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EPIDEMIOLOGY AND MANAGEMENT OF FROGEYE LEAF SPOT OF SOYBEAN: DAMAGE THRESHOLDS, EFFICACY AND PROFITABILITY OF FOLIAR FUNGICIDES

Jhonatan Paulo Barro

University of Kentucky, jhonatanbarro@gmail.com

Author ORCID Identifier:

 <https://orcid.org/0000-0002-6509-4744>

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Jhonatan Paulo Barro, Student

Dr. Carl Bradley, Major Professor

Dr. Nicole Gauthier, Director of Graduate Studies

EPIDEMIOLOGY AND MANAGEMENT OF FROGEYE LEAF SPOT OF SOYBEAN:
DAMAGE THRESHOLDS, EFFICACY AND PROFITABILITY OF FOLIAR
FUNGICIDES

DISSERTATION

A dissertation submitted in partial fulfillment of the
requirements for the degree of Doctor of Philosophy in the
College of Agriculture, Food and Environment
at the University of Kentucky

By
Jhonatan Paulo Barro
Lexington, Kentucky
Director: Dr. Dr. Carl Bradley, Professor of Plant Pathology
Lexington, Kentucky
2022

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<https://orcid.org/0000-0002-6509-4744>

ABSTRACT OF DISSERTATION

EPIDEMIOLOGY AND MANAGEMENT OF FROGEYE LEAF SPOT OF SOYBEAN: DAMAGE THRESHOLDS, EFFICACY AND PROFITABILITY OF FOLIAR FUNGICIDES

Frogeye leaf spot (FLS), caused by *Cercospora sojina*, is an economically important disease of soybean in many parts of the world where soybean is grown, including the United States. A meta-analytic approach was used to summarize a data set of 66 uniform field research trials conducted to evaluate fungicide efficacy against FLS on soybean. The dataset spanned 10 years (2012 to 2021) of experiments conducted across eight states in the U.S., including Alabama, Arkansas, Illinois, Iowa, Kentucky, Louisiana, Mississippi and Tennessee. First, the relationship between FLS severity and soybean yield was investigated. A significant negative slope obtained through random effects meta-analytic models confirmed the negative linear relationship between FLS severity and soybean yield. Additionally, the overall relative damage coefficient was calculated to be 0.51%, indicating that a 1% increase in FLS severity would result in a 0.51% yield reduction. In addition, economic damage thresholds were estimated by using the damage coefficient, for a range of soybean prices and control costs, taking into account three different fungicide efficacies representing low (25%), moderate (50%) and high (75%) levels of disease control. As expected, the threshold values increased as the control efficacy also increased and were affected by different crop prices and fungicide costs. Second, after potential yield losses caused by FLS were identified, the best fungicide options to control the disease were investigated. The results demonstrated that fungicide efficacy against FLS differ among active ingredients and is decreasing over time possibly due to fungicide resistant populations (mainly to the quinone outside inhibitors [QoIs]). The best performing fungicide reported in this study was a mixture of difenoconazole + pydiflumetofen, and the poorest performing fungicide was pyraclostrobin, a QoI fungicide. A statistically significant ($P < 0.05$) decline in performance was detected for two fungicide mixtures (azoxystrobin + difenoconazole and thiophanate-methyl + tebuconazole) and two single active ingredients (pyraclostrobin and

tetraconazole). Greater yields in trials with conditions favorable for severe epidemics were found, which could be explained by the more evident effect of the fungicides among the treated plots when compared to the nontreated control. Accordingly, the most effective treatments were more likely to be profitable under higher disease pressure and, as expected, the less effective treatment reported the higher risk of not offsetting the costs. Third, the profitability of applying fungicides was investigated in the absence or very low levels of FLS in double-crop soybean by using a different data set of 22 fungicide trials conducted between 2008 and 2021 across five states in the U.S. (Illinois, Indiana, Kentucky, Missouri, and Tennessee). The results showed no significant difference in yield response between the fungicide treatments and the nontreated control. Economic analyses indicated that, due to the lower yield responses, probabilities of breaking even were less than 50% for all the single fungicide classes, or up to 51% for mixtures, depending on fungicide cost and soybean price values. Overall, these research findings may provide useful information for regional risk assessment of potential yield loss caused by FLS, and for planning fungicide programs to control this important foliar disease. Decisions on fungicide planning must take into account, not only technical information such as control efficacy and yield return, but also profitability and strategies to mitigate fungicide resistance issues, seeking to preserve the lifespan of site-specific fungicides.

KEYWORDS: *Cercospora sojina*, chemical control, economic risk, fungicide, *Glycine max*, yield loss

Jhonatan Paulo Barro

(*Jhonatan Paulo Barro*)

11/18/2022

Date

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By
Jhonatan Paulo Barro

Carl Bradley

Director of Dissertation

Nicole Gauthier

Director of Graduate Studies

11/18/2022

Date

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CHAPTER 1. LITERATURE REVIEW

1.1 Soybean

Soybean [*Glycine max* (L.) Merr.] is an annual legume of the family *Fabaceae* and the most economically important bean in the world, being used to produce hundreds of consumer, industrial, and feedstock products such as vegetable oil, soymilk, animal feed products, and biodiesel (Knott & Lee, 2018). Brazil and the United States are the major producers of soybeans in the world and produced over 120 million metric tons each in 2020 ([FAO](#)). In Brazil, the cultivated soybean area has been increasing from 11.6 million hectares in 1994/95 to 32.1 million hectares in 2014/15, and to a total of 58.0 million hectares in 2014/15 ([Godoy et al., 2016](#)). In the 2020/2021 season, Brazil was the major soybean producer, with 135.4 million metric tons harvested across 38.5 million hectares ([EMBRAPA Soja](#)). In the United States, 33.3 million hectares were planted, and 112.5 million metric tons were produced in the 2021 growing season (United States Department of Agriculture-National Agricultural Statistics Service, 2022). Soybean production obtained during the 2021 season across the eight states where our data were collected is described in Table 1.1. Alabama, Arkansas, Illinois, Iowa, Kentucky, Louisiana, Mississippi and Tennessee accounted for almost 40% of soybean production in the United States in the 2021 season (United States Department of Agriculture-National Agricultural Statistics Service, 2022).

The growth habit of soybean is separated into two main growth stages: vegetative, which is counted by the number of nodes on the main stem; and reproductive, where R1 and R2 are based on flowering, R3 and R4 on pod development, R5 and R6 on seed

development, and R7 and R8 on maturation (Fehr et al., 1971). Soybean flowering is triggered by photoperiod, mainly for the length of the night (Cober et al., 2001). Soybean cultivars are divided into maturity groups (MG), being the early maturing cultivars adapted to the northern United States and Canada and, up to MG VIII are adapted to the southern United States (Knott & Lee, 2018). Particularly for Kentucky, MGs III, IV, and V are best suited, and soybean is used as a full-season crop or a double-crop following soft red winter wheat (*Triticum aestivum* L.) harvest (Rod et al., 2021).

Economically important soybean diseases vary annually and across locations due to multiple factors that influence disease development, including environmental conditions, crop production practices, field disease history, and cultivar selection (Bradley et al., 2021). According to the last survey investigating soybean yield losses caused by plant diseases across 29 soybean producing states in the U.S. and Ontario, Canada, between 2015 and 2019, Bradley et al. (2021) estimated that soybean cyst nematode (SCN) (*Heterodera glycines*) caused more than twice as much yield loss as any other pathogen over the duration of the survey period. Additionally, annual yield loss estimates for SCN were greater than any other diseases across the 13 northern states in the U.S. and Ontario, Canada, followed by seedling diseases (caused by various pathogens), Sclerotinia stem rot (caused by *Sclerotinia sclerotiorum*), and sudden death syndrome (SDS) (caused by *Fusarium virguliforme*). Moreover, frogeye leaf spot (FLS) (caused by *Cercospora sojina*) caused more than twice as much estimated yield loss during the 5-year period from 2015 to 2019 compared with values reported for the 5-year period from 2010 to 2014 (Allen et al., 2017; Bradley et al., 2021). In fact, foliar diseases were observed to be more yield-limiting in the southern United States, including

Cercospora leaf blight (caused by *Cercospora flagellaris*, *C. kikuchii*, *C. sigesbeckiae*), frogeye leaf spot and *Septoria* brown spot (caused by *Septoria glycines*) (Bradley et al., 2021).

1.2 Distribution of frogeye leaf spot

Cercospora sojina K. Hara is the causal agent of frogeye leaf spot (FLS) on soybean (Athow & Probst, 1952). The disease was first reported in Japan in 1915, in the United States in 1924, in Brazil in 1971, and in Argentina in 1983 (Giorda & Justh, 1983; Lehman, 1928; Melchers, 1925; Veiga & Kimati, 1974). In the United States, the disease historically has been most common in the southern soybean production region, and recently has become more common in the midwestern and northern soybean production regions of the country, including Iowa, Nebraska, North Dakota, and Wisconsin (Mengistu et al., 2002; Neves et al., 2020, 2022; Yang et al., 2001). More common observances in northern states may be explained by the combination of warm temperatures during the winter and the capability of the pathogen to survive for up to 24 months in plant debris remaining on the soil surface by the increasing use of conservation tillage practices (Cruz & Dorrance, 2009; Mian et al., 2008; Zhang & Bradley, 2014).

1.3 Economic impact of frogeye leaf spot

Epidemics of FLS have increased in frequency and severity worldwide, and thus have become a very important yield-reducing disease across the major soybean-producing countries. Soybean yield losses caused by FLS epidemics can range from 31% to up to 60% due to reduced photosynthetic leaf area, premature defoliation and reduced seed weight (Dashiehl & Akem, 1994; Mian et al., 1998). In the United States and Ontario,

Canada, the estimated average annual soybean yield losses, caused by FLS, from 2010 to 2019, ranged from 101,467 to 1,453,225 metric tons (Allen et al., 2017; Bradley et al., 2021). Additionally, losses due to FLS during the 2009/10 crop season were estimated at about \$2 billion USD in Argentina (Sepulcri et al., 2015). Average yields of non-treated plants against FLS were reduced by 37% in Zambia during the 1997/98 crop season (Mwase & Kapooria, 2000). In Brazil, the occurrence of FLS is part of a complex of late-season diseases caused by *Cercospora kikuchii*, *Colletotrichum truncatum*, and *Septoria glycines*, and up to 30% yield losses have been reported (Balardin, 2002).

1.4 Pathogen

1.4.1 Taxonomy

Domain Eukarya, kingdom Fungi, subkingdom Dikarya, phylum Ascomycota, subphylum Pezizomycotina, class Dothideomycetes, order Mycosphaerellales, family Mycosphaerellaceae, genus *Cercospora*, species *Cercospora sojina* ([NCBI](#)).

1.4.2 Identification

1.4.2.1 Morphological characterization

Although *Cercospora sojina* K. Hara is recognized as the causal agent of FLS, early literature reported *Cercospora daizu* as the causal agent of this disease (Athrow, 1987). Conidia are hyaline, elongate to fusiform and measure 6-8 x 40-60 μm (Wise & Newman, 2015). Additionally, conidia can be produced on infected parts of the plant (leaf, stem, or seeds) and from infested residue on the soil surface (Cruz & Dorrance, 2009). As

conidiophores continue to grow, conidia are formed on the tips and are pushed aside (Groenewald et al., 2012; Wise & Newman, 2015). In a single lesion, 2 to 25 conidiophores can be produced, and each conidiophore can produce 1 to 11 conidia (Lehman, 1928). Conidia can germinate on a leaf surface within an hour of deposition in the presence of water at 25 to 30°C (Phillips, 1999).

1.4.2.2 Molecular characterization

Conventional polymerase chain reaction (PCR) assays have been successfully developed and used to identify and detect several important plant pathogens. *C. sojae* can be identified by amplifying a fragment of actin, calmodulin, histone, translation elongation factor, as well as internal transcribed spacer regions and the 5.8S rRNA gene (Groenewald et al., 2013; Neves et al., 2022). However, translation elongation factor and calmodulin genes can be intermixed with other *Cercospora* species (Groenewald et al., 2013). These genes can be amplified using primers (Table 1.2), and nucleotide sequences can be compared using the BLAST search on [NCBI Genbank](#).

