FITNESS PENALTY OF QOI-RESISTANT ISOLATES OF *C. CASSIICOLA* AND SENSITIVITY PROFILE TO FIVE FUNGICIDES

M.N. Rondon
B.R. Lawaju
K.S. Lawrence
Auburn University
Auburn, AL

Abstract

The fungal pathogen known as *Corynespora cassiicola* causes a known disease on cotton and soybean plants, target spot. Fungicides are a crucial tool in disease management but reported cases of *C. cassiicola* resistance to different fungicide groups have been reported. The objectives of this study were (i) to determine if there is a fitness loss on *C. cassiicola* isolates with the G143A mutation in the cytochrome *b* gene; (ii) to determine the sensitivity profiles for *C. cassiicola* from cotton and soybean to thiophanate-methyl, prothioconazole, pyraclostrobin, mancozeb, and the combination fluxapyroxad + pyraclostrobin. Mycelial growth of twelve *C. cassiicola* isolates were compared, four isolates QoI-resistant and eight QoI-sensitive. No fitness penalty was found, whether QoI-resistant or QoI-sensitive. EC_{50} values of QoI-resistant isolates were statistically higher than QoI-sensitive isolates for all fungicides, except for thiophanate-methyl. EC_{50} values for cotton and soybean isolates were statistically different for pyraclostrobin, mancozeb, and the mixture fluxapyroxad + pyraclostrobin but not for thiophanate-methyl and prothioconazole. Our study characterized EC_{50} values for five fungicides of *C. cassiicola* isolates from cotton and soybean in the United States, and these values can be used as a reference for further studies. These results will be useful to monitor sensitivity of U.S. populations of *C. cassiicola* from cotton and soybean, and to facilitate fungicide resistance management through detection of shifts in fungicide sensitivity.

Introduction

Corynespora cassiicola (Berk. & Curt.) C.T. Wei is a widespread plant pathogenic fungus that causes target-shaped necrotic spots on plant leaves and on stems, roots, flowers, and fruits and has been recorded worldwide on up to 400 plant species (Farr and Rossman, 2020). The foliar phase of the disease is characterized as small circular spots, varying between 2 mm and 10 mm. Well-developed lesions are necrotic and show typical "target spot" symptoms, with some depression at the center of the lesion. In severe cases of infection, the leaves show severe necrosis followed by complete premature senescence and death of the leaf. The disease is known as Corynespora leaf spot or Target spot on cotton and soybean (Galbieri et al., 2014; Godoy et al., 2015). Disease management has been a concern in other countries due to increasing occurrence of target spot in soybean fields (Godoy et al., 2015), and recently concern about target spot on cotton has been rising (Sumabat et al., 2018). Severe disease symptoms and significant yield losses can occur when the pathogen is not properly controlled (Bowen et al., 2018; Faske, 2017; Hagan and Sikora, 2012; Koenning et al., 2006).

Chemical control by application of fungicides have been used for over 200 years and is far the most frequently used tool in disease management in agriculture (Brent and Hollomon, 2007). Foliar fungicide application is known to be the most effective tool to control the fungus *C. cassiicola* (Ma et al., 2020). However, the progressive development of resistance to fungicides is aggravated by the incorrect use of the fungicide groups (Asadollahi et al., 2013). The Fungicide Resistance Action Committee (FRAC; https://www.frac.info/home) constantly publishes a list of risk of resistance development by fungal pathogens, and unfortunately, there are reported cases of *C. cassiicola* isolates from cucumber, soybean, and tomato resistant to fungicides with different mode of actions (Avozani et al., 2014; Date et al., 2004; Miyamoto et al., 2009, 2010; Rondon and Lawrence, 2019; Teramoto et al., 2017; Xavier et al., 2013). *Corynespora cassiicola* is considered a high-risk pathogen for development of fungicide resistance (FRAC, 2020), and mutations associated with QoI-resistance have been detected in the cytochrome *b* (*cytb*) gene: G143A, F129L, and G137R (Duan et al., 2019; FRAC, 2020; Rondon and Lawrence, 2019).

