure 4. Mycelial growth rate (MGR) of (**A**) *Fusarium* sp., (**B**) *Penicillium* sp., (**C**) *Alternaria* sp., and (**D**) *Ulocladium* sp. on PDA amended with mefentrifluconazole. Bars are means ± standard deviation (n = 3 replicate plates) and points are individual plates; MGR = (D − 5.13 mm)/t, calculated from the final colony diameter. Within each panel, different letters indicate significant differences among concentrations (randomized complete block ANOVA followed by Tukey's HSD test, $p \leq 0.05$).

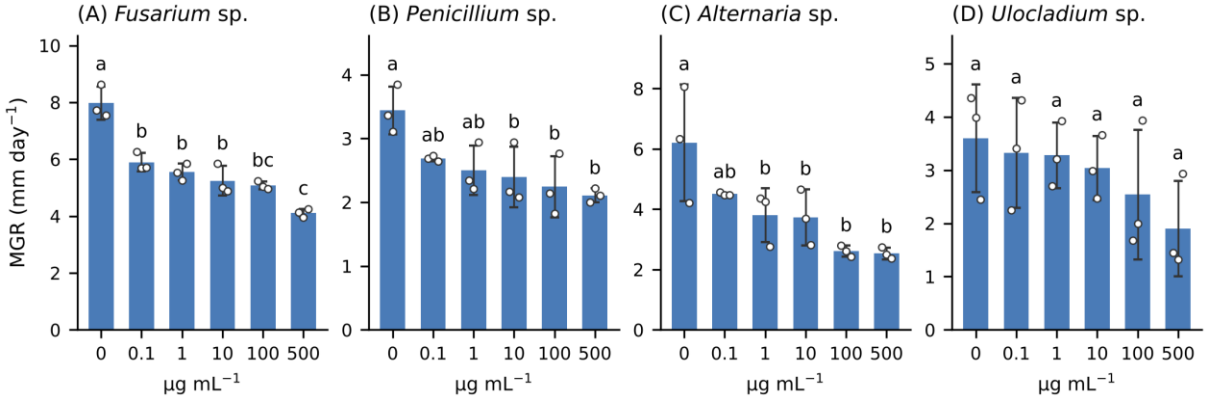


Figure 5. Mycelial growth rate (MGR) of (**A**) *Fusarium* sp., (**B**) *Penicillium* sp., (**C**) *Alternaria* sp., and (**D**) *Ulocladium* sp. on PDA amended with pyraclostrobin. Bars are means ± standard deviation (n = 3 replicate plates) and points are individual plates; MGR = (D − 5.13 mm)/t, calculated from the final colony diameter. Within each panel, different letters indicate significant differences among concentrations (randomized complete block ANOVA followed by Tukey's HSD test, $p \leq 0.05$).

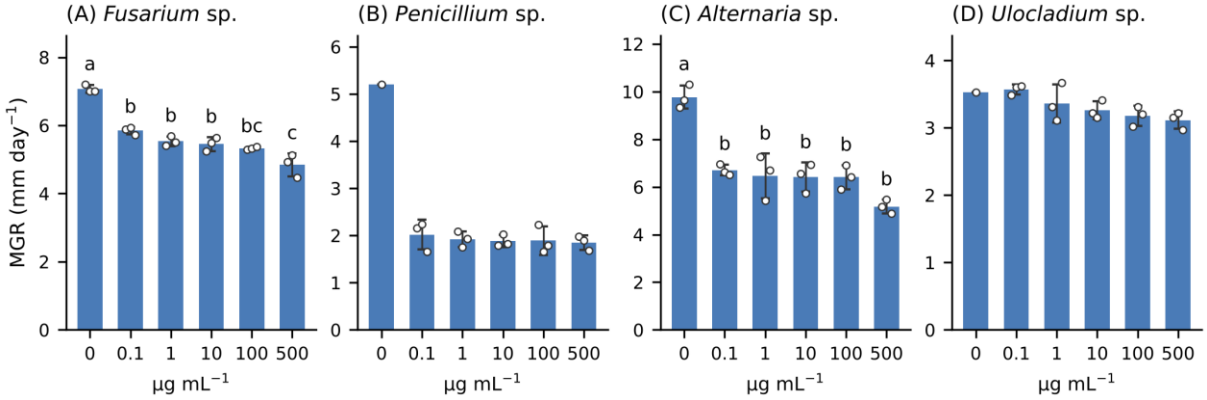


Figure 6. Mycelial growth rate (MGR) of (**A**) *Fusarium* sp., (**B**) *Penicillium* sp., (**C**) *Alternaria* sp., and (**D**) *Ulocladium* sp. on PDA amended with azoxystrobin. Bars are means ± standard deviation (n = 3 replicate plates) and points are individual plates; MGR = (D − 5.13 mm)/t, calculated from the final colony diameter. Within each panel, different letters indicate significant differences among concentrations (randomized complete block ANOVA followed by Tukey's HSD test, $p \leq 0.05$).

