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Phytopathological Note

In vitro effect of pydiflumetofen against *Fusarium virguliforme* and *Sclerotinia sclerotiorum* isolates from soybean in Iowa, USA

Edgar H Nieto-Lopez, Austin Bierl, Desiree Chang-Villatoro, Parbati Joshi, José F. González-Acuña, Stith Wiggs, and Daren Mueller*. Iowa State University, Department of Plant Pathology, Entomology, and Microbiology, Ames, IA, 50011, USA.

Corresponding Author:
Daren Mueller
dsmuelle@iastate.edu

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ABSTRACT

Background/Objective. Sudden death syndrome caused by *Fusarium virguliforme* (Fv) and White mold caused by *Sclerotinia sclerotiorum* (Ss) are the most important soybean disease in regards to yield loss in the Northern USA. Few studies have estimated for Fv and Ss isolates from Iowa, the EC_{50} mycelial growth of the fungicide pydiflumetofen for Fv and Ss; likewise, the EC_{50} sclerotial production for Ss.

Experimental development. Potato dextrose agar (PDA) at 1/3 strength was amended with Saltro®(pydiflumetofen 41.7%) at 0.0008, 0.0042, 0.0083, 0.0417, 0.0834, 0.8340, and 834 ppm from serial dilutions. Isolates were incubated in darkness at 25 °C. Fv isolates were evaluated at 7 days and Ss isolates at 48 h after inoculation, measuring the radial growth. The sclerotia number and dried-weight were measured after 21 days of incubation.

Results. Pydiflumetofen absolute EC_{50} values for mycelial growth Fv were 0.045 and 0.048 ppm. The Fv contemporary isolate (FV-RD-0024; collected in year 2024), and the historical isolate (NE205; collected in year 2006—years before pydiflumetofen introduction in the market), both presented EC_{50} mycelial growth in the range of the baseline (0.04–0.24 ppm) in the USA. EC_{50} values for the mycelial growth of Ss contemporary isolates ranged from 0.011 to 0.046 ppm, which fall in the baseline from soybean and non-soybean hosts from China. Concentrations of 0.834 and 834 ppm were statistically different (no sclerotia production) than the control, which showed 14.6 sclerotia and 0.066 g.

Conclusion. The EC_{50} mycelial growth of *F. virguliforme* Iowa isolates fall in the range of previous reported for the USA. The EC_{50} mycelial growth of *S. sclerotiorum* Iowa isolates fall in the range previous reported outside the USA. Pydiflumetofen has the potential to diminish the sclerotial production but more studies are needed.

Keywords: EC_{50} mycelial growth, EC_{50} number sclerotia, EC_{50} dried-weight sclerotia, MIC

INTRODUCTION

The United States is the second-largest producer of soybean (*Glycine max*) globally, with Iowa serving as a key contributor in the Midwest (USDA-NASS, 2022). Iowa ranks as the second-highest soybean-producing state, with a yield of 15.59 million metric ton in 3.97 million ha (USDA-NASS, 2022). Sudden death syndrome (SDS) caused by *Fusarium virguliforme*, and white mold (WM), caused by *Sclerotinia sclerotiorum*, are the second and third most impactful diseases contributing to soybean yield losses of 0.81 and 0.71 million metric ton, respectively, in the northern USA (CPN, 2025b).

Sclerotia are fungal structures produced by members of the Sclerotiniaceae family. Disease incidence can be significantly reduced if sclerotia formation is prevented or if they are destroyed in the soil or plant residues (Xia *et al*., 2019). Previous research has examined white mold sclerotia production in the field following fungicide applications (Miorini *et al*., 2017; Vieira *et al*., 2003). Similarly, other studies have investigated the impact of benzovindiflupyr and flusilazole fungicides on *in vitro* sclerotia production of WM (Huang *et al*., 2019b; Lu *et al*., 2015).