The use of fungicides continues to rise to control diseases on cotton and soybean, and *in vitro* sensitivity of *C. cassiicola* isolates associated with cotton and soybean in the United States have not been determined. It is vital to monitor *C. cassiicola* populations with EC_{50} values for their degree of sensitivity to one or more fungicides to facilitate the detection of shifts in the sensitivity of *C. cassiicola*, and to determine if resistance strategies are effective (Brent and Hollomon, 2007; Emmitt et al., 2018; Russell, 2004). Therefore, the objectives of this study were (i) to determine

if there is a fitness loss on *C. cassiicola* isolates with the G143A mutation in the cytochrome *b* gene; (ii) to determine the sensitivity profiles for *C. cassiicola* from cotton and soybean to thiophanate-methyl, prothioconazole, pyraclostrobin, mancozeb, and the combination fluxapyroxad + pyraclostrobin.

Materials and Methods

Isolates

Isolations of *C. cassiicola* were carried out using direct isolation in which small amounts of mycelia and conidia from symptomatic lesions on leaves of cotton and soybean were directly transferred onto potato dextrose agar (PDA; DIFCO Laboratories) containing 50 µg/mL of kanamycin. PDA plates were sealed and incubated at room temperature (RT, 25 ± 2°C) for mycelial growth. Pure colonies were transferred to PDA plates to establish the *C. cassiicola* Alabama collection. All isolates were identified as *C. cassiicola* based on conidiophore and conidia morphology (Ellis, 1971) and ITS sequencing (ITS1/ITS4).

Fungicides

All fungicides tested in this study were commercial formulations, with one having more than one active ingredient (Table 1). Fungicides were individually dissolved in sterile distilled water to prepare stock solutions (1,000 and 10,000 µg/mL) immediately before use.

Table 1. Description of commercial formulation of fungicides tested in this study.

Fungicide classification	Active ingredient (%)	Commercial product	Manufacturer
MBC Thiophanate (Group 1)	Thiophanate-methyl 45%	Topsin 4.5 FL	UPL
DMI Triazole (Group 3)	Prothioconazole 41.0%	Proline 480 SC	Bayer CropScience
QoI Strobilurin (Group 11)	Pyraclostrobin 23.6%	Headline EC	BASF
Dithiocarbamate (Group M3)	Mancozeb 58.1%	Manzate Pro-Stick	UPL
SDHI Carboxamides (Group 7) + QoI Strobilurin (Group 11)	Fluxapyroxad 14.33% + Pyraclostrobin 28.58%	Priaxor 500 SC	BASF

In vitro fungicide sensitivity

For this project, 12 isolates of *C. cassiicola* from cotton (n = 6) and soybean (n = 6) were selected to test the *in vitro* fungicide sensitivity. Four of these 12 isolates were reported as QoI-resistant (Rondon and Lawrence, 2019). Isolates were inoculated by placing one mycelial plug (7.0 mm) from a 10-day-old colony at the center of a PDA plate and incubated at 28 ± 2°C under a cycle of 12 h light/dark. For the *in vitro* bioassay, experiments were performed using the methods previously described with minor modifications (Ishii et al., 2007). Cooled PDA media, enriched with 50 µg/mL of kanamycin, was amended with six fungicide concentrations (0.01, 0.1, 0.5, 1, 10 and 100 µg/mL of active ingredient) and poured into Petri plates. PDA plates amended with fungicides were inoculated with one mycelial plug (7.0 mm) taken from the edges of growing PDA colonies. PDA plates without the addition of fungicide were used as control. Inoculated plates were incubated at 28 ± 2°C under a cycle of 12 h light/dark to determine the effect of each fungicide on mycelial growth. No salicylhydroxamic acid (SHAM) was added to the media, since Teramoto et al. (2017) didn't find any effect of SHAM when studying the sensitivity of *C. cassiicola* isolates to QoI fungicides. Mycelial growth was determined by measuring colony diameter of each plate along two perpendicular lines when the first colony reached the borders of the plate. The percent growth inhibition (GI %) due to the fungicide treatments at different concentrations was calculated according to Ishii et al. (2007). The GI % was used to calculate the EC_{50} values (fungicide concentration that inhibited 50% of the mycelial growth) for each isolate-fungicide and were expressed in µg/mL. The experiment was a completely randomized design with four replicates of each isolate-fungicide concentration combination. A Petri dish was used as an experimental unit and two independent experiments were conducted for each fungicide.