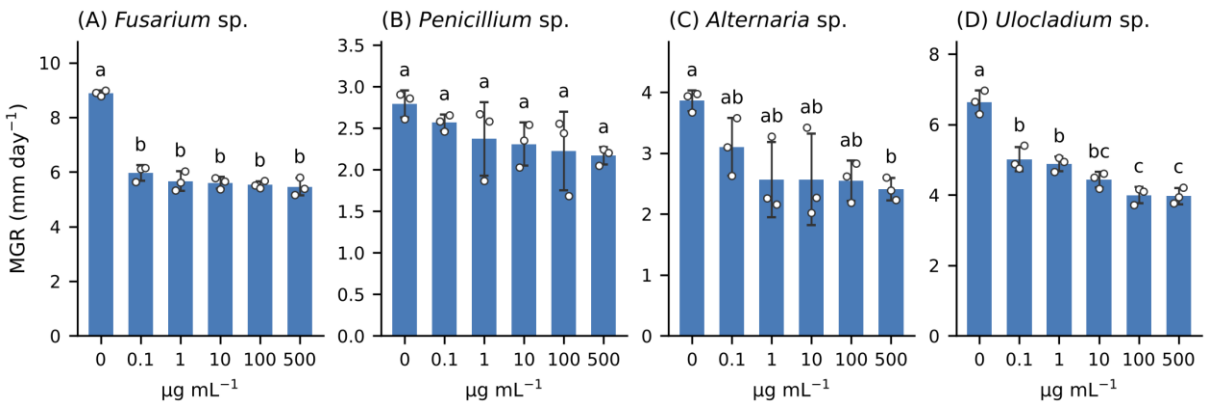


Figure 7. Mycelial growth rate (MGR) of (**A**) *Fusarium* sp., (**B**) *Penicillium* sp., (**C**) *Alternaria* sp., and (**D**) *Ulocladium* sp. on PDA amended with thiophanate-methyl. Bars are means ± standard deviation (n = 3 replicate plates) and points are individual plates; MGR = (D − 5.13 mm)/t, calculated from the final colony diameter. Within each panel, different letters indicate significant differences among concentrations (randomized complete block ANOVA followed by Tukey's HSD test, $p \leq 0.05$).

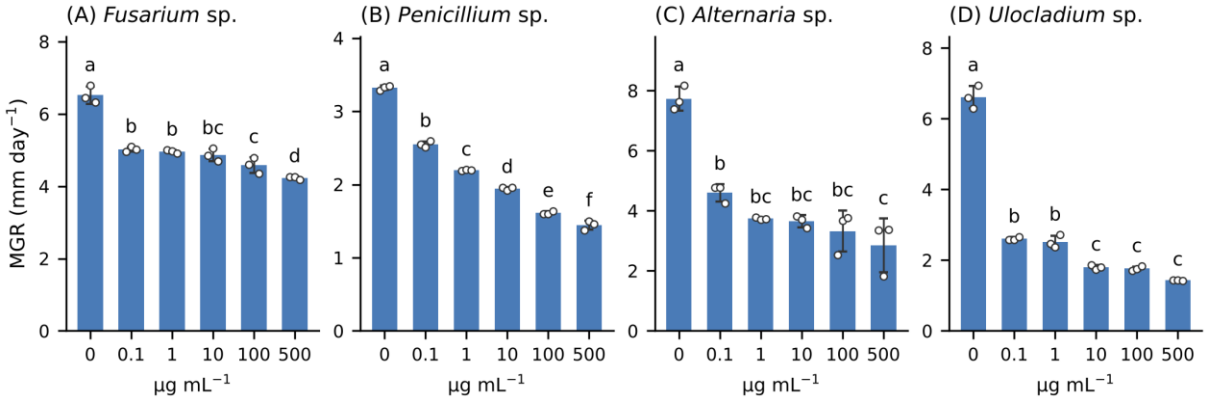


Figure 8. Mycelial growth rate (MGR) of (**A**) *Fusarium* sp., (**B**) *Penicillium* sp., (**C**) *Alternaria* sp., and (**D**) *Ulocladium* sp. on PDA amended with thiabendazole. Bars are means ± standard deviation (n = 3 replicate plates) and points are individual plates; MGR = (D − 5.13 mm)/t, calculated from the final colony diameter. Within each panel, different letters indicate significant differences among concentrations (randomized complete block ANOVA followed by Tukey's HSD test, $p \leq 0.05$).

3.2.1. Tebuconazole

Tebuconazole was the only fungicide that almost completely arrested the growth of more than one isolate. The MGR of *Fusarium* sp. fell from 7.16 ± 1.26 mm day^{-1} in the control to 3.94 mm day^{-1} at 0.1 µg mL^{-1} (45% inhibition) and to 0.08 mm day^{-1} at 500 µg mL^{-1} (99%). *Alternaria* sp. was not affected at 0.1–1 µg mL^{-1} but was inhibited by 59% at 10 µg mL^{-1} and by 98% at 500 µg mL^{-1}, whereas *Ulocladium* sp. responded mainly at the highest concentration (92% inhibition at 500 µg mL^{-1}). *Penicillium* sp. was inhibited by 55% at 0.1 µg mL^{-1}, by 59–63% at 1–100 µg mL^{-1}, and by 81% at 500 µg mL^{-1} (Figure 3).