Pydiflumetofen is a second-generation fungicide from the succinate dehydrogenase inhibitor (SDHI)–(FRAC) group 7 (FRAC, 2024), known for its broad-spectrum activity against a wide range of plant pathogens (Duan *et al*., 2019; Huang *et al*., 2019a). It has been labeled for use on soybean in the USA since 2019, both as a seed treatment (Kandel *et al*., 2019) and in commercial premix formulations for foliar application to manage diseases such as SDS and WM. While limited research has been conducted on fungicide sensitivity in the USA isolates of Fv and Ss, pydiflumetofen has demonstrated efficacy against *Fusarium graminearum* (from wheat, corn, dry bean, and soybean), *Cercospora sojina* and *Corynespora cassiicola* (from soybean) in the USA, *Sclerotinia sclerotiorum* from oil seed rape, cucumber, tomato, eggplant, kidney bean, pepper and pumpkin in China (Breunig and Chilvers, 2021; Duan *et al.*, 2019; Huang *et al*., 2019a; Neves and Bradley, 2021; Xu *et al*., 2020), *Botrytis cinerea* from tomato in China (Li *et al*., 2022) and *Bipolaris maydis* from corn in China (Sun *et al.*, 2024).

The package "drc" (Ritz *et al.*, 2015) was explicitly written to estimate dose-response relationships across many disciplines, included plant pathology. It is a robust package describing symmetric and asymmetric dose-response relationships, including those describing hormetic effects (Knezevic *et al.*, 2007). Symmetric dose-response curve describes the relationships that can be modeled, showing symmetry around an inflection point, whereas asymmetric dose-response curves do not have this property (Noel *et al*., 2018). To estimate the EC_{50}, dose-response models generally use the following parameters: the upper asymptote, lower asymptote, and slope (Noel *et al*., 2018). There are then two definitions that describe the EC_{50}: relative and absolute. The relative EC_{50} is the inflection point in a dose-response curve, whereas the absolute EC_{50} is the concentration at which 50% maximal growth occurs (more consistent with the FRAC-defined EC_{50}; Noel *et al*., 2018).

The objective was to estimate the absolute EC_{50} for mycelial growth of *Fusarium virguliforme* from Iowa. Additionally, to determine the absolute EC_{50} values for both mycelial growth and sclerotial production of *Sclerotinia sclerotiorum* from Iowa.

EXPERIMENTAL DEVELOPMENT

Media preparation. One-third strength potato dextrose agar (PDA) was prepared by dissolving 1.3 g of PDA (Difco Laboratories, Detroit, MI) and 1.0 g of agar (Bacto Laboratories, NSW, Australia) in 100 mL of distilled water. The medium was autoclaved for 1 hour. A stock solution of Saltro® (pydiflumetofen 41.7%, Syngenta; flowable concentrate formulated for seed treatment) at 417,000 ppm was diluted with sterile distilled water and incorporated into the PDA medium at 50 °C to achieve final concentrations of 0.0008, 0.0042, 0.0083, 0.0417, 0.0834, 0.8340, and 834 ppm through standard serial dilution. Medium without fungicide served as the control. Amended media were stirred thoroughly and poured into 10 cm diameter petri plates. For consistency, 20 mL of medium was dispensed into each plate using a sterile Falcon tube (Fisher Scientific, Waltham, MA).

Fungal isolates, inoculation and incubation. Monosporic isolates were isolated from roots of soybean plants exhibiting typical SDS symptoms using a modified Nash and Snyder medium by the procedure of Cho *et al*. (2001). The *F. virguliforme* isolates were morphologically and molecularly identified by the procedure of Chilvers and Brown-Rytlewski (2010). Isolations of *S. sclerotiorum* were carried out from a single sclerotium from the soybean stem with WM symptoms during the harvest season, following the procedure of Otto-Hanson *et al*. (2011). The *S. sclerotiorum* isolates grew fast with cottony-white aerial mycelia and formed irregular sclerotia on the border of the PDA petri plate.