Fitness assessment of *C. cassiicola* QoI-resistant isolates

To assess the fitness of *C. cassiicola* QoI-resistant isolates, their mycelial growth was measured on fungicide-free PDA plates with four replications and compared with *C. cassiicola* QoI-sensitive isolates (Zhang and Bradley, 2017). The isolates were cultured as described above, and mycelial growth measurements were obtained for all isolates. The experiments were conducted in a completely randomized design and repeated six times.

Data analysis

The EC_{50} values were estimated by the Gauss-Newton iterative method in the non-linear regression procedure using PROC NLIN in SAS 9.4 (SAS Institute Inc., Cary, NC). Diagnostics plots were generated to check for normality and equal variance assumptions. Data from two trials for each fungicide were combined for statistical analysis representing eight replications per isolate-fungicide concentration. EC_{50} values for each fungicide were subjected to analysis of variance using PROC GLM, and means were separated with Tukey's HSD test ($\alpha = 0.05$). Two-sample Student's t-tests were performed using PROC TTEST ($\alpha = 0.05$) for detecting significant EC_{50} by the origin of the isolates (cotton or soybean) for each fungicide. Mycelial growth data were combined after visual diagnostics of normality/equal variance assumptions and subjected to analysis of variance using PROC GLM, and means were separated with Tukey's HSD test ($\alpha = 0.05$).

Results and Discussion

***In vitro* fungicide sensitivity**

The sensitivity of 12 *C. cassiicola* isolates obtained from cotton and soybean infected leaves in Alabama were tested to establish a baseline sensitivity to all fungicides described in Table 1. EC_{50} values for the fungicides analyzed in this study were calculated based on the mycelial growth inhibition of *C. cassiicola* isolates (Table 2).

Table 2. Sensitivity of *Corynespora cassiicola* isolates obtained from symptomatic cotton and soybean leaves to five fungicides.

Isolate	Origin	EC_{50} (µg/mL)[x]				
		Thiophanate-methyl	Prothioconazole	Pyraclostrobin	Mancozeb	Fluxapyroxad + Pyraclostrobin
BRW03	Cotton	0.81 bc	0.72 bc	4.61 d	6.05 bc	0.41 cdef
FHP01	Cotton	1.06 bc	0.50 c	9.73 d	4.96 c	0.57 cde
FHP22	Cotton	0.98 bc	1.50 b	18.50 cd	4.20 c	0.25 ef
HSV01	Cotton	0.57 c	0.37 c	12.32 cd	5.86 bc	0.14 f
HSV12	Cotton	0.63 c	0.34 c	6.60 d	7.43 bc	0.29 def
MAC01	Cotton	0.90 bc	0.32 c	23.24 bcd	9.31 bc	0.52 cde
ELM04[y]	Soybean	0.58 c	0.52 c	75.50 a	9.24 bc	1.08 a
ELM06[y]	Soybean	0.71 c	0.87 bc	59.02 ab	11.85 b	1.00 ab
ELM07[y]	Soybean	0.64 c	0.49 c	49.96 abc	8.21 bc	1.28 a
LIM02	Soybean	1.99 a	0.43 c	14.51 cd	5.44 bc	0.59 cd
LIM13	Soybean	0.72 c	0.31 c	13.85 cd	9.97 bc	0.47 cde
LIM14[y]	Soybean	1.55 ab	2.73 a	76.48 a	18.63 a	0.73 bc
Mean		0.93	0.76	30.36	46.38	0.61
Range		0.57 – 1.99	0.31 – 2.73	4.61 – 76.48	4.20 – 18.63	0.14 – 1.28
CV (%)[z]		53.40	63.47	76.93	46.38	31.66
F value		6.12	17.23	10.75	8.17	26.30

[x] LS-mean of EC_{50} values (estimated fungicide concentration that inhibited 50% of the mycelial growth) followed by the same letter in the columns were not significantly different in Tukey's HSD test ($P < 0.05$).
[y] Isolates with G143A mutation that confers resistance to QoI fungicides (Rondon and Lawrence, 2019).
[z] Coefficient of variation.