3.2.2. Mefentrifluconazole

Mefentrifluconazole produced a graded, concentration-dependent response in all four isolates, but never complete inhibition. *Ulocladium* sp. and *Alternaria* sp. were the most affected, with 63% and 52% inhibition already at 0.1 µg mL^{-1} and 87% and 76% at 500 µg mL^{-1}, respectively. In contrast, inhibition of *Fusarium* sp. and *Penicillium* sp. exceeded 50% only at ≥100 and 500 µg mL^{-1}, respectively (60% and 58% at 500 µg mL^{-1}) (Figure 4).

3.2.3. Pyraclostrobin

Pyraclostrobin caused only partial inhibition, which typically reached a plateau at low concentrations. At 500 μg mL^{-1}, inhibition was 48% for *Fusarium* sp., 39% for *Penicillium* sp., and 59% for *Alternaria* sp., whereas the MGR of *Ulocladium* sp. was not significantly affected (47% at 500 μg mL^{-1}, with high variability among replicates) (Figure 5).

3.2.4. Azoxystrobin

Azoxystrobin also produced a plateau-shaped response. Inhibition of *Fusarium* sp. increased only from 17% at 0.1 μg mL^{-1} to 32% at 500 μg mL^{-1}, and that of *Alternaria* sp. from 31% to 47%, with no significant differences among 0.1–100 μg mL^{-1} in *Alternaria* sp. *Penicillium* sp. was inhibited by 61% at 0.1 μg mL^{-1}, and inhibition remained at 63–64% up to 500 μg mL^{-1}, whereas inhibition of *Ulocladium* sp. did not exceed 12% at any concentration (Figure 6).

3.2.5. Thiophanate-Methyl

Thiophanate-methyl caused only partial inhibition of all isolates; at 500 μg mL^{-1}, inhibition reached 39% in *Fusarium* sp., 38% in *Alternaria* sp., and 40% in *Ulocladium* sp., and the effect on *Penicillium* sp. (22%) was not significant (Figure 7).

3.2.6. Thiabendazole

Thiabendazole was more active than thiophanate-methyl. *Ulocladium* sp. was inhibited by 61% at 0.1 μg mL^{-1} and by 78% at 500 μg mL^{-1}, and *Alternaria* sp. by 41% and 63%, respectively; *Penicillium* sp. showed a steady concentration-dependent decline to 57% inhibition at 500 μg mL^{-1}, whereas *Fusarium* sp. was inhibited by only 23–35% across the whole range (Figure 8).

3.3. Median Effective Concentration (EC_{50})

Among the 21 replicated combinations, the EC_{50} could be estimated within the tested range for 8, was below the lowest concentration tested (<0.1 μg mL^{-1}) for 3, and exceeded 500 μg mL^{-1} for 10 (Table 5; Figure S1). Tebuconazole was highly active against *Fusarium* sp. (EC_{50} = 0.24 μg mL^{-1}; 95% CI 0.11–0.54) and moderately active against *Alternaria* sp. (7.41 μg mL^{-1}; 4.85–11.3), whereas *Ulocladium* sp. showed low sensitivity (44.9 μg mL^{-1}; 9.6–209). Mefentrifluconazole was highly active against *Alternaria* sp. and *Ulocladium* sp. (EC_{50} < 0.1 μg mL^{-1}), but *Fusarium* sp. (59.0 μg mL^{-1}; 31.1–112) and *Penicillium* sp. (206 μg mL^{-1}; 112–378) fell in the low- and very-low-sensitivity categories, respectively. Thiabendazole was highly active against *Ulocladium* sp. (<0.1 μg mL^{-1}), active against *Alternaria* sp. (2.32 μg mL^{-1}; 0.23–23.4), and less active against *Penicillium* sp. (79.7 μg mL^{-1}; 57.8–110). Pyraclostrobin reached 50% inhibition only for *Alternaria* sp. (36.0 μg mL^{-1}, with a wide CI of 1.5–841). In the remaining 10 combinations—azoxystrobin with *Fusarium* sp. and *Alternaria* sp.; pyraclostrobin with *Fusarium* sp., *Penicillium* sp., and *Ulocladium* sp.; thiophanate-methyl with all four isolates; and thiabendazole with *Fusarium* sp.—mean inhibition at 500 μg mL^{-1} remained between 22% and 48%; the EC_{50} is therefore reported as >500 μg mL^{-1}, without extrapolation. The EC_{50} was <0.1 μg mL^{-1} for *Penicillium* sp. with tebuconazole (model estimate 0.02 μg mL^{-1}) and with azoxystrobin (inhibition plateau of 61–64%), and >500 μg mL^{-1} for *Ulocladium* sp. with azoxystrobin (12% inhibition at 500 μg mL^{-1}). Overall, 5 of the 24 combinations had EC_{50} < 0.1 μg mL^{-1} and 11 had EC_{50} > 500 μg mL^{-1}.