Two isolates of *F. virguliforme* and three isolates of *S. sclerotiorum* were used in total (Table 1). Historical isolates are considered isolates collected before pydiflumetofen introduction in the market (2019) ($Fv1^{his}$), whereas contemporary isolates are considered isolates collected currently and after pydiflumetofen introduction in the market (2019). We were able to obtain historical isolates for *F. virguliforme*, but not for *S. sclerotiorum*. Actively growing cultures of *F. virguliforme* (seven days after inoculation, DAI) and *S. sclerotiorum* (48 hours after inoculation, HAI) on 1/3 strength PDA were used. Agar plugs (0.6 cm diameter) were taken from the colony edge and placed the center of each amended medium-plate. Plates were sealed with film parafilm® (Amcor corporate, Zürich, Switzerland) and placed in an incubator at 25 °C dark. After the incubation period (7 DAI for *F. virguliforme* and 48 HAI for *S. sclerotiorum*), plates were scanned to quantify the mycelial growth at 400 dpi with a blue background and a small ruler as a scale—without removing the parafilm®. Colony diameters (equatorial and polar) were measured using ImageJ (National Institutes of Health, Bethesda, MD), and the *S. sclerotiorum* plates were further kept at 25 °C dark.

Sclerotia Production. These aforementioned *S. sclerotiorum* plates were incubated at 25 °C dark for 21 days to allow sclerotia formation. All sclerotia were per plate were collected, dried in an oven at 50 °C for 48 hours, and counted and weighed, following the procedure of Lu *et al*. (2015).

Data Analysis. EC_{50} values for mycelial growth of both fungus (*F. virguliforme* and *S. sclerotiorum*) and for sclerotial production of *S. sclerotiorum* were estimated by fitting three models: the three-parameter log-logistic (LL.3), four-parameter log-logistic (LL.4), and four-parameter Weibull type I (W1.4) and type II (W2.4) dose-response models using the "drc" package in R (Ritz *et al*., 2015). The best-fitting model was selected based on Akaike's Information Criterion (AIC), F-tests for lack-of-fit, and examination of residual

variance, following Noel *et al*. (2018), using the "mselect" function in the same package. All fungicide concentrations, including controls, had three replications of a media plate (experimental unit). The petri plates were incubated in a completely random design, and the experiment was performed twice. ANOVA and Fisher's Least Significant Difference (LSD) post hoc tests were conducted using the "agricolae" package (de Mendiburu, 2023). All data analyses were performed in R studio version (R Development Core Team, 2024).

The best-fit models for mycelial growth were the Weibull type II (W2.4) for Fv1 and the four-parameter log-logistic (LL.4) for Fv3. For *S. sclerotiorum*, the best models were W2.4 for Ss1, and Weibull type I (W1.4) for both Ss2 and Ss4 (Table 1). The absolute EC_{50} values of pydiflumetofen for mycelial growth of *F. virguliforme* isolates were 0.045 ppm (Fv1) and 0.048 ppm (Fv3) (Table 1). For *S. sclerotiorum*, the absolute EC_{50} values for mycelial growth ranged from 0.011 to 0.046 ppm (Table 1).

Isolate Ss2 did not produce sclerotia in the control and was therefore excluded from subsequent sclerotia analyses. The best-fit models for sclerotia number and dried-weight were LL.3 for Ss1 and Ss4. Absolute EC_{50} values for sclerotia number ranged from 0.004 to 0.019 ppm, and for dried-weight sclerotia ranged from 0.101 to 0.137 ppm (Table 1).

The historical isolate *F. virguliforme* Fv1 isolate (NE305) showed a lower EC_{50} value than the contemporary Fv3 isolate (FV-RD-0024), indicating greater sensitivity to pydiflumetofen, as expected. Notably, the highest tested concentrations (0.834 and 834 ppm) did not completely inhibit *F. virguliforme* mycelial growth (Figure 1).