1.5 Disease symptoms

Although disease symptoms most commonly appear during reproductive growth stages, FLS lesions can affect leaves, pods and stems at any stage of development (Wise & Newman, 2015). Symptoms include small, dark lesions that evolve from tan to brown spots surrounded by a narrow, purple-brown margin (Wise & Newman, 2015) (Fig. 1.1A). The lesion diameters range from 1 to 5 mm (Grau et al., 2004). On the abaxial surface, the formation of clusters of conidia can be observed in the center of mature

lesions (Wise & Newman, 2015) (Fig. 1.1B). Stem lesions, which are two to four-times longer and wider than leaf lesions, are less common, but they can appear later in the season (Bisht & Sinclair, 1985). Additionally, the fungus can penetrate through the pod walls and infect the seeds (Phillips, 1999). Symptoms on seeds include light to dark gray or brown areas that can range from specks to large blotches covering the entire seed coat (Bisht & Sinclair, 1985).

1.6 Disease cycle and epidemiology

Initial inoculum can be produced on infected plant debris where the pathogen can overwinter for up to 24 months, or it can survive on infected seeds (Cruz & Dorrance, 2009; Singh & Sinclair, 1985; Zhang & Bradley, 2014). Conidia are then dispersed throughout the crop canopy from the infested residue by wind or splashing rain. The lesions are not visible for nearly 14 days after infection. Fully expanded leaves are more resistant to infection than young expanding leaves which are highly susceptible. For plants grown in warm (25–30°C) and humid conditions (>90% relative humidity), sporulation can occur within 48 h of the appearance of visible symptoms (Wise & Newman, 2015). Under favorable conditions, secondary infection of leaves, stems, and pods continue throughout the soybean growing season, characterizing the disease as polycyclic (Fig. 1.2) (Wise & Newman, 2015). In seeds, the fungus can penetrate indirectly through pores and cracks in the seed coat or directly through the hilar tracheid, and may grow into seedling tissues during germination and emergence (Singh & Sinclair, 1985). Seed transmission can play an important role in disease spread, as the disease has been found in fields never planted with soybean or under soybean rotation in Argentina, indicating that the pathogen was likely introduced via infected seeds (Sautua et al., 2018).

1.7 Pathogenicity

C. soja infects the plants by branched hyphae through open stomata. Luo et al. (2018) assembled the genome of *C. soja* race 1 and obtained a total assembly size around 40.84 Mb. Additionally, the genome of *C. soja* contained 11,655 protein-coding genes, of which a total of 233 proteins were predicted as the putative small (400 amino acids) cysteine-rich proteins (Luo et al., 2018). The authors found 141 putative effectors and more than one third of them were upregulated during starvation, suggesting that *C. soja* can probably deploy effectors to promote infection (Luo et al., 2018). Despite the fact that most of the species across the *Cercospora* genus can produce a toxin called cercosporin, it has been disputed if *C. soja* produces it (Goodwin et al., 2001). Luo et al. (2018) identified a gene cluster with eight cercosporin biosynthesis genes in the *C. soja* genome and observed the increased transcription of the eight genes during infection. These results imply that *C. soja* may produce cercosporin during infection. However, authors were unable to detect cercosporin in either cultured mycelium or infected plant tissue (Luo et al., 2018). Finally, in the *C. soja* genome, there were around 23.5% potential secreted proteins that were predicted as putative carbohydrate-active enzymes (CAZymes), demonstrating that *C. soja* may employ a large group of CAZymes to digest host cell walls during invasion (Luo et al., 2018). Another study sequenced Race 15 of *C. soja* and analyzed the comparative genome with respect to Race 1 (Gu et al., 2020). The authors found that the pathogenic reaction patterns of Race 1 and Race 15 were similar.

1.8 Genetic diversity

Bradley et al. (2012) used amplified fragment length polymorphism (AFLP) markers to better understand the genetic diversity of a historical collection of 62 *C. soja* isolates from Brazil, China, Nigeria, and the United States. The authors found a high degree of genetic diversity with no clear separation of isolates based on their origin. Only two isolates collected from Georgia and two isolates from China were clustered together among the two major clusters and seven sub-clusters obtained. Another study investigated the genetic diversity of a subset of 186 isolates of *C. soja*, including historical isolates, which were genotyped for 49 single nucleotide polymorphism (SNP) markers, revealing 35 unique genotypes (Shrestha et al., 2017).

1.8.1 Sexual recombination

Sexual reproduction is a key mechanism through which genetic diversity is produced in many plant-pathogenic fungi (Glass & Kulda, 1992). Although for most *Cercospora* spp., including *C. soja*, a sexual stage has not been observed in either field or laboratory conditions, molecular analyses have shown that *Cercospora* spp. form a monophyletic group within the teleomorphic genus *Mycosphaerella* (Goodwin et al., 2001; Crous & Braun, 2003; Crous et al., 2004a). In fact, comparative genome analysis of *C. soja* with plant pathogen members of the genus *Mycosphaerella* (*M. pini*, *M. Populorum*, *Z. tritici* [*M. graminicola*] and *M. fijiensis*) on different plant hosts (pine, poplar and banana, respectively) found considerable conserved synteny, higher average exon numbers per gene and gene density between *C. soja* and *Z. tritici* compared to the genomes of the other three fungal species in the genus *Mycosphaerella* (Zeng et al., 2017). These genome features can be linked by the fact that the hosts of *C. soja* and *Z.*

tritici, soybean and wheat, have similar characteristics of growing conditions and pathogen resistance, compared with perennial tree species pine, poplar, and banana as hosts of *M. pini*, *M. populorum* and *M. fijiensis*, respectively (Zeng et al., 2017).

When the sexual stage is not known, which is the case of *C. sojina*, several approaches have been used to provide evidence of cryptic sexual reproduction, including quantification of genetic diversity, population differentiation, and mating-type frequencies (Kim et al., 2013). Typically, populations undergoing sexual reproduction exhibit high genetic diversity and equal mating-type frequencies compared with populations solely or predominantly reproducing asexually (Milgroom, 1996). Kim et al. (2013) developed a multiplex PCR assay with specific primers for *C. sojina* aiming to determine mating types for a collection of 132 *C. sojina* isolates collected from six fields in Arkansas. Of the 132 *C. sojina* isolates, 68 isolates had the *MATI-1-1* idiomorph, and 64 isolates had the *MATI-2* idiomorph. No isolates possessed both idiomorphs. An equal proportion of mating-type loci in all populations analyzed and high genotypic diversity (26 to 79%) suggested that populations of *C. sojina* in Arkansas are most likely undergoing cryptic sexual reproduction (Kim et al., 2013). Another study investigated the genetic diversity of a subset of 186 isolates of *C. sojina*, including historical isolates, which were genotyped for 49 single nucleotide polymorphism (SNP) markers, revealing 35 unique genotypes (Shrestha et al., 2017). Both mating type alleles (*MATI-1-1* and *MATI-2*) were found in individual lesions suggesting opportunity for sexual recombination (Shrestha et al., 2017).

1.9 Management

1.9.1 *Host genetic resistance and races*

In the United States, a total of 12 races of *C. sojina* were reported from various states (Grau et al., 2004) (Table 1.3). In Brazil, 22 races have been reported (Yorinori, 1992), and in Argentina, races 11 and 12 were identified during the 2008/09 and 2009/10 growing seasons (Scandiani et al., 2012) (Table 1.3). In China, 11 races of *C. sojina* were identified and, among them, races 1, 7, and 10 were considered the major ones (Huo et al., 1988). However, the total number of races in China increased to 14 and, more recently, a race 15 was reported to be the dominant, occurring at a frequency of 36%, higher than the previously dominant race 1 (Gu et al., 2020) (Table 1.3). This has led to a loss of host resistance in many cultivars in China (Gu et al., 2020).

Grau et al. (2004) stated that different sets of soybean differential cultivars were used to identify the *C. sojina* races in the United States, Brazil, and China. Additionally, Mian et al. (2008) pointed out the lack of a universally accepted set of soybean differential cultivars for the classification of *C. sojina* isolates into races as well as to identify, designate and compare races of this pathogen. Hence, the later authors created a new set of soybean differential lines and revised the *C. sojina* race designations to advance the characterization of *C. sojina* races and to identify additional FLS resistance genes in soybean (Mian et al., 2008). A total of 93 *C. sojina* isolates were analyzed for their reaction on 38 putative soybean differential lines, resulting in 3,534 isolate–differential combinations (Mian et al., 2008). The authors initiated the new race structure with race 5, since there are no known existing cultures of races 1 to 4, and identified 11 unique isolates which were designated as races 5 to 15 (Mian et al., 2008).

The approach used by Mian et al. (2008) does not account for the range of disease severity reaction in each of the differentials. Therefore, Mengistu et al. (2020) proposed a new approach, known as Pathogenicity Group, to address and simplify the current system of *C. soja* race designations. The authors evaluated the diversity of 83 *C. soja* isolates collected from 2006 to 2009 by using pathogenicity groups among 12 soybean differentials (Davis, Peking, Kent, CNS, Palmetto, Tracy, Lincoln, S-100, Richland, Blackhawk, Hood, and Lee). The set of 83 isolates grouped into five pathogenicity groups (PG1 through PG5), representing the virulence diversity present in those isolates collected from various geographical regions (Mengistu et al., 2020). The pathogenicity group PG1 did not infect eight of the differentials except Blackhawk, Lincoln, S-100, and Lee; PG2 showed low virulence on all differentials except on Davis (hypersensitive reaction); PG3 produced hypersensitive reaction on Davis but with less than moderate reaction to the rest of the differentials; PG4 caused no infection on Davis but moderate infection on Peking; and, PG5 was the most virulent pathotype that infected all genotypes except Davis (Mengistu et al., 2020). Therefore, even the most virulent pathogenicity group could not overcome the resistant *Rcs3* gene in Davis and, to date there are no *Rcs3*-virulent races reported in the literature. Similarly, a previous study screened 40 isolates of *C. soja* collected in 2018 and 2019 across six counties in Georgia, and found no isolates virulent on Davis, suggesting that the *Rcs3* gene is still an effective source of resistance in Georgia (Harrelson et al., 2021).

The *Rcs3* gene is one of the three single dominant genes conditioning resistance to *C. soja* recognized by the Soybean Genetics Committee (Mian et al., 2009). The first gene found was *Rcs1* in Lincoln, which conferred resistance to race 1 of *C. soja*

(Athow & Probst, 1952). *Rcs2* was identified in Kent for resistance to race 2 (Athow et al., 1962). Finally, *Rcs3* from Davis was found to condition resistance to race 5 and to all other known races of *C. sojina* in the United States (Phillips & Boerma, 1982; Boerma & Phillips, 1983) as well as to all known isolates of *C. sojina* in Brazil (Yorinori, 1992) (Table 1.3). Although other dominant genes for resistance to race 5 were found in the cultivars Ransom, Stonewall and Lee in 1993 (Pace et al., 1993), they were not considered to be important sources of resistance, because, currently, race 5 is not seen as an economic threat to soybean in the United States (Baker et al., 1999). Additionally, another single dominant gene nonallelic to *Rcs3* was found from the cultivar Peking and provided resistance against many *C. sojina* isolates (Baker et al., 1999).

In China, the gene *Rcsc7* conditions dominant resistance to Chinese race 7 (Table 1.3), but it has not been officially approved by the Soybean Genetics Committee as the allelism between *Rcsc7* and other resistance genes is not known (Zou et al., 1999). In Brazil, F₁ plants were obtained from the diallel mating of seven soybean cultivars (Bossier, Cristalina, Davis, Kent, Lincoln, Paraná, and Uberaba), and their reactions were evaluated against *C. sojina* race 4 using a multivariate variable developed from soybean reactions to infection degree, mean lesion diameter, percent of lesioned leaf area, lesions per square centimeter, and disease index (Gravina et al., 2004). The authors reported that Davis, Cristalina, and Uberaba were free of FLS symptoms (Gravina et al., 2004). Mengistu et al. (2011) assessed resistance to *C. sojina* race 11 by field screening soybean accessions in maturity groups I to VI across two locations (Missouri and Illinois). A total of 260 accessions, including 12 differentials, resulted in 20 remaining resistant accessions

that might contain novel loci for FLS resistance as the presence of *Rcs3* allele was not found using molecular markers (Mengistu et al., 2011).