For the fungicide thiophanate-methyl (MBC fungicide), no difference was found for the mean EC_{50} for *C. cassiicola* isolated from cotton ($\bar{X} = 0.82$ µg/mL) or soybean ($\bar{X} = 1.03$ µg/mL) ($P = 0.1006$). Our results were equivalent to the EC_{50} of wild-type strains of *C. cassiicola* in cucumber, which were extremely sensitive to benzimidazoles (Duan et al., 2019). Recently, benzimidazole-resistant isolates were reported with EC_{50} values > 192 µg/mL for carbendazim, > 78 µg/mL for benomyl, and > 18 µg/mL for thiabendazole (Duan et al., 2019). *Corynespora cassiicola* isolates from cotton and soybean from our collection did not exhibit reduced sensitivity to benzimidazoles; however, we do not recommend the use of MBC fungicides as a sole fungicide to control target spot; if needed, the MBC fungicide should

be used in a mixture with other modes of action that are effective to control *C. cassiicola*. Duan et al. (2019) stated that the use of benzimidazoles to control *C. cassiicola* in cucumber should be restricted in China.

The highest EC_{50} value to prothioconazole (DMI fungicide) was 2.73 µg/mL for a soybean *C. cassiicola* isolate, and no significant difference was found for the mean EC_{50} for *C. cassiicola* isolated from cotton ($\bar{X}$ = 0.62 µg/mL) or soybean ($\bar{X}$ = 0.89 µg/mL) (P = 0.110). Despite the intensive use of DMI fungicides to control target spot, there are few reports about the resistance of *C. cassiicola* to DMI fungicides (FRAC, 2020). Our EC_{50} values for *C. cassiicola* isolates from Alabama to prothioconazole were within the range of what was found in previous studies in Brazil for DMI fungicides (Xavier et al., 2013; Avozani et al., 2014; Teramoto et al., 2017). Usually, China is the first place to report resistance of *C. cassiicola* isolates to different fungicides because of their intensive use of fungicides to control cucumber Corynespora leaf spot; however, so far no *C. cassiicola* isolate with resistance to DMI fungicides has been reported in China suggesting this as a good option to control the disease (Zhu et al., 2020). Although, it is still important to follow basic strategies to delay fungicide resistance development (Ishii and Hollomon, 2015).

The emergence of *C. cassiicola* isolates resistant to pyraclostrobin (QoI fungicide) (Rondon and Lawrence, 2019) will become a limitation to the management of target spot in the field. In this study, the mean EC_{50} for *C. cassiicola* isolated from cotton ($\bar{X}$ = 12.50 µg/mL) was statistically lower than the mean EC_{50} for *C. cassiicola* isolated from soybean ($\bar{X}$ = 48.22 µg/mL) ($P < 0.0001$) for pyraclostrobin. The high values of EC_{50} for *C. cassiicola* isolated from soybean found in this study suggest loss of sensitivity to pyraclostrobin in Alabama but not for *C. cassiicola* isolated from cotton. All four *C. cassiicola* isolates with the G143A mutation reported in Rondon and Lawrence (2019) exhibited statistically higher EC_{50} values for pyraclostrobin (EC_{50} > 50 µg/mL. In Brazil, among 34 *C. cassiicola* isolates sampled from soybean Teramoto et al. (2017) reported EC_{50} < 0.16 µg/mL for 10 isolates, considering them as sensitive to pyraclostrobin. Only one isolate was considered as highly non-sensitive with an EC_{50} = 36.55 µg/mL. Additionally, 14 isolates exhibited EC_{50} > 28 µg/mL to azoxystrobin, 21 isolates exhibited EC_{50} > 28 µg/mL to trifloxystrobin, and 10 isolates with EC_{50} > 28 µg/mL to picoxystrobin. All of them were considered as highly non-sensitive to QoI fungicides (Teramoto et al., 2017). EC_{50} values > 100 µg/mL were found for azoxystrobin on *C. cassiicola* isolates from tomato in Florida, which limited the use of QoI fungicides to control the disease on tomatoes (MacKenzie et al., 2020).