A significant effect of pydiflumetofen concentration on sclerotia number was observed ($p < 0.001$). Concentrations of 0.0042, 0.0083, 0.0417, 0.0834, 0.834, and 834 ppm significantly reduced the number of sclerotia compared to the control (14.6 sclerotia). The highest concentrations, 0.834 and 834 ppm, resulted in the lowest sclerotia counts recorded (0) in the study (Table 2; Figure 2). Due to noticeable variation in both the number and size of sclerotia within the same treatment, sclerotia dried-weight was also assessed as a more consistent metric. Pydiflumetofen concentration had a significant effect on sclerotia dried-weight as well ($p < 0.001$), with concentrations of 0.834 and 834 ppm significantly reducing sclerotia dried-weight (0 g) compared to the control (0.066 g) (Table 2; Figure 2).

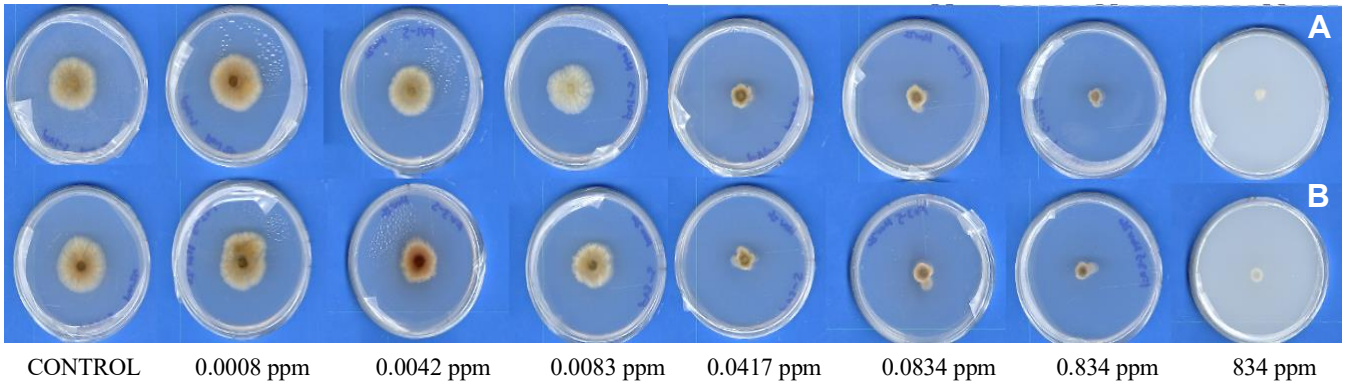

Figure 1. *Fusarium virguliforme* isolates on 1/3 strength PDA petri plates incubated for 7 days after inoculation (DAI) at 25 °C dark. Petri plates amended with pydiflumetofen fungicide through standard serial dilutions. A) *Fusarium virguliforme* isolate Fv1 (NE305); B) *Fusarium virguliforme* isolate Fv3 (FV-RD-0024).

Table 1. Isolates of *Fusarium virguliforme* (Fv) and *Sclerotinia sclerotiorum* (Ss) used in the study including their background information and their absolute EC_{50} estimated per variable on petri plates amended with pydiflumetofen fungicide (Saltro®; Syngenta) at 0.0008, 0.0042, 0.0083, 0.0417, 0.0834, 0.8340, and 834 ppm through standard serial dilutions. The isolate Ss2 (#630) shows one model since it did not produce sclerotia in the control.

Fungus	ID	Isolate	EC_{50}^{M} (ppm)	SE^{M}	EC_{50}^{N} (ppm)	SE^{N}	EC_{50}^{D} (ppm)	SE^{D}	Model	Collection location	Collection Year
Fusarium virguliforme	$Fv1^{his}$	NE305 (Gongora-Canul *et al.*, 2012) Mueller's lab	0.045	0.004	-	-	-	-	$W2.4^{Md}$	Nevada	2006
F. virguliforme	$Fv3^{con}$	FV-RD-0024	0.048	0.006	-	-	-	-	$LL.4^{Mb}$	Roland	2024
S. sclerotiorum	Ss1	Leandro's lab WM-2024	0.011	0.001	0.004	0.005	0.101	0.014	$W2.4^{Md}$; $LL.3^{Na}$; $LL.3^{Da}$	Unknown in IA	Unknown
S. sclerotiorum	Ss2	#630 Mueller's lab	0.046	0.004	-	-	-	-	$W1.4^{Mc}$	Unknown in IA	2020
S. sclerotiorum	Ss4	WM-CO-0024	0.041	0.005	0.019	0.012	0.137	0.019	$W1.4^{Mc}$; $LL.3^{Na}$; $LL.3^{Da}$	Colo	2024