Quantitative resistance to race 2 of *C. sojina* was identified in the greenhouse using recombinant inbred lines derived from the cross of the cultivars Essex and Forrest (Sharma & Lightfoot, 2014). Essex is known to be partially resistant while Forrest is partially susceptible to mixed races of *C. sojina*. The authors inferred that quantitative resistance to *C. sojina* race 2 involved two major quantitative trait loci (QTL). The two loci were effective at different stages of seedling development, suggesting they were conditional QTL, and, according to the location of the QTL, the loci were not allelic to *Rcs3* (Sharma & Lightfoot, 2014). Recently, McAllister et al. (2021) also screened 91 recombinant inbred lines from the crossing between Essex and Forrest under greenhouse conditions for FLS resistance to *C. sojina* race 15 and used single nucleotide polymorphism (SNP) markers to identify associated QTL. Two QTL were mapped, being one QTL reported on chromosome 13 coinciding with the QTL previously reported (Pham et al., 2015), and the QTL on chromosome 19 was novel (McAllister et al. 2021).

1.9.2 Biocontrol

The use of beneficial microorganisms to control plant diseases is an alternative or a supplemental way of reducing the use of chemicals. In the U.S., *Lysobacter enzymogenes* strain C3 (LeC3) was tested against *C. sojina*, which effectively inhibited its vegetative mycelial growth and conidial germination on plates (Nian et al., 2021). Moreover, a previous study reported that the application of *Trichoderma virens* conidial suspensions

as a foliar treatment significantly reduced frog-eye leaf spot severity of soybean compared to a nontreated control (Lacey, 2018). A previous study in Argentina reported reduced mycelial growth of *C. sojae* *in vitro* by testing a cell suspension of three indigenous bacterial strains, including BNM297 (*Pseudomonas fluorescens*), BNM340 and BNM122 (*Bacillus amyloliquefaciens*) (Simonetti et al., 2012). The authors found that *Bacillus* BNM122 and BNM340 inhibited the fungus to a similar degree (52–53%). Additionally, a significant inhibition of conidial germination was observed after 24 and 72 h of co-cultivation with cell suspension from BNM297, BNM340 or BNM122, (~79%, 79% and 89%, respectively). Biocontrol tests *in vivo* were conducted and both spray-applied bacteria, BNM340 and BNM122, significantly reduced the disease severity to a similar degree with respect to positive control plants, showing no significant differences between them, while *P. fluorescens* BNM297 did not have any effect on FLS severity on soybean plants (Simonetti et al., 2012).

Induced systemic resistance (ISR) consists of the activation of a plant defense upon pathogen attack by triggering a cascade of reactions that spread from the site of induction to distant parts of the plant (Kloepper et al., 1992). Previous studies showed that soybean plants inoculated with *Bacillus* sp. CHEP5 showed reduced FLS severity, with healthier and greener leaves compared to non-inoculated plants (Tonelli & Fabra, 2014). Additionally, as *Bacillus* sp. CHEP5 was applied onto the roots and the response was detected in the shoot system, the bacterial induction of resistance in the plant was considered to be systemic, hence, attributed to ISR (Tonelli & Fabra, 2014). The authors also investigated if the mechanism to induce systemic resistance of *Bacillus* sp. CHEP5 involved the priming of the jasmonic acid dependent pathway. The increased expression

of the defense related gene *GmAOS* in *Bacillus* sp. CHEP5 plus pathogen challenged plants strongly suggest that the enhanced soybean resistance to *C. sojina* attack induced by this bacterium occurs in a jasmonic acid dependent pathway (Tonelli & Fabra, 2014). Additionally, a following study showed a mutualistic behavior between *Bacillus* sp. CHEP5 with the nitrogen fixing strain *Bradyrhizobium japonicum* E109 being more effective in reducing frogeye leaf spot severity than the inoculation of *Bacillus* sp. CHEP5 alone (Tonelli et al., 2017).

1.9.3 Cultural practices

Cultural practices such as crop rotation and tillage can help to reduce FLS incidence (Grau et al., 2004; Wise & Newman, 2015). A previous study recommended that crop rotation with a nonhost of a minimum of two years would be more effective to reduce the level of viable *C. sojina* inoculum, regardless of the depth of the crop residue in the soil (Zhang & Bradley, 2014). Tillage can reduce the inoculum by burying infested plant residues (Mengistu et al., 2014). However, recent studies in Tennessee have not found significant differences in FLS severity, in the absence of fungicide application, between tilled and non-tilled plots across field trials conducted from 2007 to 2010 (Mengistu et al., 2014), and from 2014 to 2016 (Mengistu et al., 2018). Although conventional tillage alone did not significantly affect disease, fungicide efficacy was greater in tilled compared to non-tilled plots (Mengistu et al., 2014). Moreover, early planting seems to be favorable to avoid higher FLS pressure, as a previous study reported higher yield reduction due to FLS when soybean planting was delayed two weeks after the optimum planting date (Akem & Dashiell, 1994).

1.9.4 *Chemical control*

The regular use of fungicides in the United States started in 2005 driven by an increase in soybean prices and the potential threat of soybean rust (caused by *Phakopsora pachyrhizi*) (Phillips et al., 2021). Fungicide applications aiming to control FLS are recommended during reproductive growth stages (Floyd et al., 2021). Active ingredients from different fungicide classes available for managing FLS include demethylation inhibitors (DMI; FRAC 3), quinone outside inhibitors (QoI; FRAC 11), methyl benzimidazole carbamates (MBC; FRAC 1), succinate dehydrogenase inhibitors (SDHI, FRAC 7) and chloronitriles (FRAC M5) (Crop Protection Network, 2022). MBCs act in the cytoskeleton by inhibiting the formation of the β tubulin assembly during mitosis (Fungicide Resistance Action Committee, 2020). QoIs and SDHIs are fungicides that inhibit respiration (Sierotzki & Scalliet, 2013; Fungicide Resistance Action Committee, 2020). The QoIs act in the complex III in the mitochondria, binding the activity of the quinol oxidation (Qo) site of the *Cytochrome b*, which avoid electron transfer between *Cytochrome b* and *Cytochrome c*, interrupting ATP synthesis (Bartlett et al., 2002). On the other hand, the SDHIs act in the complex II of the electron transport chain in the mitochondria, also inhibiting the production of ATP (Sierotzki & Scalliet, 2013). The DMIs are compounds that inhibit the sterol biosynthesis in membranes, which can cause cell rupture and electrolyte leakage (Kumar et al., 2021). Finally, chlorothalonil is a multi-site fungicide that belongs to the chloronitriles group and is used as a protectant fungicide (Battaglin et al., 2011; Miles et al., 2007).

QoI fungicides, mainly azoxystrobin, pyraclostrobin and trifloxystrobin, have been commercially available and largely used on soybean in the United States, including for FLS management (Dorrance et al., 2010; Mengistu et al., 2018; Nelson et al., 2010; Sauter et al., 1999). However, after the emergence of QoI-resistant *C. sojae* isolates, studies have shown that DMIs, MBCs, SDHIs and premixes can be effective for managing FLS (Backman et al., 1978; Butler et al., 2018; Dorrance et al., 2010; Floyd et al., 2021; Mengistu et al., 2018; Phillips et al., 2021; Viggers et al., 2022). For instance, benomyl (MBC) was very effective in reducing FLS severity among susceptible cultivars in Alabama, U.S. (Backman et al., 1978) and Zambia (Mwase & Kapooria, 2000). However, another study conducted in Zimbabwe in 1996 and 1997 reported that the DMI flusilazole and the mixture of flusilazole + carbendazim were more effective against FLS than benomyl applied alone or as a premix with mancozeb (Galloway, 2008). Recently, Mengistu et al. (2018) showed significantly higher efficacies (>70%) for flutriafol (DMI), thiophanate-methyl (MBC) and the premix azoxystrobin + difenoconazole (QoI + DMI), compared to the single application of pyraclostrobin (27%) and chlorothalonil (30%). Additionally, a previous study summarized data from 66 uniform fungicide trials conducted from 2012 to 2021 across the major soybean-producing states in the U. S. using a meta-analytic approach (Barro et al., 2022). On average, the authors found the most effective fungicides to be the premixes difenoconazole + pydiflumetofen, thiophanate-methyl + tebuconazole, azoxystrobin + difenoconazole and trifloxystrobin + prothioconazole, all with percent control greater than 50%. The poorly performing fungicide was pyraclostrobin (11%). A statistically significant decline in performance over the years was detected for two dual premixes (azoxystrobin + difenoconazole and

thiophanate-methyl + tebuconazole) and two single active ingredients (pyraclostrobin and tetraconazole), which can be linked to fungicide resistance issues (Barro et al., 2022).

Fungicide application timing and coverage are critical for optimal disease control. Floyd et al. (2021) investigated different application times (R3, R4, R5, R6 and R3 + R5) on soybean yield by applying single and double premixes including different chemical classes during the 2017 and 2018 growing seasons in Mississippi (Floyd et al., 2021). The authors found no effect of timing on grain yield confirming the value of current management strategies (applications at R3) while also allowing flexibility in the application timing in situations that warrant application during later growth stages (Floyd et al., 2021). However, Akem (1995) evaluated applications of the fungicide benomyl at six different growth stages, starting from V3 (fully developed leaves, beginning with trifoliolate nodes) to R5 (beginning seed), to determine the effect of the fungicide timing on frogeye leaf spot severity and found that applications at R1 (beginning bloom) and R3 (beginning pod) significantly reduced disease severity. Regarding coverage, Butler et al. (2018) conducted field experiments in 2014 and 2015 in Tennessee to evaluate the influence of droplet size on foliar fungicide efficacy. The authors found no significant differences among the industry recommended standard flat fan XR11002VS (XR) nozzle and the drift-reduction nozzle type TTI11002-VP (TTI), but found significant disease reduction after application of azoxystrobin + difenoconazole compared to the nontreated control (Butler et al., 2018). Additionally, results from ten field trials conducted from 2017 to 2020 in Iowa by applying fluxapyroxad + pyraclostrobin using a traditional ground sprayer with an overhead spray boom and a ground sprayer with 360 undercover

sprayers showed no statistical difference between fungicide application methods on FLS severity (Viggers et al., 2022).

As mentioned previously, the primary inoculum sources of the disease are infected seeds and plant debris. Therefore, the use of pathogen-free or fungicide-treated seeds is crucial to prevent the introduction and further spread of the disease (Sautua et al., 2018). A previous study in Argentina evaluated the effect of fungicide seed treatments in reducing FLS incidence and found that pyraclostrobin + thiophanate-methyl and carbendazim + thiram, were the most effective treatments evaluated to eradicate the pathogen in seeds (Sautua et al., 2018).

1.10 Fungicide resistance

Cercospora sojina isolates with reduced sensitivity to quinone outside inhibitor (QoI) fungicides were first reported from Tennessee in 2010 (Zhang et al., 2012a). The resistance mechanism involved is an amino acid substitution (glycine is replaced with alanine at the codon 143) caused by the G143A mutation in the *Cytochrome b* gene (Zeng et al., 2015). However, other mutations associated with resistance to QoI fungicides such as the F129L (change from phenylalanine to leucine at codon 129) and G137R (change from glycine to arginine at codon 137), have not been reported in *C. sojina* (Zeng et al., 2015). Since the first confirmation in 2010, QoI-resistant isolates have become widespread across more than 20 soybean-producing states in the U.S. (Harrelson et al., 2021; Mathew et al., 2019; Neves et al., 2020, 2021, 2022; Standish et al., 2015; Zeng et al., 2015; Zhang et al., 2012a, 2012b, 2018; Zhou & Mehl, 2020) (Fig. 1.3).