Within each FRAC group, the two active ingredients frequently differed: tebuconazole was more active than mefentrifluconazole against *Fusarium* sp. (0.24 vs. 59.0 μg mL^{-1}) and *Penicillium* sp. (<0.1 vs. 206 μg mL^{-1}), whereas the opposite ranking was found for *Alternaria* sp. and *Ulocladium* sp., and thiabendazole was more active than thiophanate-

methyl against *Alternaria* sp., *Penicillium* sp., and *Ulocladium* sp. The sensitivity categories were unchanged when the best model according to AIC was used and when only the colony diameter perpendicular to the axis joining the two colonies was considered (Table S1).

Table 5. EC_{50} (μg mL^{-1}; 95% confidence interval) and sensitivity category of the four isolates for the six fungicides, estimated with the three-parameter log-logistic model.

Fungicide	FRAC Group	*Fusarium* sp.	*Penicillium* sp.	*Alternaria* sp.	*Ulocladium* sp.
Tebuconazole	DMI	0.24 (0.11–0.54) HS	<0.1 [c] HS	7.41 (4.85–11.3) MS	44.9 (9.6–209) LS
Mefentrifluconazole	DMI	59.0 (31.1–112) LS	206 (112–378) VLS	<0.1 [b] HS	<0.1 [b] HS
Pyraclostrobin	QoI	>500 [a] VLS	>500 [a] VLS	36.0 (1.5–841) LS	>500 [a] VLS
Azoxystrobin	QoI	>500 [a] VLS	<0.1 [c,d] HS	>500 [a] VLS	>500 [a,c] VLS
Thiophanate-methyl	MBC	>500 [a] VLS	>500 [a] VLS	>500 [a] VLS	>500 [a] VLS
Thiabendazole	MBC	>500 [a] VLS	79.7 (57.8–110) LS	2.32 (0.23–23.4) S	<0.1 [b] HS

HS, highly sensitive (<1 μg mL^{-1}); S, sensitive (1–5); MS, moderately sensitive (>5–20); LS, low sensitivity (>20–100); VLS, very low sensitivity (>100 μg mL^{-1}). [a] Mean inhibition at 500 μg mL^{-1} < 50%; no extrapolated value is given. [b] Growth was reduced by >50% already at 0.1 μg mL^{-1}, the lowest concentration tested (model estimates 0.001–0.082 μg mL^{-1}). [c] Combination without replication; the EC_{50} was estimated without confidence interval, lack-of-fit test or analysis of variance (see Tables S1–S3). [d] Inhibition reached a plateau of 61–64% from 0.1 to 500 μg mL^{-1} without a dose-dependent increase; the value reflects partial, concentration-independent inhibition. Model fit statistics, alternative models, and a sensitivity analysis are given in Table S1.

4. Discussion

The main finding of this study is that sensitivity was specific to each isolate–active ingredient combination and could not be predicted from the FRAC group alone. Only tebuconazole (against *Fusarium* sp., *Penicillium* sp., and *Alternaria* sp.), mefentrifluconazole (against *Alternaria* sp. and *Ulocladium* sp.), and thiabendazole (against *Alternaria* sp. and *Ulocladium* sp.) produced EC_{50} values below 10 μg mL^{-1}, whereas the QoIs and thiophanate-methyl rarely halved mycelial growth even at 500 μg mL^{-1}. The four isolates were pathogenic on white onion, and the symptoms they reproduced are consistent with those described for these genera on *Allium* crops: the root reddening caused by *Fusarium* sp. agrees with the reddish to violet discoloration that accompanies the root and basal rot caused by *F. oxysporum* and *F. proliferatum* [3,4]; the necrotic leaf spots caused by *Alternaria* sp. resemble the early lesions of purple blotch caused by *A. porri* [5,6]; the root rot caused by *Penicillium* sp. is consistent with the soft rot that *Penicillium* spp. cause in underground and stored tissues, usually through wounds [7,8]; and the leaf spots caused by *Ulocladium* sp. agree with the necrotic foliar lesions reported for *Ulocladium* spp., which are generally regarded as weak or opportunistic pathogens [10,11].

4.1. Response to DMI Fungicides

DMIs inhibit sterol 14α-demethylase (CYP51) and thus ergosterol biosynthesis [28]. Tebuconazole was highly active against *Fusarium* sp. and *Penicillium* sp. and almost completely inhibited *Alternaria* sp. at ≥100 μg mL^{-1}, in agreement with the activity of triazoles against *Fusarium* spp. reported in other pathosystems [15]. Mefentrifluconazole, in contrast, was the most active DMI against *Alternaria* sp. and *Ulocladium* sp. but showed low activity against *Fusarium* sp. and *Penicillium* sp. Mefentrifluconazole has been reported to be active against several *Fusarium* species [29], and in *A. alternata* only low-level resistance, associated with *CYP51* point mutations combined with gene overexpression, has been described [30]; the low sensitivity of the *Fusarium* and *Penicillium* isolates observed here

therefore deserves confirmation with more isolates. Differences in intrinsic activity among DMIs against a given fungus are well known, and incomplete cross-sensitivity within this group is consistent with our observations [14,28].