The mean EC_{50} for *C. cassiicola* isolated from cotton ($\bar{X}$ = 6.30 µg/mL) was statistically lower than the mean EC_{50} for *C. cassiicola* isolated from soybean ($\bar{X}$ = 10.56 µg/mL) ($P < 0.0001$) for mancozeb. Multisite fungicides are classified with a low risk of development of fungicide resistance, and dithiocarbamates fungicides (mancozeb, maneb and propineb) are among them (Brent and Hollomon, 2007; FRAC, 2020). Here, we demonstrated that the majority of *C. cassiicola* isolates exhibited EC_{50} < 10 µg/mL, with two of them (17%) with EC_{50} > 12 µg/mL for mancozeb. *Cercospora* species from soybean in Argentina exhibited EC_{50} > 10 µg/mL to the fungicide mancozeb (Sautua et al., 2020). Multisite fungicides are a good fit to be used in combination with another mode of action fungicide, usually a systemic fungicide to reduce the selection pressure on one fungicide, and inhibit the growth of resistant populations (FAO, 2012). According to MacKenzie et al. (2018), the control of *C. cassiicola* on tomatoes in Florida relies on constant applications of protectant fungicides which avoid $3.5 million in potential revenue lost in fields without protectant fungicides applications.

For the fungicide mixture (fluxapyroxad + pyraclostrobin), *C. cassiicola* isolated from cotton exhibited statistically lower EC_{50} mean ($\bar{X}$ = 0.36 µg/mL) than *C. cassiicola* isolated from soybean ($\bar{X}$ = 0.86 µg/mL) ($P < 0.0001$). Teramoto et al. (2017) reported the sensitivity of *C. cassiicola* soybean isolates to fluxapyroxad only (SDHI fungicide) with EC_{50} < 1 µg/mL for the majority of the isolates (85%); however, 3 isolates exhibited EC_{50} > 91 µg/mL to fluxapyroxad and one isolate with EC_{50} > 100 µg/mL to boscalid. These isolates were classified as highly non-sensitive to SDHI fungicides. Zhu et al. (2019) reported sensitive isolates with EC_{50} = 0.92 to 2.12 µg/mL, and highly-resistant isolates with EC_{50} > 50 µg/mL for boscalid. Fungicide resistance in *C. cassiicola* has developed in a short period of time when SDHI was used as a sole fungicide, causing severe problems in the disease management (Zhu et al., 2019). Our results suggest that the combination of fungicides with different modes of action provide an adequate control of the pathogen but SDHI should not be used as a sole fungicide. Fungicide mixtures are recommended to prevent the development of fungicide resistance (Brent and Hollomon, 2007; FAO, 2012; Ghini and Kimati, 2000).

Fitness assessment of *C. cassiicola* QoI-resistant isolates

Mycelial growth of 12 *C. cassiicola* isolates were compared and among those isolates, four were QoI-resistant, and eight were QoI-sensitive (Rondon and Lawrence, 2019). Significant differences in mycelial growth was observed

among the isolates ($P < 0.0001$), separating the isolates into several statistical groups. QoI-resistant isolates of *C. cassiicola* were placed in different statistical groups (Figure 1). We could not correlate mycelial growth of *C. cassiicola* isolates with the presence of G143A mutation (QoI-resistant). The fitness of fungicide-resistant isolates was described as important to develop helpful anti-resistance strategies because the competitive ability of these isolates defines their persistence in the fungal population when there is no fungicide selection pressure (Ishii, 2015). In this study, no correlation was observed between the mycelial growth and sensitivity to pyraclostrobin, with no clear separation of QoI-resistant and -sensitive isolates. Our results suggest that there is no fitness penalty of *C. cassiicola* isolates from cotton and soybean associated with resistance to pyraclostrobin based on mycelial growth. Investigations are still needed on different resistance mechanisms that *C. cassiicola* might express to other fungicides groups. Torriani et al. (2017) emphasized that extra research is required to know the possible fitness cost associated with fungicide resistance.