^{his}Historical isolate = Collected before pydiflumetofen introduction in the market; ^{con}Contemporary isolate = Collected currently and after pydiflumetofen introduction in the market; ^{M}Mycelial growth; ^{N}Number sclerotia; ^{D}Dried-weight sclerotia.
SE = Standard Error; ^{a}Three-parameter log-logistic (symmetric curve); ^{b}Four-parameter log-logistic (symmetric curve); ^{c}Four-parameter Weibull type I (asymmetric curve); ^{d}Four-parameter Weibull type II (asymmetric curve).

Table 2. Average of number and dried-weight of sclerotia produced of *Sclerotinia sclerotiorum* isolates Ss1 (WM-2024) and Ss4 (WM-CO-0024) incubated for 21 days in darkness 25 °C. Petri plates amended with pydiflumetofen fungicide. Metrics between both isolates were combined.

Concentration (ppm)	Number sclerotia	Groups^{z}	Dried-weight sclerotia (g)	Groups^{z}
0	14.600	a	0.066	a
0.0008	10.00	ab	0.071	a
0.0042	7.167	b	0.068	a
0.0083	7.333	b	0.064	a
0.0417	7.333	b	0.057	a
0.0834	8.667	b	0.051	a
0.834	0	c	0	c
834	0	c	0	c

^{z}Concentrations with the same letter are not significantly different by Fisher's Least Significant Difference (LSD) at $p<0.05$