4.2. Response to QoI Fungicides

The QoIs, which block electron transfer at the Qo site of the cytochrome bc_1 complex [31], produced only partial inhibition of mycelial growth, usually as a plateau that did not increase with concentration. Incomplete inhibition of mycelial growth by QoIs has also been reported in in vitro studies with *Alternaria alternata* [32]. This pattern is expected in mycelial assays, because the alternative oxidase allows fungi to bypass the inhibited cytochrome pathway on nutrient-rich media; QoI sensitivity is therefore usually assessed through conidial germination or in media amended with salicylhydroxamic acid [33]. Consequently, our QoI EC_{50} values (>500 μg mL^{-1} in most combinations) are not comparable with baseline values obtained by spore germination, such as those described for *Alternaria* spp. on citrus [34], and must not be interpreted as evidence of QoI resistance, which in *A. alternata* is associated with target-site mutations [17] that were not investigated here. The inhibition plateau of 61–64% from 0.1 to 500 μg mL^{-1} observed for *Penicillium* sp. with azoxystrobin is consistent with this partial, concentration-independent inhibition of mycelial growth, in which alternative oxidase-mediated respiration sustains part of the growth once the cytochrome pathway is blocked [33]; for this reason, its $EC_{50} < 0.1$ μg mL^{-1} reflects the magnitude of the plateau rather than a steep dose–response. Because both active ingredients share the same target site and are cross-resistant, differences in their in vitro activity do not justify alternating them as if they were independent modes of action [14].

4.3. Response to MBC Fungicides

The MBCs bind β-tubulin and interfere with microtubule assembly [35]. Thiabendazole was consistently more active than thiophanate-methyl, which is a pro-fungicide that must be converted to carbendazim (methyl benzimidazol-2-ylcarbamate) to exert its activity [36]; its lower activity in agar assays does not by itself indicate reduced sensitivity to the MBC group. The weak response of *Fusarium* sp. to both MBCs (≤39% inhibition at 500 μg mL^{-1}) contrasts with the use of benzimidazoles such as carbendazim to control *Fusarium* species such as *F. graminearum* [35] and, given the frequent occurrence of benzimidazole resistance in *Penicillium* spp. [16] and other plant-pathogenic fungi [12], warrants follow-up with more isolates and analysis of the β-tubulin gene.

4.4. Limitations and Implications for Disease Management

These results must be interpreted within clear limits. First, a single isolate per genus from one locality was evaluated in a single experiment with three replicate plates per concentration, so the values are not representative of the fungal populations of the region and no baseline distribution is available; high EC_{50} values therefore indicate low phenotypic sensitivity of that isolate, which may reflect intrinsic insensitivity as much as acquired resistance [13]. Second, no resistance mechanism was investigated, and no mechanistic inference can be drawn from EC_{50} differences [20]. Third, the isolates were identified only to genus level, so species-specific comparisons with the literature are not possible. Finally, mycelial growth assays do not capture effects on spore germination, the stage most affected by QoIs, and in vitro activity cannot be directly extrapolated to field efficacy, which also depends on dose, timing, coverage, and environmental conditions.

From a practical standpoint, the data provide preliminary reference values for fungi associated with white onion in Santa Fe de Galán and indicate that the choice of active ingredient should consider the target fungus rather than the chemical group alone. Future

work should include larger collections of isolates from several production areas and seasons, replicated experiments with a single colony per plate, species-level molecular identification, quantitative assessment of the aggressiveness of the isolates, spore-germination assays (particularly for QoIs), and molecular analysis of target genes (*CYP51*, *cytb*, and β-tubulin) in the least sensitive isolates. Such information is required before specific recommendations on fungicide rotation can be made, which in any case should alternate or combine different FRAC groups [14].

5. Conclusions

Under in vitro conditions, the sensitivity of four fungal isolates obtained from symptomatic white onion depended on the specific isolate–active ingredient combination. Tebuconazole was highly active against *Fusarium* sp. and *Penicillium* sp., and active against *Alternaria* sp.; mefentrifluconazole was highly active against *Alternaria* sp. and *Ulocladium* sp.; and thiabendazole was highly active against *Ulocladium* sp., and active against *Alternaria* sp.

Fusarium sp. and *Penicillium* sp. showed low sensitivity to mefentrifluconazole, and the QoIs and thiophanate-methyl did not halve the mycelial growth of most isolates within 0.1–500 μg mL^{-1}, a result that partly reflects the limitations of mycelial assays for QoIs and pro-fungicides. Active ingredients of the same FRAC group did not produce equivalent responses.