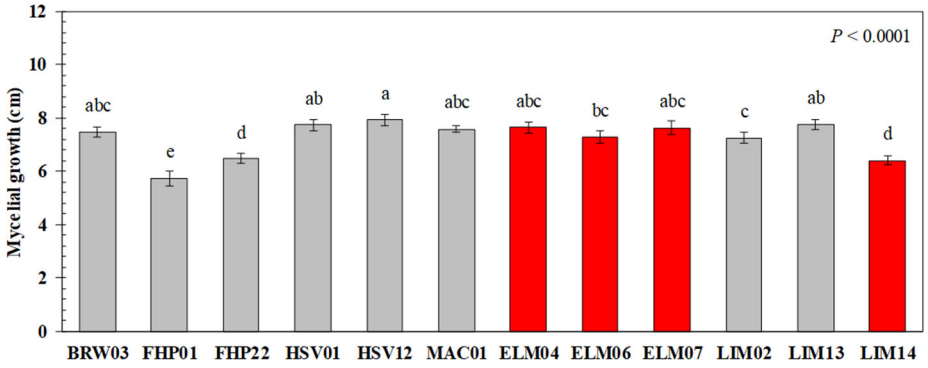

Figure 1. Mycelial growth on fungicide-free PDA of *Corynespora cassiicola* isolates. QoI-resistant isolates are highlighted in red, while QoI-sensitive are represented by gray bars only. Data represent means of replicate samples ($n = 28$), and vertical bars indicate 95% C.L. Bars labeled with different letters are significantly different according to Tukey's HSD test ($\alpha = 0.05$).

Summary

Our study described baseline results for important fungicides used for *C. cassiicola* management. The importance of baseline data for fungal pathogens was stated by Russell (2004) as essential to explain shifts in sensitivity, and further to provide evidence that resistant populations were responsible for the disease control failures. Our study characterized EC_{50} values of *C. cassiicola* isolates for five fungicides on cotton and soybean in the United States, and these values can be used as a reference for further studies. Furthermore, it is imperative to develop disease-resistant varieties, use crop rotation, and even possible biological control options. These strategies will complement the management of target spot in the field in combination with chemical control, prolonging the life expectancy of fungicides. To avoid the rapid development of *C. cassiicola* populations non-sensitive to fungicides, single-site fungicides should be applied in combination with fungicides that have different modes of action, and the number of applications should be limited for each crop cycle (Ghini and Kimati, 2000). Additionally, we recommend that applications with QoI fungicides should be avoided when not combined with another mode of action in areas where resistant populations have been reported.

References

Asadollahi, M., Szojka, A., Fekete, E., Karaffa, L., Takács, F., Flipphi, M., Sándor, E., 2013. Resistance to QoI fungicide and cytochrome *b* diversity in the Hungarian *Botrytis cinerea* population. J. Agric. Sci. Technol. 15, 397–407.

Avozani, A., Reis, E.M., Tonin, R.B., 2014. Sensitivity loss by *Corynespora cassiicola* isolated from soybean, to the fungicide carbendazim. Summa Phytopathol. 40, 273–276. https://doi.org/10.1590/0100-5405/1928

Bowen, K.L., Hagan, A.K., Pegues, M., Jones, J., Miller, H.B., 2018. Epidemics and yield losses due to *Corynespora cassiicola* on cotton. Plant Dis. 102, 2494–2499. https://doi.org/10.1094/PDIS-03-18-0382-RE

Brent, K.J., Hollomon, D.W., 2007. Fungicide resistance in crop pathogens: How can it be managed? FRAC Monograph No.1, 2nd ed. Fungicide Resistance Action Committee 2007, Brussels, Belgium.

Date, H., Kataoka, E., Tanina, K., Sasaki, S., Inoue, K., Nasu, H., Kasuyama, S., 2004. Sensitivity of *Corynespora cassiicola*, causal agent of Corynespora leaf spot of cucumber, to thiophanate-methyl, diethofencarb and azoxystrobin. Japanese J. Phytopathol. 70, 10–13. https://doi.org/10.3186/jjphytopath.70.10

Duan, Y., Xin, W., Lu, F., Li, T., Li, M., Wu, J., Wang, J., Zhou, M., 2019. Benzimidazole- and QoI-resistance in *Corynespora cassiicola* populations from greenhouse-cultivated cucumber: An emerging problem in China. Pestic. Biochem. Physiol. 153, 95–105. https://doi.org/10.1016/j.pestbp.2018.11.006

Emmitt, R.S., Stevenson, K.L., Martinez-Espinoza, A.D., Buck, J.W., 2018. Fungicide sensitivity profiles of *Puccinia hemerocallidis* to pyraclostrobin, flutolanil, and thiophanate-methyl. Crop Prot. 110, 103–107. https://doi.org/10.1016/j.cropro.2018.04.008

FAO, 2012. Guidelines on prevention and management of pesticide resistance. Rome, Italy.