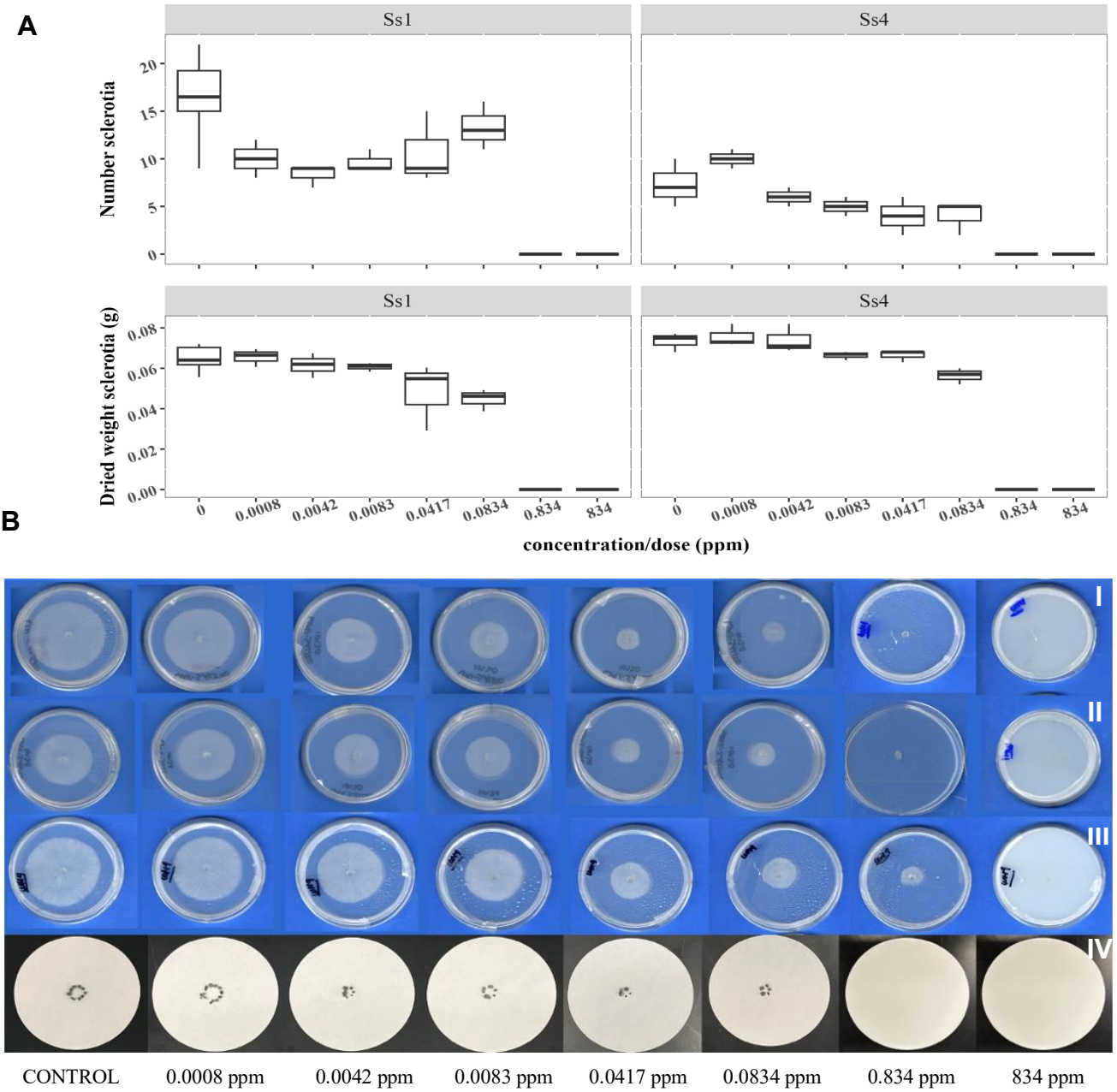

Figure 2. Mycelial growth and sclerotia production of *Sclerotinia sclerotiorum* isolates petri plates incubated at 25 °C dark. Petri plates amended with pydiflumetofen fungicide serial dilutions. A) Boxplot. B) Image in which a same isolate lays on each panel, with different concentrations increase from left to right along the row. Panels B-I to B-III: Mycelial growth at 48 hours after inoculation (HAI); B-I) *Sclerotinia sclerotiorum* isolate Ss1 – labeled WM1 (WM-2024); B-II) *Sclerotinia sclerotiorum* isolate Ss2 – labeled WM2 (#630); B-III) *Sclerotinia sclerotiorum* isolate Ss4 – labeled WM4 (WM-CO-0024); Panel B-IV: Sclerotia production at 21 days of incubation (DAI); B-IV) *Sclerotinia sclerotiorum* isolate Ss4 (WM-CO-0024).

Pydiflumetofen reduced the mycelial growth of *Fusarium virguliforme*. The EC_{50} mycelial growth values for *F. virguliforme* in this study (0.045–0.048 ppm) fall within the previously reported range of 0.011–0.4602 ppm from soybean isolates in the USA (Hamilton *et al*., 2024; Olaya *et al*., 2021). The best-fitting models for mycelial growth were four-parameter types: The Weibull type II (asymmetric) and the log-logistic (symmetric) distributions. The use of the Weibull type II model is consistent with Hamilton *et al*. (2024), who studied the same fungus and active ingredient. However, their maximum

tested concentration was 100 ppm, whereas this study used concentrations up to 834 ppm. This difference may be attributed to our use of a commercial formulation, while Hamilton *et al*. (2024) used the pure active ingredient. Nevertheless, the highest concentrations tested (0.834 and 834 ppm) in this study cannot be considered the minimum inhibitory concentration (MIC).

The historical isolate Fv1 (NE205), collected in 2006—prior to the introduction of pydiflumetofen in the USA—and the contemporary isolate Fv3 (FV-RD-0024) had EC_{50} values of 0.045 and 0.048 ppm, respectively. Both values fall within the baseline range of 0.04–0.24 ppm for unexposed isolates in the USA (Hamilton *et al*., 2024).