These findings are preliminary reference values obtained from one isolate per genus. They do not establish resistance status or predict field efficacy, which require population-level studies and field evaluation, but they provide the first baseline against which future sensitivity monitoring of white onion pathogens in Ecuador can be compared.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ijpb17110121/s1. Table S1: EC_{50} (μg mL^{-1}) of the four isolates for six fungicides, estimated with the three-parameter log-logistic model (lower limit fixed at 0) fitted to plate-level mycelial growth rate (MGR; n = 18 plates per replicated combination); Table S2: Analysis of variance of MGR for each isolate–fungicide experiment (randomized complete block design; 6 concentrations × 3 blocks; df = 5 for concentration, 2 for blocks and 10 for error) and assumption tests (Shapiro–Wilk test on residuals; Levene test, median-centered); Table S3: Mean mycelial growth rate (MGR, mm day^{-1} ± SD, n = 3 plates) and mean percentage inhibition (PI) at each concentration (μg mL^{-1}); Figure S1: Plate-level MGR (points) and fitted three-parameter log-logistic dose–response curves for each isolate–fungicide combination (rows, fungicides; columns, isolates).

Author Contributions: Conceptualization, P.I.A.-R.; methodology, P.I.A.-R. and J.A.M.-M.; formal analysis, P.I.A.-R.; investigation, J.A.M.-M., E.G.-G. and D.A.R.-R.; resources, E.G.-G. and D.A.R.-R.; data curation, J.A.M.-M.; validation, E.G.-G., D.A.R.-R. and A.F.T.A.F.e.F.; writing—original draft preparation, J.A.M.-M.; writing—review and editing, P.I.A.-R., E.G.-G., D.A.R.-R., E.P.S.-C. and A.F.T.A.F.e.F.; supervision, P.I.A.-R. All authors have read and agreed to the published version of the manuscript.

Funding: This study was supported by the FIASA Project No. FIASA-CA-2024-029, "Microbioma como estrategia para mitigar futuras pandemias vegetales, buscando un mecanismo de resiliencia en papa y maíz al cambio climático en la provincia de Chimborazo", funded by the Fondo de Investigación para la Agrobiodiversidad, Semillas y Agricultura Sustentable (FIASA), Ecuador.

Data Availability Statement: The original measurement records are available from the corresponding author upon request.

Acknowledgments: The authors thank the Fondo de Investigación para la Agrobiodiversidad, Semillas y Agricultura Sustentable (FIASA), Ecuador, for supporting this study through Project No.

FIASA-CA-2024-029. The authors also thank Danny Fernando Manchay Paucar (ESPOCH) for conducting the laboratory work as part of his undergraduate thesis at the Escuela Superior Politécnica de Chimborazo (ESPOCH, 2025), which generated the experimental data analyzed in this study.