Farr, D.F., Rossman, A.Y., 2020. Fungal Databases, U.S. National Fungus Collections, ARS, USDA [WWW Document]. URL https://nt.ars-grin.gov/fungaldatabases/ (accessed 6.10.20).

Faske, T., 2017. Target spot of soybean: What do we know? [WWW Document]. Arkansas Row Crop. URL http://www.arkansas-crops.com/2016/11/02/target-spot-of-soybean-what-do-we-know/ (accessed 6.10.20).

FRAC, 2020. FRAC Code List 2020: Fungal control agents sorted by cross resistance pattern and mode of action (including FRAC code numbering). CropLife International, Brussels, Belgium.

Galbieri, R., Araújo, D.C.E.B., Kobayasti, L., Girotto, L., Matos, J.N., Marangoni, M.S., Almeida, W.P., Mehta, Y.R., 2014. Corynespora leaf blight of cotton in Brazil and its management. Am. J. Plant Sci. 5, 3805–3811. https://doi.org/10.4236/ajps.2014.526398

Ghini, R., Kimati, H., 2000. Resistência de fungos a fungicidas, 2nd ed, Embrapa Meio Ambiente. Embrapa Meio Ambiente, Jaguariúna, SP.

Godoy, C. V., 2015. Target spot, in: Hartman, G.L., Rupe, J.C., Sikora, E.J., Domier, L.L., Davis, J.A., Steffey, K.L. (Eds.), Compendium of Soybean Diseases and Pests. American Phytopathological Society, St. Paul, MN, pp. 62–63.

Hagan, A.K., Sikora, E.J., 2012. Leaf spot management in Alabama cotton - Control of potash-incited leaf spot diseases and Corynespora leaf spot. Alabama Cooperative Extension System, Auburn University, Alabama, AL.

Ishii, H., 2015. Stability of resistance, in: Hideo, I., Hollomon, D.W. (Eds.), Fungicide Resistance in Plant Pathogens. Springer Japan, pp. 35–48. https://doi.org/10.1007/978-4-431-55642-8_3

Ishii, H., Hollomon, D.W., 2015. Fungicide resistance in plant pathogens - Principles and a guide to practical management. Springer Japan. https://doi.org/10.1007/978-4-431-55642-8

Ishii, H., Yano, K., Date, H., Furuta, A., Sagehashi, Y., Yamaguchi, T., Sugiyama, T., Nishimura, K., Hasama, W., 2007. Molecular characterization and diagnosis of QoI resistance in cucumber and eggplant fungal pathogens. Phytopathology 97, 1458–1466. https://doi.org/10.1094/PHYTO-97-11-1458

Koenning, S.R., Creswell, T.C., Dunphy, E.J., Sikora, E.J., Mueller, J.D., 2006. Increased occurrence of target spot of soybean caused by *Corynespora cassiicola* in the southeastern United States. Plant Dis. 90, 974–974. https://doi.org/10.1094/PD-90-0974C

Ma, D., Jiang, J., Zhu, J., Zhang, L., Li, B., Mu, W., Liu, F., 2020. Evaluation of sensitivity and resistance risk of *Corynespora cassiicola* to isopyrazam and mefentrifluconazole. Plant Dis. PDIS-02-20-0384-RE. https://doi.org/10.1094/pdis-02-20-0384-re

MacKenzie, K.J., Sumabat, L.G., Xavier, K. V., Vallad, G.E., 2018. A review of *Corynespora cassiicola* and its increasing relevance to tomato in Florida. Plant Heal. Prog. 19, 303–309. https://doi.org/10.1094/php-05-18-0023-rv