Pydiflumetofen also had an effect on the mycelial growth of *Sclerotinia sclerotiorum*. The EC_{50} values obtained in this study were within the baseline ranges previously reported: 0.0058–0.09 ppm (soybean isolates; Duan *et al*., 2019) and 0.0015–0.0350 ppm (non-soybean isolates from six different crops; Huang *et al*., 2019a), both from China. However, it is worth noting that Huang *et al*. (2019a) used broth media for *in vitro* testing, while our study used solid media. The best fit models for *S. sclerotiorum* mycelial growth were the W1.4 and W2.4 distributions (asymmetric), but these model types were not reported in previous studies using this fungus and fungicide, limiting direct comparisons.

In addition to inhibiting mycelial growth, pydiflumetofen significantly reduced sclerotia production in *S. sclerotiorum*. The EC_{50} values for sclerotia number and dried-weight (0.004–0.019 ppm and 0.101–0.137 ppm, respectively) overlap with the ranges reported by Gao *et al*. (2020) in China for non-soybean baseline isolates (mean EC_{50} values of 0.023 ppm for number and 0.003 ppm for dried-weight). In this study, sclerotia number was significantly reduced starting at 0.0042 ppm, while sclerotia dried-weight was significantly reduced starting at 0.834 ppm. These results are consistent with previous findings that concentrations between 0.2 and 0.8 ppm significantly reduced both the number and dried-weight of sclerotia when using benzovindiflupyr, another second-generation SDHI fungicide (Huang *et al*., 2019b). Based on our results, the sclerotial inhibitory concentration *in vitro* appears to be 0.834 ppm.

The EC_{50} values for mycelial growth of *Fusarium virguliforme* isolates from Iowa fall within the previously reported range for the USA soybean isolates (Hamilton *et al*., 2024; Olaya *et al*., 2021). Similarly, EC_{50} values for *Sclerotinia sclerotiorum* isolates from Iowa are consistent with those reported for isolates from outside the USA (Duan *et al*., 2019; Huang *et al*., 2019a). These findings suggest that pydiflumetofen has potential to reduce sclerotia production, an important survival structure in the disease cycle of *S. sclerotiorum*. Therefore, additional studies under field conditions (*in planta*) are warranted to evaluate its effectiveness in disrupting disease progression by targeting sclerotia formation. Commercial fungicide formulations containing this active ingredient (7%) in mixture with more active ingredients, are currently labeled for foliar application on soybean to manage *S. sclerotiorum* in the USA (CPN, 2025a).

CONCLUSIONS

The EC50 mycelial growth of *F. virguliforme* Iowa isolates fall in the range of previous reported for the USA. The EC50 mycelial growth of *S. sclerotiorum* Iowa isolates fall in the range previous reported outside the USA. Pydiflumetofen has the potential to diminish the sclerotial production but more studies are needed. This study did not assess the environmental or non-target effects of pydiflumetofen, which should be addressed in future research. To our knowledge, this is the first report estimating the EC_{50} of pydiflumetofen

on both mycelial growth and sclerotia production of *S. sclerotiorum* soybean isolates from the USA.

Limitations

This fungicide sensitivity study was based on a limited number of fungal isolates. As such, caution should be exercised when extrapolating these results to broader populations. A larger sample size for future studies would strengthen conclusions and provide more robust baseline data for resistance monitoring.

Conflicts interest

The authors declare no conflicts of interest related to this research.

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Author contributions

Conception and conceptualization: Nieto-Lopez and Mueller; Methodology: Nieto-Lopez, Bierl, Chang-Villatoro, Joshi, and González-Acuña; Software: Nieto-Lopez; Validation: Nieto-Lopez and Parbati Joshi; Formal analysis: Nieto-Lopez; Investigation: Nieto-Lopez; Resources: Mueller; Data curation: Nieto-Lopez; Writing—original draft: Nieto-Lopez and Gonzalez- González-Acuña; Writing—review and editing: Mueller; Visualization: Nieto-Lopez; Supervision: Joshi; Project administration: Nieto-Lopez; Funding acquisition: Mueller.

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