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

References

1. Padula, G.; Xia, X.; Hołubowicz, R. Welsh Onion (*Allium fistulosum* L.) Seed Physiology, Breeding, Production and Trade. *Plants* **2022**, *11*, 343. https://doi.org/10.3390/plants11030343.
2. Cabrera Bravo, D.A.; Álvarez Sanchez, A.R.; Arroyo Barragán, A.P.; Monge Freile, M.F. Análisis socioeconómico de la producción de cebolla blanca y papa en la parroquia Santa Fe de Galán, cantón Guano, provincia de Chimborazo. *Cienc. Lat. Rev. Cient. Multidiscip.* **2025**, *9*, 869–887. https://doi.org/10.37811/cl_rcm.v9i1.15803.
3. Le, D.; Audenaert, K.; Haesaert, G. Fusarium Basal Rot: Profile of an Increasingly Important Disease in *Allium* spp. *Trop. Plant Pathol.* **2021**, *46*, 241–253. https://doi.org/10.1007/s40858-021-00421-9.
4. Sakane, K.; Ueno, T.; Shigyo, M.; Sasaki, K.; Ito, S.-I. Pathogenicity Differentiation of *Fusarium* spp. Causing Fusarium Basal Rot and Wilt Disease in *Allium* spp. *Pathogens* **2024**, *13*, 591. https://doi.org/10.3390/pathogens13070591.
5. Koike, S.T.; Henderson, D.H. Purple Blotch, Caused by *Alternaria porri*, on Leek Transplants in California. *Plant Dis.* **1998**, *82*, 710. https://doi.org/10.1094/PDIS.1998.82.6.710B.
6. Abdel-Hafez, S.I.I.; Abo-Elyousr, K.A.M.; Abdel-Rahim, I.R. Leaf Surface and Endophytic Fungi Associated with Onion Leaves and Their Antagonistic Activity against *Alternaria porri*. *Czech Mycol.* **2015**, *67*, 1–22. https://doi.org/10.33585/cmy.67101.
7. Valdez, J.G.; Makuch, M.A.; Ordovini, A.F.; Frisvad, J.C.; Overy, D.P.; Masuelli, R.W.; Piccolo, R.J. Identification, Pathogenicity and Distribution of *Penicillium* spp. Isolated from Garlic in Two Regions in Argentina. *Plant Pathol.* **2009**, *58*, 352–361. https://doi.org/10.1111/j.1365-3059.2008.01960.x.
8. Duduk, N.; Vasić, M.; Vico, I. First Report of *Penicillium polonicum* Causing Blue Mold on Stored Onion (*Allium cepa*) in Serbia. *Plant Dis.* **2014**, *98*, 1440. https://doi.org/10.1094/PDIS-05-14-0550-PDN.
9. Woudenberg, J.H.C.; Groenewald, J.Z.; Binder, M.; Crous, P.W. Alternaria Redefined. *Stud. Mycol.* **2013**, *75*, 171–212. https://doi.org/10.3114/sim0015.
10. Köhl, J.; Molhoek, W.M.L.; Goossen-van de Geijn, H.M.; Lombaers-van der Plas, C.H. Potential of *Ulocladium atrum* for Biocontrol of Onion Leaf Spot through Suppression of Sporulation of *Botrytis* spp. *BioControl* **2003**, *48*, 349–359. https://doi.org/10.1023/A:1023614622179.
11. Esfahani, M.N. Identification of *Ulocladium atrum* Causing Potato Leaf Blight in Iran. *Phytopathol. Mediterr.* **2018**, *57*, 112–114. https://doi.org/10.14601/Phytopathol_Mediterr-22282.
12. Yin, Y.; Miao, J.; Shao, W.; Liu, X.; Zhao, Y.; Ma, Z. Fungicide Resistance: Progress in Understanding Mechanism, Monitoring, and Management. *Phytopathology* **2023**, *113*, 707–718. https://doi.org/10.1094/PHYTO-10-22-0370-KD.
13. Lucas, J.A.; Hawkins, N.J.; Fraaije, B.A. The Evolution of Fungicide Resistance. *Adv. Appl. Microbiol.* **2015**, *90*, 29–92. https://doi.org/10.1016/bs.aambs.2014.09.001.
14. Corkley, I.; Fraaije, B.; Hawkins, N. Fungicide Resistance Management: Maximizing the Effective Life of Plant Protection Products. *Plant Pathol.* **2022**, *71*, 150–169. https://doi.org/10.1111/ppa.13467.
15. Avozani, A.; Reis, E.M.; Tonin, R.B. In Vitro Sensitivity Reduction of *Fusarium graminearum* to DMI and QoI Fungicides. *Summa Phytopathol.* **2014**, *40*, 358–364. https://doi.org/10.1590/0100-5405/1970.
16. Kanetis, L.; Förster, H.; Adaskaveg, J.E. Baseline Sensitivities for New Postharvest Fungicides Against *Penicillium* spp. on Citrus and Multiple Resistance Evaluations in *P. digitatum*. *Plant Dis.* **2008**, *92*, 301–310. https://doi.org/10.1094/PDIS-92-2-0301.
17. Chitolina, G.M.; Silva-Junior, G.J.; Feichtenberger, E.; Pereira, R.G.; Amorim, L. Distribution of *Alternaria alternata* Isolates with Resistance to Quinone Outside Inhibitor (QoI) Fungicides in Brazilian Orchards of Tangerines and Their Hybrids. *Crop Prot.* **2021**, *141*, 105493. https://doi.org/10.1016/j.cropro.2020.105493.
18. Ramírez-Jiménez, G.; Alvarado-Gómez, O.G.; Torres-de la Cruz, M.; Mayo-Hernández, M.Á.; Huamán-Pilco, Á.F.; Díaz-Valderrama, J.R. Characterization and Fungicide Sensitivity of Fungi Causing Postharvest Deterioration in *Allium sativum*, Nuevo León, Mexico. *Mex. J. Phytopathol.* **2025**, *43*, 2. https://doi.org/10.18781/R.MEX.FIT.2406-2.
19. Ritz, C.; Baty, F.; Streibig, J.C.; Gerhard, D. Dose-Response Analysis Using R. *PLoS ONE* **2015**, *10*, e0146021. https://doi.org/10.1371/journal.pone.0146021.