MacKenzie, K.J., Xavier, K. V., Wen, A., Timilsina, S., Adkison, H.M., Dufault, N.S., Vallad, G.E., 2020. Widespread QoI fungicide resistance revealed among *Corynespora cassiicola* tomato isolates in Florida. Plant Dis. 104, 893–903. https://doi.org/10.1094/PDIS-03-19-0460-RE

Miyamoto, T., Ishii, H., Seko, T., Kobori, S., Tomita, Y., 2009. Occurrence of *Corynespora cassiicola* isolates resistant to boscalid on cucumber in Ibaraki Prefecture, Japan. Plant Pathol. 58, 1144–1151. https://doi.org/10.1111/j.1365-3059.2009.02151.x

Miyamoto, T., Ishii, H., Stammler, G., Koch, A., Ogawara, T., Tomita, Y., Fountaine, J.M., Ushio, S., Seko, T., Kobori, S., 2010. Distribution and molecular characterization of *Corynespora cassiicola* isolates resistant to boscalid. Plant Pathol. 59, 873–881. https://doi.org/10.1111/j.1365-3059.2010.02321.x

Rondon, M.N., Lawrence, K.S., 2019. *Corynespora cassiicola* isolates from soybean in Alabama detected with G143A mutation in the cytochrome *b* gene. Plant Heal. Prog. 20, 247–249. https://doi.org/10.1094/PHP-07-19-0046-BR

Russell, P.E., 2004. Sensitivity baselines in fungicide resistance research and management. FRAC Monogr. 3, 56. https://doi.org/10.1564/17jun07

Sautua, F.J., Doyle, V.P., Price, P.P., Porfiri, A., Fernandez, P., Scandiani, M.M., Carmona, M.A., 2020. Fungicide resistance in *Cercospora* species causing Cercospora leaf blight and Purple seed stain of soybean in Argentina. Plant Pathol. 00, 1–17. https://doi.org/10.1111/ppa.13261

Sumabat, L.G., Kemerait, R.C., Brewer, M.T., 2018. Phylogenetic diversity and host specialization of *Corynespora cassiicola* responsible for emerging target spot disease of cotton and other crops in the southeastern United States. Phytopathology 108, 892–901. https://doi.org/10.1094/phyto-12-17-0407-r

Teramoto, A., Meyer, M.C., Suassuna, N.D., Cunha, M.G. da, Da Cunha, M.G., 2017. *In vitro* sensitivity of *Corynespora cassiicola* isolated from soybean to fungicides and field chemical control of target spot. Summa Phytopathol. 43, 281–289. https://doi.org/10.1590/0100-5405/2195

Torriani, S.F.F., Frey, R., Buitrago, C., Wullschleger, J., Waldner, M., Kuehn, R., Scalliet, G., Sierotzki, H., 2017. Succinate-Dehydrogenase Inhibitor (SDHI) resistance evolution in plant pathogens, in: Deising, H.B., Fraaije, B., Mehl, A., Oerke, E.C., Sierotzki, H., Stammler, G. (Eds.), Modern Fungicides and Antifungal Compounds. Lebenshilfe Braunschweig gemeinnützige GmbH, Friedrichroda, Germany, pp. 89–94.

Xavier, S.A., Canteri, M.G., Barros, D.C.M., Godoy, C. V, 2013. Sensitivity of *Corynespora cassiicola* from soybean to carbendazim and prothioconazole. Trop. Plant Pathol. 38, 431–435. https://doi.org/10.1590/S1982-56762013005000020

Zhang, G., Bradley, C.A., 2017. Comparison of quinone outside inhibitor fungicide-resistant and -sensitive isolates of *Cercospora sojina*. Crop Prot. 94, 59–63. https://doi.org/10.1016/j.cropro.2016.12.007

Zhu, J., Zhang, L., Li, T., Ma, D., Gao, Y., Mu, W., Liu, F., 2020. Baseline sensitivity of *Corynespora cassiicola* to metconazole and efficacy of this fungicide. Crop Prot. 130, 1–7. https://doi.org/10.1016/j.cropro.2019.105056

Zhu, F., Shi, Y., Xie, X., Chai, A., Li, B., 2019. Occurrence, distribution, and characteristics of boscalid-resistant *Corynespora cassiicola* in China. Plant Dis. 103, 69–76. https://doi.org/10.1094/PDIS-11-17-1760-RE