Several methods have been used to identify whether *C. soja* isolates are sensitive or resistant to QoI fungicides. First, the effect of fungicide *in vitro* is a standard bioassay to evaluate the influence of chemistries and determine the effective concentration that reduces fungal growth or conidia germination by 50% relative to the non-amended control (EC₅₀) (Fungicide Resistance Action Committee, 2020). Based on EC₅₀, the discriminatory dose assay determined for *C. soja* was 1 µg/ml of azoxystrobin, 0.1 µg/ml for pyraclostrobin and 1 µg/ml for trifloxystrobin (Zhang et al., 2018). Conidia that germinated on the discriminatory dose are considered to be resistant to QoI fungicides. Second, molecular methods also can be used to identify QoI-sensitive and -resistant isolates of *C. soja*. Zeng et al. (2015) developed specific primers for PCR assay to recognize a mutation point that confers resistance to QoI fungicides. The primers used to identify QoI-sensitive isolates (Cs-2F/Cs-5R-2) produce a 359 bp fragment whereas the primers used to identify QoI-resistant isolates (Cs-1F/Cs-1R-2) produce a 207 bp fragment (Table 1.4). Additionally, Mut4-F/Mut4-R primers can amplify a fragment of the *Cytochrome b* gene that spans the area of F129L, G137R and C143A mutations (Zeng et al., 2015) (Table 1.4). Standish et al. (2015) developed a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) to identify the G143A mutation in *C. soja* using the restriction enzyme *AluI*. With that, PCR products from QoI-resistant isolates will produce two fragments, while QoI-sensitive isolates will remain intact upon digestion with restriction enzymes. Zhou & Mehl (2020) designed PCR (FLS-F2/FLS-R2) and pyrosequencing (FLS-S2) primers that target the presence of the G143A mutation in the *Cytochrome b* gene of *C. soja* (Table 1.4).

Since QoI resistant populations of *C. soja* in the U.S. have become widespread, growers have relied more on demethylation inhibitor (DMI) and methyl benzimidazole carbamate (MBC) fungicides, applied alone or as mixtures (Zhang et al., 2021). Although DMI and MBC fungicides are classified as medium and high risk, respectively, for fungicide resistance development (Fungicide Resistance Action Committee, 2020), *C. soja* isolates resistant to these fungicide classes have not yet been reported in the United States. Zhang et al. (2021) investigated the sensitivity to the DMI fungicides, flutriafol and tetraconazole, and the MBC fungicide, thiophanate-methyl, for 145 *C. soja* isolates collected prior to 2001 (baseline isolates), and from 2007 to 2012, representing 12 states (AL, AR, GA, IA, IL, KY, LA, MS, NY, SC, TN, and WI). No shift towards reduced sensitivity to the DMI and MBC fungicides was found between baseline isolates versus isolates collected from 2007 to 2012 (Zhang et al., 2021).

1.11 Hypothesis and objectives of this dissertation

The study of yield losses caused by plant diseases has been performed through the understanding of the relationships between disease intensity (e.g. severity) and yield. The relationship between FLS severity and soybean yield had never being analyzed using meta-analytic methods applied to data obtained from numerous site-years. In this dissertation, I focused on the hypothesis that FLS foliar symptoms and yield were negatively correlated. To test that, I explored and summarized the relationship between soybean yield and FLS severity to further estimate damage coefficients and economic damage thresholds. To avoid yield losses due to FLS, chemical control is still the most used strategy to manage this disease. Despite several options available to farmers, the decision of which fungicides to utilize in spray programs should account for current

information on their efficacy and yield response. My hypothesis is that fungicide efficacy against FLS differ among active ingredients and is decreasing over time due to reported fungicide resistant populations (mainly to QoIs). In my dissertation, I estimated FLS control efficacy and yield response to different fungicides, evaluated if those responses varied over time and, calculated the profitability of fungicides using the meta-analytic estimates of yield response. Furthermore, the use of unnecessary fungicide applications in low-disease environments does not only increase the risk of fungicide resistance development due selection pressure, but also might not be profitable. To test my hypothesis that applying fungicides in the absence or very low levels of disease lead to a high risk of not offsetting the costs, I calculated fungicide profitability in the absence or under very low levels of disease in a double-crop system (wheat-soybean).

1.12 Tables

Table 1.1. Soybean area planted and production per state during the 2021 crop season (United States Department of Agriculture-National Agricultural Statistics Service, 2022).

| State | Harvested Hectares | Production (metric tons) | Production (USD) |
|--------------|--------------------|--------------------------|-----------------------|
| Alabama | 123,429 | 381,833 | 175,375,000 |
| Arkansas | 1,218,103 | 4,177,851 | 1,949,577,000 |
| Illinois | 4,253,246 | 18,306,233 | 8,878,848,000 |
| Iowa | 4,058,996 | 16,924,230 | 8,084,180,000 |
| Kentucky | 744,621 | 2,804,285 | 1,339,520,000 |
| Louisiana | 428,966 | 1,500,118 | 694,512,000 |
| Mississippi | 882,214 | 3,203,808 | 1,495,044,000 |
| Tennessee | 615,122 | 206,837 | 965,200,000 |
| Total | 12,324,697 | 47,505,195 | 23,582,256,000 |

Table 1.2. Primers for molecular identification of *Cercospora sojina*.

| Definition | Primers | Primer DNA sequences (5'to 3') | Reference |
|--|----------|--------------------------------|--------------------------|
| Actin | Act-512F | ATGTGCAAGGCCGGTTTCGC | Carbone & Kohn (1999) |
| | Act-783R | TACGAGTCCTTCTGGCCCAT | |
| Calmodulin | Cal-228F | GAGTTCAAGGAGGCCTTCTCCC | Carbone & Kohn (1999) |
| | Cal-737R | CATCTTTCTGGCCATCATGG | |
| Translation elongation factor 1-alpha | EF1-728F | CATCGAGAAGTTCGAGAAGG | Carbone & Kohn (1999) |
| | EF1-986R | TACTTGAAGGAACCCTTACC | |
| ITS region and 5.8S rRNA gene | ITS1 | TCCGTAGGTGAACCTGCGG | White et al. (1990) |
| | ITS4 | TCCTCCGCTTATTGATATGC | |
| Histone | CylH3F | AGGTCCACTGGTGGCAAG | Crous et al. (2004b) |
| | CylH3R | AGCTGGATGTCCTTGGACTG | |

Table 1.3. Race identification for *Cercospora sojina* with respective resistant genes (Rcs).

| Race | <i>Rcs</i> virulence | Country | Reference |
|--------------|---------------------------------|----------------|--|
| 1 | <i>Rcs1</i> | United States | Athow & Probst, 1952 |
| 2 | <i>Rcs2</i> | United States | Athow et al., 1962 |
| 3-4 | <i>Rcs3</i> | United States | Ross, 1968 |
| 5-12 | <i>Rcs3</i> | United States | Phillips & Boerma, 1981; Grau et al., 2004 |
| 1-15 | <i>Rsc7*</i> | China | Huo et al., 1988; Gu et al., 2020 |
| 11-12 | <i>Rcs3</i> | Argentina | Scandiani et al., 2012 |
| 1-22 | <i>Rcs3</i> | Brazil | Yorinori, 1992 |

*Not officially approved by the Soybean Genetics Committee.

Table 1.4. Primers for molecular characterization of resistant isolates of *Cercospora sojina* to quinone outside inhibitor (QoI) fungicides.

| Definition | Primers | Primer DNA sequences (5'to 3') | Reference |
|--------------------------|---------|--------------------------------|----------------------|
| QoI-sensitive | Cs-2F | GGTTCACTATTAGGATTTTGTCTTGTA | |
| (specific primers) | Cs-5R-2 | CTCATTA AATTAGTAATAACTGTGGCCG | Zeng et al., 2015 |
| QoI-resistant | Cs-1F | TAATACAGCTTCAGCATTTTTCTTCT | |
| (specific primers) | Cs-1R-2 | CTCATTA AATTAGTAATAACTGTGGCCG | Zeng et al., 2015 |
| <i>Cytochrome b</i> gene | Mut4-R | GATTCACCTCAGCCTTCAA | |
| | Mut4-F | CTCAACTATGTCCTGTCCTACTCA | Zeng et al., 2015 |
| | FLS-F2 | CTTACAAAGCACCTAGAACATTGG | |
| Pyrosequencing | FLS-R2 | TCCTACTCATGGTATTGCACTCA | Zhou & Mehl, 2020 |
| | FLS-S2 | TTACGGACAAATGTCTTTAT | |

1.13 Figures

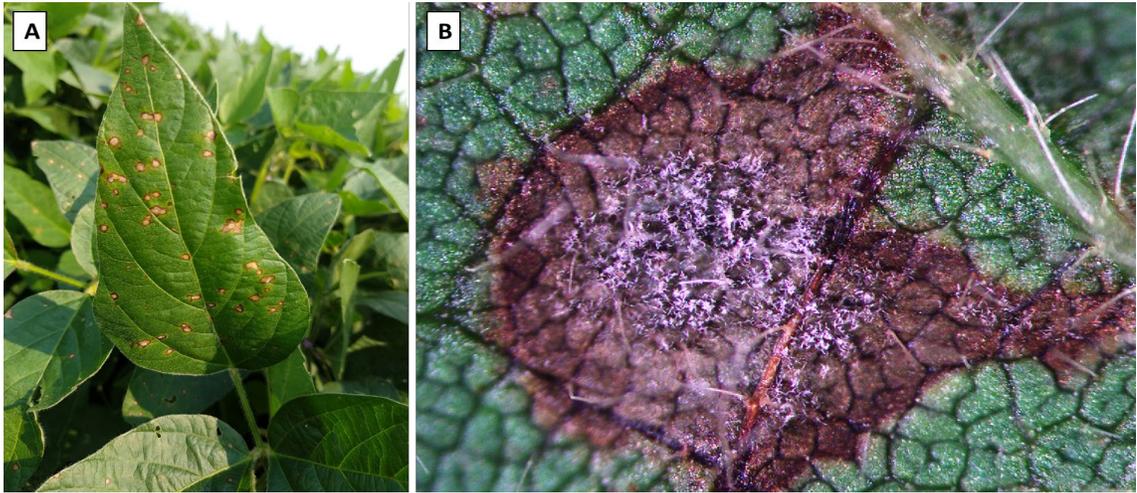


Fig. 1.1. Frogeye leaf spot symptoms include round to angular lesions with a dark-brown margin and a tan to light brown center (**A**); and fuzzy gray sporulation of *C. sojina* in the center of the lesion on the underside of the leaf (**B**).

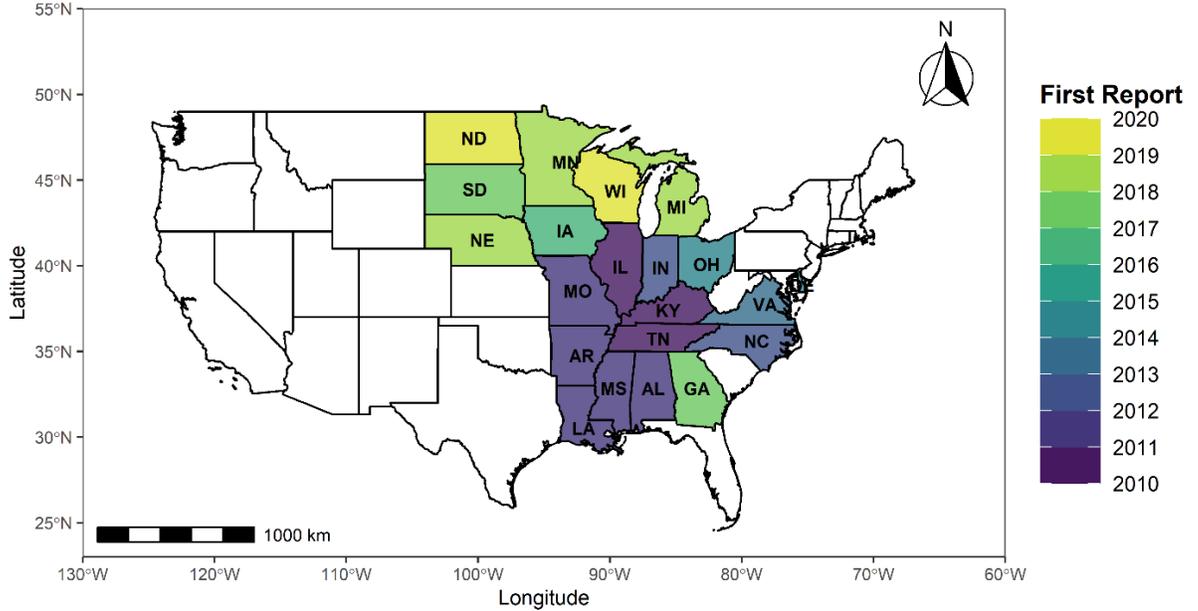


Fig. 1.3. States where QoI-resistant isolates of *C. sojae* were detected due to the amino acid substitution (glycine is replaced with alanine at the codon 143) caused by the G143A mutation in the *Cytochrome b* gene. Results from Harrelson et al. (2021), Mathew et al. (2019), Neves et al. (2020, 2021, 2022), Standish et al. (2015), Zeng et al. (2015), Zhang et al. (2012a, 2012b, 2018) and Zhou & Mehl (2020).