20. Hu, M.; Chen, S. Non-Target Site Mechanisms of Fungicide Resistance in Crop Pathogens: A Review. *Microorganisms* **2021**, *9*, 502. https://doi.org/10.3390/microorganisms9030502.
21. Leslie, J.F.; Summerell, B.A. (Eds.) *The Fusarium Laboratory Manual*; Blackwell Publishing: Ames, IA, USA, 2006. https://doi.org/10.1002/9780470278376.
22. Visagie, C.M.; Houbraken, J.; Frisvad, J.C.; Hong, S.-B.; Klaassen, C.H.W.; Perrone, G.; Seifert, K.A.; Varga, J.; Yaguchi, T.; Samson, R.A. Identification and Nomenclature of the Genus *Penicillium*. *Stud. Mycol.* **2014**, *78*, 343–371. https://doi.org/10.1016/j.simyco.2014.09.001.
23. Alves, K.S. *ec50estimator: An Automated Way to Estimate EC50 for Stratified Datasets*, Version 0.1.0; R Package; R Foundation for Statistical Computing: Vienna, Austria, 2020. https://doi.org/10.32614/CRAN.package.ec50estimator.
24. Virtanen, P.; Gommers, R.; Oliphant, T.E.; Haberland, M.; Reddy, T.; Cournapeau, D.; Burovski, E.; Peterson, P.; Weckesser, W.; Bright, J.; et al. SciPy 1.0: Fundamental Algorithms for Scientific Computing in Python. *Nat. Methods* **2020**, *17*, 261–272. https://doi.org/10.1038/s41592-019-0686-2. Correction in *Nat. Methods* **2020**, *17*, 352.
25. Harris, C.R.; Millman, K.J.; van der Walt, S.J.; Gommers, R.; Virtanen, P.; Cournapeau, D.; Wieser, E.; Taylor, J.; Berg, S.; Smith, N.J.; et al. Array Programming with NumPy. *Nature* **2020**, *585*, 357–362. https://doi.org/10.1038/s41586-020-2649-2.
26. Gudmestad, N.C.; Arabiat, S.; Miller, J.S.; Pasche, J.S. Prevalence and Impact of SDHI Fungicide Resistance in *Alternaria solani*. *Plant Dis.* **2013**, *97*, 952–960. https://doi.org/10.1094/PDIS-12-12-1176-RE.
27. Edgington, L.V.; Khew, K.L.; Barron, G.L. Fungitoxic Spectrum of Benzimidazole Compounds. *Phytopathology* **1971**, *61*, 42–44. https://doi.org/10.1094/Phyto-61-42.
28. Price, C.L.; Parker, J.E.; Warrilow, A.G.S.; Kelly, D.E.; Kelly, S.L. Azole Fungicides—Understanding Resistance Mechanisms in Agricultural Fungal Pathogens. *Pest Manag. Sci.* **2015**, *71*, 1054–1058. https://doi.org/10.1002/ps.4029.
29. Liu, Y.; Ma, T.; Dong, Y.; Mao, C.; Wu, J.; Zhang, C. Bioactivity of Mefentrifluconazole against Different *Fusarium* spp. *Pestic. Biochem. Physiol.* **2022**, *186*, 105169. https://doi.org/10.1016/j.pestbp.2022.105169.
30. Li, G.; Li, X.; Zeng, Y.; Liao, S.; Chen, Y.; Miao, J.; Peng, Q.; Liu, X. Three Point Mutations in AaCYP51 Combined with Induced Overexpression of AaCYP51 Conferred Low-Level Resistance to Mefentrifluconazole in *Alternaria alternata*. *Pestic. Biochem. Physiol.* **2023**, *197*, 105677. https://doi.org/10.1016/j.pestbp.2023.105677.
31. Bartlett, D.W.; Clough, J.M.; Godwin, J.R.; Hall, A.A.; Hamer, M.; Parr-Dobrzanski, B. The Strobilurin Fungicides. *Pest Manag. Sci.* **2002**, *58*, 649–662. https://doi.org/10.1002/ps.520.
32. Achilonu, C.C.; Gryzenhout, M.; Ghosh, S.; Marais, G.J. In Vitro Evaluation of Azoxystrobin, Boscalid, Fentin-Hydroxide, Propiconazole, Pyraclostrobin Fungicides against *Alternaria alternata* Pathogen Isolated from *Carya illinoinensis* in South Africa. *Microorganisms* **2023**, *11*, 1691. https://doi.org/10.3390/microorganisms11071691.
33. Wood, P.M.; Hollomon, D.W. A Critical Evaluation of the Role of Alternative Oxidase in the Performance of Strobilurin and Related Fungicides Acting at the Qo Site of Complex III. *Pest Manag. Sci.* **2003**, *59*, 499–511. https://doi.org/10.1002/ps.655.
34. Mondal, S.N.; Bhatia, A.; Shilts, T.; Timmer, L.W. Baseline Sensitivities of Fungal Pathogens of Fruit and Foliage of Citrus to Azoxystrobin, Pyraclostrobin, and Fenbuconazole. *Plant Dis.* **2005**, *89*, 1186–1194. https://doi.org/10.1094/PD-89-1186.
35. Zhou, Y.; Xu, J.; Zhu, Y.; Duan, Y.; Zhou, M. Mechanism of Action of the Benzimidazole Fungicide on *Fusarium graminearum*: Interfering with Polymerization of Monomeric Tubulin but Not Polymerized Microtubule. *Phytopathology* **2016**, *106*, 807–813. https://doi.org/10.1094/PHYTO-08-15-0186-R.
36. Vonk, J.W.; Kaars Sijpesteijn, A. Methyl Benzimidazol-2-ylcarbamate, the Fungitoxic Principle of Thiophanate-Methyl. *Pestic. Sci.* **1971**, *2*, 160–164. https://doi.org/10.1002/ps.2780020406.

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.