CHAPTER 2. META-ANALYTIC MODELING OF THE SEVERITY-YIELD RELATIONSHIPS IN SOYBEAN FROGEYE LEAF SPOT EPIDEMICS

Abstract

Frogeye leaf spot (FLS), caused by *Cercospora sojina*, is an important foliar disease affecting soybean in the United States. A meta-analytic approach including 39 fungicide trials conducted from 2012 to 2021 across eight states (Alabama, Arkansas, Illinois, Iowa, Kentucky, Louisiana, Mississippi, and Tennessee) was used to assess the relationship between FLS severity and soybean yield. Correlation and regression analyses were performed separately to determine Fisher's transformation of correlation coefficients (Z_r), intercept (β_0) and slope (β_1). Disease pressure (low severity = ≤ 34.5 and high severity = $> 34.5\%$) and yield class (low = $\leq 3,352$ and high = $> 3,352$ kg/ha) were included as categorical moderators. The Pearson's \bar{r} , obtained from back-transforming the \bar{Z}_r estimated by an overall random-effects model, showed a significant negative linear relationship between FLS severity and yield ($\bar{r} = -0.60$). The \bar{Z}_r was affected by disease pressure ($P = 0.0003$) but not by yield class ($P = 0.8141$). A random-coefficient model estimated a slope of -19 kg/ha for each % severity for a mean attainable yield of 3,719.9 kg/ha. Based on the overall mean (95% CI) of the intercept and slope estimated by the random-coefficients model, the overall relative damage coefficient was estimated to be 0.51% (0.36-0.69), indicating that a percent increase in FLS severity would result in a 0.51% yield reduction. The best model included yield class as a covariate and population-average intercepts differed significantly between low (3,455.1 kg/ha) and high (3,842.7 kg/ha) yield classes. The current results highlight the potential impact of FLS on soybean yield if not well managed and may be helpful in disease management decisions.

Keywords: *Cercospora sojina*, damage coefficient, management, yield loss

2.1 Introduction

Frogeye leaf spot (FLS) of soybean is caused by the fungus *Cercospora sojina* (Athow & Probst, 1952). Symptoms on the leaves, usually detected sometime during soybean reproductive development stages, include small, dark lesions that develop into light gray to tan circular to irregular-shaped spots surrounded by a narrow, purple-brown margin (Wise & Newman, 2015). The disease was first reported in the United States in 1924, and historically has been most common in the southern soybean production region (Melchers, 1925). However, FLS recently has become more common in the midwestern and northern soybean production regions of the country, including Iowa, Nebraska, North Dakota, and Wisconsin (Yang et al., 2001; Mengistu et al., 2002; Neves et al., 2020, 2022). Following its larger geographical footprint in North America, yield losses due to FLS epidemics, from 2010 to 2019, were estimated to range from 101,467 to 1,453,225 metric tons across the United States and Ontario, Canada (Allen et al., 2017; Bradley et al., 2021). Yield losses, which can reach up to 60%, are mainly due to reduced photosynthetic leaf area, premature defoliation and reduced seed weight (Dashiell & Akem, 1994).

The disease is best managed by integrating multiple tactics such as planting resistant soybean cultivars, applying foliar fungicides, and rotating to non-host crops (Wise & Newman, 2015). However, since the first confirmation in 2010 of *C. sojina* isolates with resistance to quinone outside inhibitors (QoI), due to the presence of the G143A mutation in the *Cytochrome b* gene, have been detected across more than 20 soybean-producing states in the U.S. (Harrelson et al. 2021; Mathew et al. 2019; Neves et al. 2020; 2021; 2022; Standish et al. 2015; Zeng et al. 2015; Zhang et al. 2012a; 2012b;

2018; Zhou and Mehl 2020). Hence, an integrated disease management program based on scouting and epidemiological criteria to base fungicide use, including to estimate the economic damage threshold, is critical (Carmona et al., 2015).

The intensity of FLS is usually quantified as severity, or the proportion of tissue area affected by the disease (Bock et al., 2022). A standard area diagram also has been developed to aid and standardize visual assessment of FLS severity in soybean (Price et al., 2016). The study of yield losses has been performed through the understanding of the relationships between disease intensity (in this case, severity) and yield from field experiments, where disease levels are assessed and the corresponding yield measured (Savary et al., 2006). Uniform field trials (UFTs) have been conducted since 2008 in multiple U.S. states to evaluate fungicide efficacy of products that are labeled or marketed for FLS management in soybean. The availability of such data provides an opportunity to explore and analyze large amounts of data on the relationship between FLS epidemics and soybean yield.

In this study, we used a meta-analytic framework to analyze these data, similar to the approach performed in previous studies on wheat (Paul et al., 2005, 2006). Meta-analysis is a statistical technique that has gained popularity by plant pathologists and accepted as a valid and powerful research methodology to quantitatively integrate the results of a collection of primary studies in a given topic (Madden et al., 2016; Madden & Paul, 2011). In plant pathology, meta-analysis has been used to summarize fungicide efficacy (Ascari et al., 2021; Barro et al., 2019; 2021a; 2021b; Dalla Lana et al., 2018; Machado et al., 2017; 2021; Paul et al., 2008; 2010), and relationships between variables such as disease and yield (Dalla Lana et al., 2015; Duffeck et al., 2020; Edwards Molina

et al., 2019; Kandel et al., 2020; Lehner et al., 2016; Madden & Paul, 2009), and disease and mycotoxins (Paul et al., 2005; 2006).

Therefore, we gathered FLS severity and soybean yield data from UFTs conducted over 10 years (2012 to 2021) and across eight states in the United States. Our objectives were to: i) explore and summarize the relationship between soybean yield and FLS severity; ii) identify variables affecting this relationship; and iii) estimate economic damage thresholds for different scenarios of soybean prices, fungicide costs and efficacies. This information is essential to estimate yield loss at the field level due to FLS and to assist farmers with making informed decisions about disease management.

2.2 Material and Methods

2.2.1 Data source and criteria for inclusion of trials in the analysis

Data were obtained from 66 uniform fungicide trials (UFTs) conducted during 10 crop seasons (2012 - 2021) across eight states in the U.S. (Alabama [AL], Arkansas [AR], Illinois [IL], Iowa [IA], Kentucky [KY], Louisiana [LA], Mississippi [MS] and Tennessee [TN]). The UFTs were conducted following the same experimental design and a common set of treatments. Each trial also utilized a susceptible cultivar that was regionally adapted for each location. Field plots were four rows wide, with interrow spacings of 38, 76, or 101 cm, and plot lengths of at least 6 m long. Plots were arranged in a randomized complete block design, with four replications. Fungicide treatments were applied when soybean plants were at the R3 developmental stage (beginning pod development; Fehr et al., 1971) by using a CO₂-pressurized backpack sprayer (276 kPa),

which was calibrated to deliver volumes between 141 and 200 liters/ha. All nutrient, weed, and insect management practices followed regional suggestions.

Disease severity was visually assessed as percent leaf area exhibiting FLS symptoms at the plot level, 4 weeks post-fungicide application (approximately at the R6 growth stage; full seed) focusing specifically on the upper third of the canopy. A standard area diagram, when needed, was used as reference during the ratings (Price et al., 2016). Yield was obtained by harvesting the 2 central rows of each plot after full maturity (R8) with a small plot combine. Grain weight and moisture were obtained, and soybean yield was calculated as kilograms/hectare standardized to 13% moisture.

To be included in the analysis, trials needed to have within-study differences between the minimum and maximum severity of at least 5 percentage points. The data were explored, and 26 trials were excluded, as these were considered inappropriate to reliably quantify the relationship (Duffeck et al., 2020; Lehner et al., 2016). One trial did not provide information on FLS severity. Hence, data from 39 trials remained and were used to model the yield–severity relationship.

2.2.2 Effect sizes and meta-analytic modeling

2.2.2.1 Correlation coefficients

A standard univariate random-effect meta-analysis was performed using the *rma.uni* function of the *metafor* package of R (R Core Team, 2020), with parameters estimated via maximum likelihood, to obtain the Fisher’s Z_r and respective sampling variances

(Dalla Lana et al., 2015; Lehner et al., 2016). The Fisher's Z_r transformation of r was used in the analysis, which is given by Equation 1:

$$Z_i = \frac{1}{2} \times \ln \left(\frac{1+r_i}{1-r_i} \right) \quad (1)$$

where Z_i is the Fisher's Z transformation of the i th study ($i = 1, 2, \dots, n$) and r is the Pearson's correlation coefficient of the i th study. The analysis was conducted to account for the sampling variance for each study given by Equation 2:

$$V_Z = \{1 \div (n_i - 3)\} \quad (2)$$

where n_i is the number of pairs of FLS severity and yield in each i th study.

Overall means and 95% confidence interval (95% CI) were calculated. Heterogeneity among the true effect sizes was evaluated based on significance of the Cochran Q test and the I^2 index that measures the extent of heterogeneity of the true effect sizes (Higgins & Thompson, 2002).

2.2.2.2 *Regression coefficients*

Intercepts (β_0) and slopes (β_1) (using ordinary least square regression modeling) were estimated for the relationship between FLS severity and soybean yield at the trial level (Dalla Lana et al., 2015; Duffeck et al., 2020; Madden & Paul, 2009). The distribution of the linear coefficients estimated independently for each trial was summarized by the calculation of the interdecile (ID) range (90–10%), or 80% of the estimated intercepts and

slope values (Madden & Paul, 2009). Then, the data were aggregated using a multilevel meta-analytic model with corresponding random effects, and the population-average intercept and slope were estimated assuming a linear relationship between disease and yield (Lehner et al., 2016; Madden & Paul, 2009). The *lmer* function of the *lme4* package of R was used to estimate the mean effect based on the between-study and within-study variance and also to predict the study specific intercept (β_0) and slopes (β_1) coefficients as performed elsewhere (Duffeck et al., 2020; Lehner et al., 2016).

2.2.3 Analysis of moderator effects

Moderator variables that could account for at least part of the heterogeneity in the true effects were included as a fixed effect and expanded the model from a random to a mixed effects model (Madden & Paul, 2009). Moderator variables were all categorical, including disease pressure (low = ≤ 34.5 and high = $> 34.5\%$) and yield class (low = $\leq 3,352$ and high = $> 3,352$ kg/ha). Baseline severity was defined based on the median of the data in the nontreated check, while yield class was based on the median of the data. The among-study variance was estimated using maximum likelihood in both random and mixed models. The mean effect was estimated using weights based on the among-study variance and within study variance, the latter being held fixed for each study (Madden & Paul, 2011). Wald-type tests were performed to determine whether the inclusion of the covariates in the model significantly affected the coefficients at 5% probability.

2.2.4 *Relative yield loss estimation*

The scale of the estimated slopes was kg/ha per unitary increase in percent FLS severity. Because damage functions are commonly reported in relative terms (% increase in yield loss or % yield reduction), and for the purpose of comparison with other studies, the percentage yield loss was calculated by dividing the estimated slope ($\bar{\beta}_1$) (kg/ha/%) with the estimated intercept ($\bar{\beta}_0$) (kg/ha), both derived from the fit of the random-effects model, and multiplying by 100 (Dalla Lana et al., 2015; Lehner et al., 2016; Madden & Paul, 2009).

2.2.5 *Economic damage threshold*

Economic damage threshold (*EDT*) was calculated using the Mumford & Norton (1984) formula modified for plant diseases (Casa et al., 2009; Carmona et al., 2015) as described in Equation 3:

$$EDT = \left(\frac{F_c}{S_p * D_c} \right) * \lambda \quad (3)$$

where *EDT* = disease intensity (% in disease severity); F_c = cost of control (USD/ha); S_p = soybean price (USD/ton); D_c = damage coefficient (calculated based on the potential yield); λ = control efficacy of fungicide.

Using equation 3, response surfaces of *EDT* were obtained using the damage coefficient estimated and standardized to metric tons (0.0051) and three control efficacy values (= {25%, 50% 75%}). Each response surface was a function of 200 equally spaced values of F_c and S_p varying from 30 to 80 (USD/ha) and 200 to 700 (USD/ton),

respectively. The soybean prices were based on the average prices from 2017 to 2021 (United States Department of Agriculture-National Agricultural Statistics Service, 2022).

2.3 Results

2.3.1 Study-level variables

Disease severity values ranged from 0.22 to 86.2% (mean = 19.6%), and soybean yield ranged from 1,175 to 5,309 kg/ha (mean = 3,355 kg/ha) among the studies (Fig. 2.1). Linear regressions fitted at the study level showed that, in general, yield decreased as FLS severity increased (Fig. S2.1).

2.3.2 Yield-severity relationship

2.3.2.1 Correlation coefficients

Pearson's correlation coefficients (r) ranged from -0.94 to 0.08 (Fig. 2.2A). The estimated \bar{Z}_r by the random-effects model varied from -1.74 to 0.08 (Fig. 2.2B) and the mean was -0.69 (95% CI = -0.85 to -0.53), corresponding to a mean back-transformed correlation coefficient across all studies of -0.60 (95% CI = -0.69 to -0.49) (Table 2.1). The between-study variability (τ^2) estimated using maximum likelihood was high ($\tau^2 = 0.155$, SE = 0.0801) and confirmed by the significance of the Q test ($Q = 109.01$, d.f. = 38, $P = < 0.0001$), and high values of the I^2 (63.23%). In the mixed-model, only disease pressure covariate significantly affected \bar{Z}_r ($P = 0.0001$) while yield class was not affected (\bar{Z}_r ($P = 0.4888$)) (Table 2.1).

2.3.2.2 Regression coefficients

The intercept (β_0), which corresponds to the attainable yield in absence of disease, ranged from 1,282 to 5,119 kg/ha (mean = 3,741 kg/ha) (Fig. 2.3A-C). The distribution of β_1 ranged from -68.6 to 6.7 kg/ha, with negative values in nearly 90% of the studies (Fig. 2.3D). Estimates of the population-average intercept and slope were $\bar{\beta}_0 = 3,719.9$ kg/ha (SE = 161.5 kg/ha) and $\bar{\beta}_1 = -19.1$ kg/ha for each % severity (SE = 2.3), respectively (Table 2.2; Fig. 2.3B). Both parameters were statistically different from 0 ($P < 0.001$). The estimated best linear unbiased predictions (BLUPs) for the intercepts ($\bar{\beta}_0$) ranged from 1,386 to 5,160 kg/ha, and the interdecile (ID) was 2,488.2 kg/ha. The BLUPs for the slopes ($\bar{\beta}_1$) ranged from -39.8 to -4.5 kg/ha for each % severity, and the ID was -18 kg/ha for each % severity. The best model, defined based on a likelihood ratio test ($P < 0.001$) and lowest Akaike information criterion (7,607.92), included yield class as a covariate. A Wald-type test showed that effect of yield class on the slope ($\bar{\beta}_1$) did not differ from 0 ($P > 0.30$), suggesting that the slope was not affected by yield class. However, as expected, effect of yield class on the intercept ($\bar{\beta}_0$) differed from zero ($P < 0.001$). The study-specific parameters estimated for the high class were $\bar{\beta}_0 = 3,842.7$ kg/ha (SE = 141.6), $\bar{\beta}_1 = -16.2$ kg/ha for each % severity (SE = 2.4), and for the low class were $\bar{\beta}_0 = 3,455.1$ kg/ha (SE = 54.3) and $\bar{\beta}_1 = -14.3$ kg/ha for each % severity (SE = 1.8) (Table 2.2; Fig. 2.4).

2.3.3 *Model-predicted yield losses*

Based on the overall mean (95% CI) of the intercept (3,719.9 kg/ha) and slope (-19.1 kg/ha) estimated by the random-coefficients model, the overall relative damage coefficient was estimated to be 0.51% (95% CI = 0.36-0.69) (Table 2.2), indicating that a percent increase in FLS severity would result in a 0.51% yield reduction. The relative damage coefficient estimated for each of the yield classes was 0.42% (95% CI = 0.26-0.60) for high yield and 0.41% (95% CI = 0.13-0.76) for low yield (Table 6).

2.3.4 *Economic damage threshold*

In general, the economic damage thresholds (*EDT*) were affected by a range of scenarios of costs and soybean prices, and ranged from 2.1 to 58.8%. For the lower control efficacy (25%), *EDT* values were between 2.1 and 19.6% (mean = 6.7%) (Fig. 2.5). As the control efficacy increased, *EDT* values also increased. For instance, the *EDT* values for the intermediary control efficacy (50%) ranged from 4.2 to 39.2% (mean = 13.5%) (Fig. 2.5). Higher *EDT* values were estimated for the higher efficacy used here (75%) and ranged from 6.3 to 58.8% (mean = 20.2%) (Fig. 2.5).

2.4 Discussion

Our modeling of yield losses confirms that FLS is an important yield-limiting disease of soybean in the United States. This is the first study where numerous site-years of data were analyzed using meta-analytic methods and found that FLS foliar symptoms and

yield were negatively correlated. A significant negative slope obtained through random effects meta-analytic models confirmed the negative linear relationship between FLS severity and soybean yield. From our study, we calculated that for each unit increase in disease severity, yield declined 0.51% or, in other words, the average reduction of the attainable yield is expected to be 51% when there is maximum observed disease (FLS severity = 100%). An example of using this relationship is as follows, a field with an expected yield of 4,000 kg/ha (intercept) and an FLS severity of 10% could experience a yield reduction greater than 200 kg/ha.

Our meta-analytic estimate of yield (population-average intercept) was lower (3,719.91 kg/ha) compared to the estimates for soybean in the United States for another meta-analysis study (4,130 kg/ha) that evaluated the damage coefficient for sudden death syndrome (caused by *Fusarium virguliforme*) over 52 uniform field experiments conducted from 2013 to 2017 (Kandel et al., 2020). However, the slope obtained in the latter study (-21 kg/ha) was very similar to the slope obtained in this study (-19 kg/ha) and, the damage coefficient was the same for frogeye leaf spot and sudden death syndrome (0.51%) (Kandel et al., 2020). Similar negative relationships between disease severity-yield and damage coefficients have been estimated in Brazil for other soybean diseases with the same meta-analytic approach, including for white mold (caused by *Sclerotinia sclerotiorum*) (0.49%), target spot (caused by *Corynespora cassiicola*) (0.48%) and soybean rust (caused by *Phakopsora pachyrhizi*) (0.60%) (Dalla Lana et al., 2015; Edwards Molina et al., 2019; Lehner et al., 2016). Nonetheless, the damage coefficient reported here for FLS was lower comparing to the findings reported in individual studies conducted in the mid-western U. S., where the mean estimated yield

reduction caused by white mold was of 0.63% (Chun et al., 1987; Hoffman et al., 1998; Yang et al., 1999).

We also estimated economic damage thresholds for a range of soybean prices and control costs taking into account three different fungicide efficacies representing low (25%), moderate (50%) and high (75%) disease control. As expected, the threshold values increased as the control efficacy also increased and were affected by different crop prices and fungicide simulated costs. The economic damage thresholds should never be exceeded throughout the crop season (Carmona et al., 2015). Currently in the U.S., fungicide products available for managing FLS may contain one or more active ingredients from different fungicide classes, which include demethylation inhibitors (DMI), quinone outside inhibitors (QoI), methyl benzimidazole carbamates (MBC) and succinate dehydrogenase inhibitors (SDHI) (Mengistu et al., 2018; Wise & Newman, 2015). It is important to highlight that the economic damage thresholds obtained in this study are just an example, calculated with the values previously indicated. These thresholds will vary depending on the potential grain yield, cost of fungicide application and soybean price.

In the analyses presented here, disease pressure influenced the correlation coefficients significantly. A stronger negative correlation between FLS severity and yield was observed for trials with more FLS than the trials with less disease. Similarly, Dalla Lana et al. (2015) found significant negative correlation between soybean rust severity and yield with highest estimated mean correlation reported for studies conducted under higher disease pressure. Although yield class did not affect the population-average slope in our study, intercept values differed between trials representing a low or a high yield.

The fact that slope was not influenced by the yield classes means that fields with high or low yields will experience yield loss at a similar rate of FLS increase. Similar effects of yield class on only intercepts and not slopes were obtained in a previous study that summarized the relationship between Fusarium head blight (caused by *Fusarium graminearum*) and wheat (*Triticum aestivum*) yield over 37 studies conducted during nine years in Brazil (Duffeck et al., 2020).

The overall mean damage caused by FLS on soybean yield was estimated, and variables that explained a portion of the variability in the disease-yield relationship was identified by using multilevel and random-effects meta-analytic models following approaches previously used to address related questions in plant pathology (Dalla Lana et al., 2015; Duffeck et al., 2020; Edwards Molina et al., 2019; Kandel et al., 2020; Lehner et al., 2016; Madden & Paul, 2009). However, a significant portion of the variability remained unexplained and might be related to unknown factors or factors inherent in the study design. The disease-yield relationship was examined in trials that used several commercial fungicides with different effects on the disease and yield. However, if only specific treatments had been selected from a single trial, fewer disease-yield pairs would be left to model the relationship than using the whole set of fungicides evaluated in the primary studies.

In summary, there was a significant negative linear relationship between soybean yield and FLS severity, and intercept and slopes were highly variable. The damage coefficient estimated was determined based on a large dataset from fungicide trials conducted over 10 years under a range of conditions. As such, these models may provide more general estimations of yield loss based on FLS severity, and the large variability

encountered may preclude accurate site-specific prediction of actual yield due to FLS. Yet, results from this study may provide useful information for regional risk assessment of potential yield loss if FLS severity is measured on site. For instance, as long as damage functions are available, they can be incorporated in interactive web apps for risk assessment by simulating different scenarios of disease pressure, potential yields and fungicide efficacies (Alves et al., 2021).

2.5 Tables

Table 2.1. Estimates, related statistics, and heterogeneity measures of the transformed (\bar{Z}_r) and back-transformed Pearson (\bar{r}) correlation coefficients for the relationship between FLS severity and soybean yield based on a separate random-effects model (overall) and, for each level of moderator variables included in separate mixed-effects meta-analytic models.

| Effect-size | Statistics ^a | | | | | | Heterogeneity ^b | |
|--|-------------------------|----------|--------|-----------------------|-----------------------|----------------|----------------------------|---------------------------|
| | <i>k</i> ^c | Estimate | SE | <i>CI_L</i> | <i>CI_U</i> | <i>P value</i> | τ^2 | <i>I</i> ² (%) |
| \bar{Z}_r | 39 | -0.6954 | 0.0801 | -0.8525 | -0.5384 | <0.0001 | 0.1556 | 63.23 |
| \bar{r} | 39 | -0.6015 | | -0.6924 | -0.4918 | ... | ... | ... |
| Moderator effect on Fisher's \bar{Z}_r | | | | | | | | |
| Disease baseline | ... | ... | ... | ... | ... | ... | 0.0930 | 50.51 |
| High | 21 | -0.9263 | 0.0936 | -1.1097 | -0.7429 | <0.0001 | ... | ... |
| Low | 18 | -0.4237 | 0.1799 | -0.8771 | 0.0297 | 0.0003 | ... | ... |
| Yield class | ... | ... | ... | ... | ... | ... | 0.1528 | 62.70 |
| High | 19 | -0.7288 | 0.0931 | -0.9113 | -0.5463 | <0.0001 | ... | ... |
| Low | 20 | -0.6043 | 0.1601 | -0.6832 | -0.0692 | 0.4888 | ... | ... |

^a Mean estimate, standard error (SE), lower (*CI_L*) and upper (*CI_U*) limits of the 95% confidence interval.

^b Between-studies variance estimates (τ^2) and *I*² statistic.

^c Total number of studies used in each analysis.

Table 2.2. Estimates, related statistics, and heterogeneity measures of the linear regression intercept ($\bar{\beta}_0$) and slope ($\bar{\beta}_1$) for the relationship between FLS severity and soybean yield based on a separate random-effects model (overall) and, for each level of moderator variables included in separate mixed-effects meta-analytic models.

| | Estimated intercept ($\bar{\beta}_0$) | | | | Estimated slope ($\bar{\beta}_1$) | | | |
|---|---|-----------------------|----------|----------|-------------------------------------|-----------------------|----------|----------|
| | k^a | Estimate ^b | CI_L^b | CI_U^b | k^a | Estimate ^b | CI_L^b | CI_U^b |
| Overall | 39 | 3,719.9 | 3,403.2 | 4,036.5 | 39 | -19.0 | -23.5 | -14.5 |
| Moderator effect | | | | | | | | |
| Disease baseline ($P = 0.1806$) | | | | | | | | |
| High | 21 | 3,983.0 | 3,554.4 | 4,413.4 | 21 | -18.7 | -24.0 | -13.4 |
| Low | 18 | 3,337.8 | 2,372.2 | 4,343.7 | 18 | -20.9 | -37.5 | -4.3 |
| Yield class ($P < 0.0001$) | | | | | | | | |
| High | 19 | 3,842.6 | 3,557.1 | 4,126.2 | 19 | -16.2 | -21.4 | -10.8 |
| Low | 20 | 3,455.1 | 3,060.1 | 3,847.6 | 20 | -14.3 | -23.2 | -5.3 |

^a Total number of studies used in each analysis.

^b Mean estimate, lower (CI_L) and upper (CI_U) limits of the 95% confidence interval.

2.6 Figures

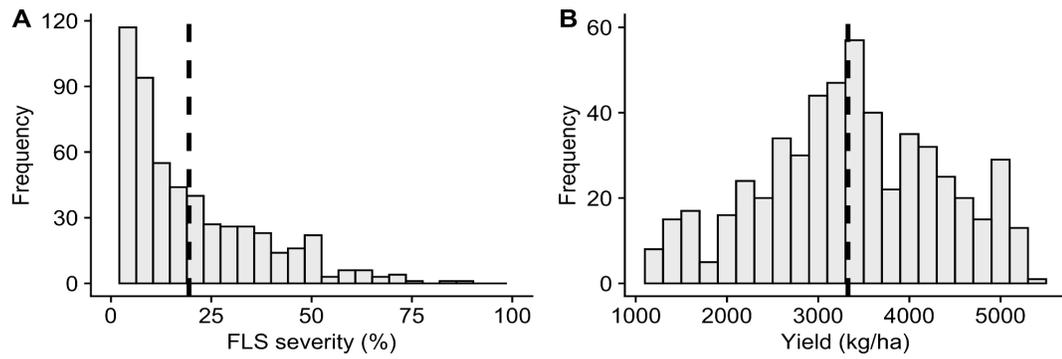


Fig. 2.1. Histograms for the distribution of FLS severity (**A**) and soybean yield (**B**) raw data. The vertical dashed thick lines represent the means of the respective variable.

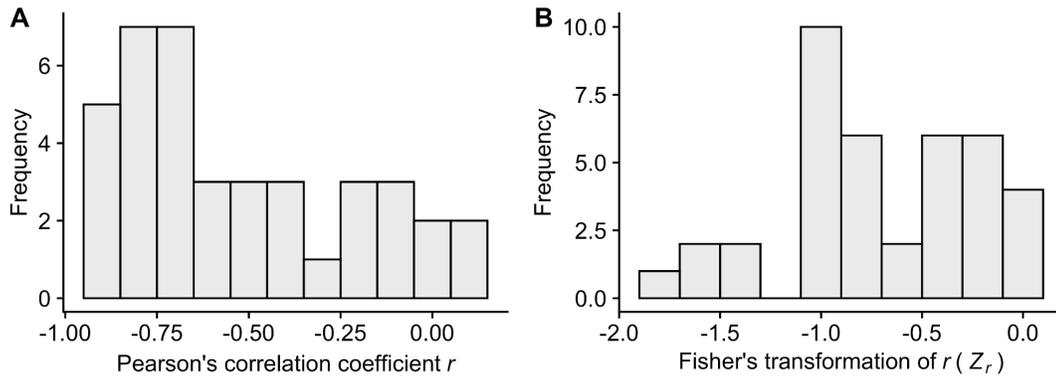


Fig. 2.2. Frequency of the Pearson's correlation coefficient (**A**) and their respective Fisher's transformation of r (Z_r) (**B**) between FLS severity and soybean yield in 39 field trials conducted across eight states over 10 years (2012 to 2021).

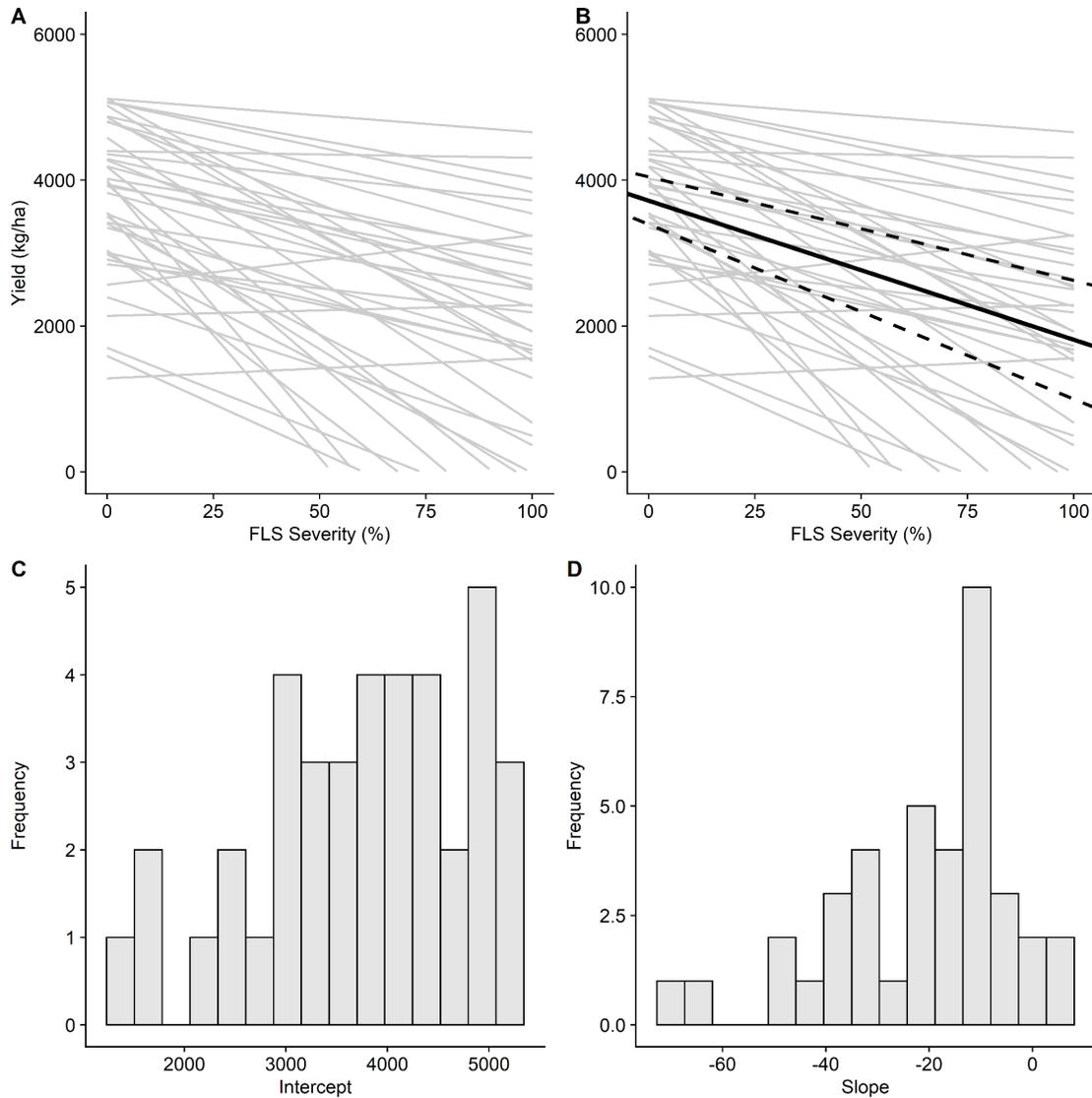


Fig. 2.3. Study-specific prediction regression lines (gray line) of a simple linear model fit (A); and study-specific prediction lines (gray line) and population-average predictions (black solid line) of yield and respective 95% confidence interval (dashed black line) (B). Frequency of the linear regression coefficients: intercepts (β_0) (C) and slopes (β_1) (D) obtained from the prediction regression lines between FLS severity and soybean yield in 39 field trials conducted across eight states over 10 years (2012 to 2021).

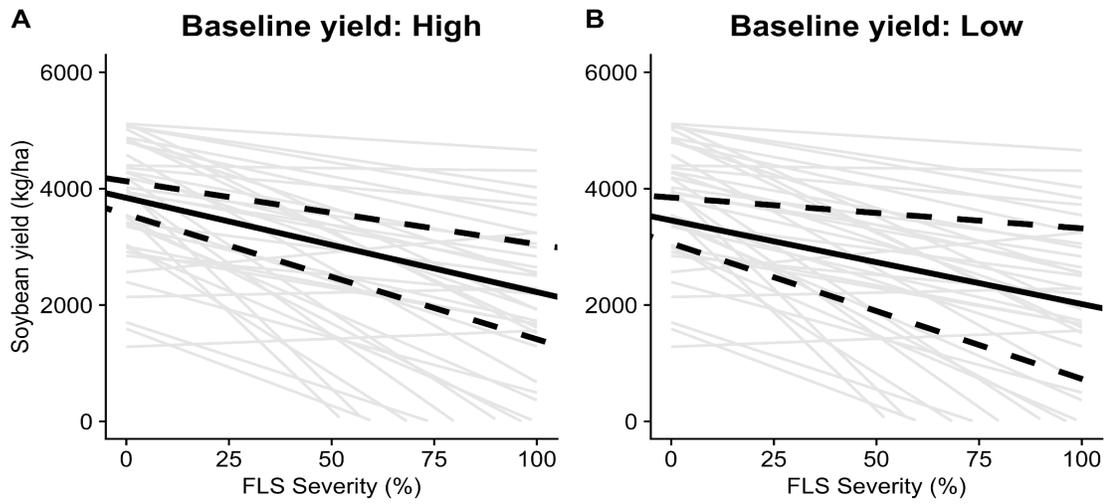


Fig. 2.4. Results for the fit of a random-coefficient model to absolute soybean yield (kg/ha) and FLS severity (%) with the population-average predictions (thick solid black) and respective 95% confidence interval (thick dashed black) for high (A) and low yield (B). Yield class was determined by the median of yield data (3,352 kg/ha).

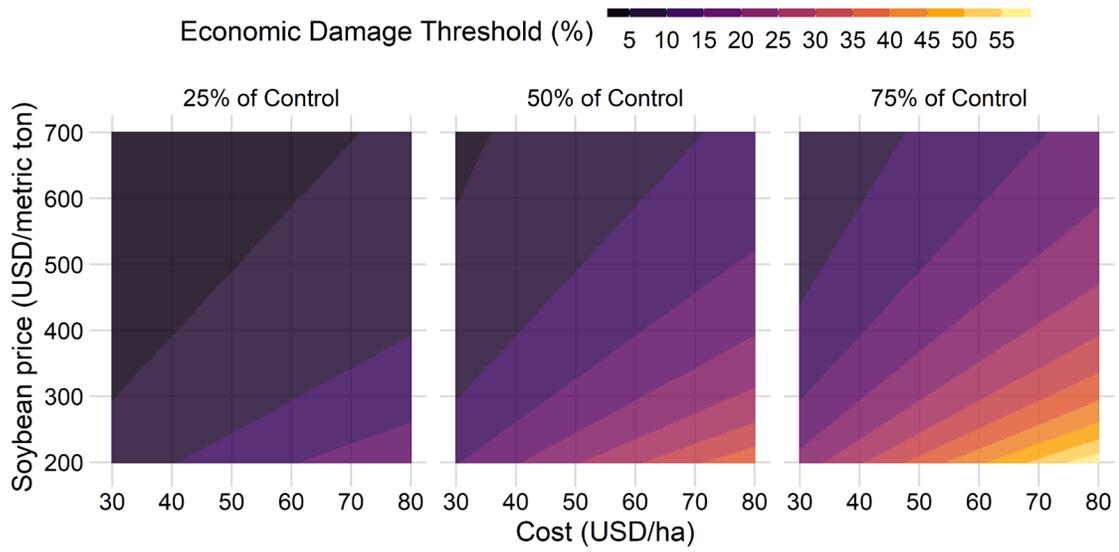


Fig. 2.5. Response curves of economic damage thresholds obtained using the damage coefficient estimated and three control efficacy values (25%, 50% and 75%) for a range of soybean prices and control costs.

2.7 SUPPLEMENTARY MATERIAL

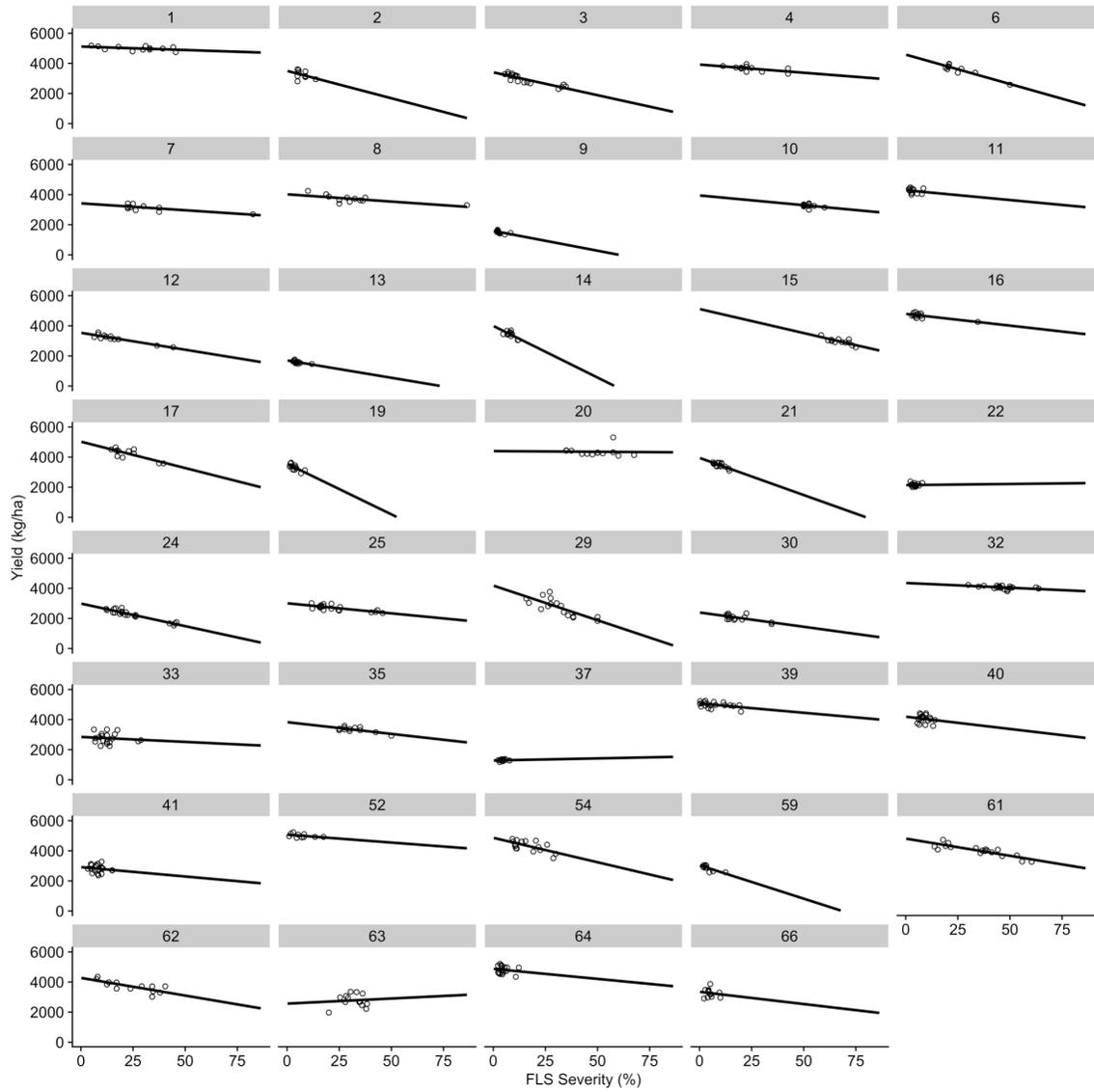


Fig. S2.1. Relationship between FLS severity (%) and soybean yield (kg/ha), for each one of the 39 studies included in this analysis.

CHAPTER 3. EFFICACY AND PROFITABILITY OF FUNGICIDES FOR MANAGEMENT OF FROGEYE LEAF SPOT ON SOYBEAN IN THE UNITED STATES: A 10-YEAR QUANTITATIVE SUMMARY

Abstract

Frogeye leaf spot (FLS), caused by *Cercospora sojina*, is an economically important disease of soybean in the United States. Data from 66 uniform fungicide trials (UFTs) conducted from 2012 to 2021, across eight states (Alabama, Arkansas, Illinois, Iowa, Kentucky, Louisiana, Mississippi, and Tennessee), were gathered and analyzed to determine the efficacy and profitability of the following fungicides applied at the beginning pod developmental stage (R3): azoxystrobin + difenoconazole (AZOX + DIFE), difenoconazole + pydiflumetofen (DIFE + PYDI), pyraclostrobin (PYRA), pyraclostrobin + fluxapyroxad + propiconazole (PYRA + FLUX + PROP), tetraconazole (TTRA), thiophanate-methyl (TMET), thiophanate-methyl + tebuconazole (TMET + TEBU), and trifloxystrobin + prothioconazole (TFLX + PROT). A network meta-analytic model was fitted to the log of the means of FLS severity data and to the non-transformed mean yield for each treatment, including the nontreated. The percent reduction in disease severity (%) and the yield response (kg/ha) relative to the nontreated was the lowest for PYRA (11%; 136 kg/ha) and the greatest for DIFE+PYDI (57%; 441 kg/ha). A significant decline in efficacy over time was detected for PYRA (18 percentage points [p.p.]), TTRA (27 p.p.), AZOX + DIFE (18 p.p.) and TMET + TEBU (19 p.p.), by using year as a continuous covariate in the model. Finally, probabilities of breaking even were the greatest (>65%) for the most effective fungicide DIFE+PYDI, and the lowest (<55%) for PYRA. Results of this meta-analysis may be useful to support fungicide application decisions.

Keywords: *Cercospora sojina*, chemical control, management, severity

3.1 Introduction

Frogeye leaf spot (FLS), caused by *Cercospora sojina*, is a yield-reducing disease of soybean (*Glycine max*) (Camera et al., 2018; Carmona et al., 2015; Mengistu et al., 2014; 2018). The disease was first reported in Japan in 1915, in the United States in 1924, and in Brazil in 1971 (Melchers, 1925; Veiga & Kimati, 1974). Lesions caused by FLS appear on leaves, pods, and stems, and are most prevalent during important reproductive developmental stages generally between beginning pod and beginning seed developmental stages (R3 and R5 stages, respectively) (Fehr et al., 1971; Wise & Newman, 2015). Leaf symptoms include small, dark lesions that develop into light gray to tan circular to irregular-shaped spots surrounded by a narrow, purple-brown margin (Phillips, 1999). Epidemics of FLS have been reported to cause soybean yield losses up to 60% (Dashiell & Akem, 1994). In the U.S. and Ontario, Canada, the estimated average annual soybean yield losses caused by FLS, from 2010 to 2019, ranged from 101,467 to 1,453,225 metric tons (Allen et al., 2017; Bradley et al., 2021). In Argentina, losses due to FLS during the 2009/2010 crop season were estimated to be approximately 2 billion U. S. dollars (Sepulcri et al., 2015). In Brazil, the occurrence of FLS is part of a complex of late-season diseases caused by *Cercospora kikuchii*, *Septoria glycines*, and *Colletotrichum truncatum*, and up to 30% yield losses have been reported (Balardin, 2002).

The disease is best managed by integrating multiple tactics such as planting certified pathogen-free seed of resistant cultivars, applying foliar fungicides, and rotating to non-host crops (Wise & Newman, 2015). Planting resistant cultivars, based on three single genes (*Rcs1*, *Rcs2*, and *Rcs3*), can be an effective measure for managing the

disease (Mian et al., 2009). However, *C. soja* has a high evolutionary potential and high genetic diversity, allowing it to rapidly overcome host genetic resistance through recombination (Gu et al., 2020; Kim et al., 2013).

Nevertheless, when resistant cultivars are not available, foliar fungicide applications can effectively manage FLS (Mengistu et al., 2014, 2018). Currently in the U.S., fungicide products available for managing FLS may contain one or more active ingredients from different fungicide classes, which include demethylation inhibitors (DMI), methyl benzimidazole carbamate (MBC), quinone outside inhibitors (QoI) and succinate dehydrogenase inhibitors (SDHI) (Crop Protection Network, 2022). Hence, several options are available for farmers and the decision of which fungicide to incorporate in a fungicide program should take into account current information on their efficacy and yield response. Continuous evaluation of fungicide performance is essential to help monitor for the development of fungicide resistance, and even more critical when single active ingredients, classified as medium or high risk of resistance development (Fungicide Resistance Action Committee, 2020), are applied alone across large areas and during consecutive crop seasons. Therefore, temporal changes, such as decline in performance over time, are dependent on which chemical is used and how they are deployed (space and time); and, the capability of the pathogen to adapt and build resistant populations (Hollomon, 2015).

In fact, *C. soja* isolates with resistance to QoIs, due to the presence of the G143A base pair substitution in the *cytochrome b* gene, have been detected across more than 20 soybean-producing states in the U.S. (Harrelson et al., 2021; Mathew et al., 2019; Neves et al., 2020; 2021; 2022; Standish et al., 2015; Zeng et al., 2015; Zhang et al.,

2012a; 2018; Zhou & Mehl, 2020). Since the initial confirmation in 2010 (Zhang et al., 2012b), QoI-resistant populations of *C. soja* in the U.S. have become widespread, which have forced growers to rely more on additional fungicide classes, applied alone or as premix fungicide products that contain more than one active ingredient. DMI and SDHI fungicides are classified as medium risk, while MBC is classified as a high risk for fungicide resistance development (Fungicide Resistance Action Committee, 2020). Thus far, *C. soja* isolates resistant to DMI and MBC fungicides have not been reported in the U.S. Zhang et al. (2021) evaluated the sensitivity of *C. soja* isolates to the DMI fungicides, flutriafol and tetraconazole, and the MBC fungicide, thiophanate methyl, and reported no shift towards reduced sensitivity to those fungicides in isolates collected from 2007 to 2012 compared to isolates collected prior to 2001. Even so, continued monitoring of *C. soja* population sensitivity to QoI, DMI and MBC fungicides is critical to support decision making in selecting fungicides for optimizing FLS management.

To help define the best options and the economic benefits from using fungicides to manage FLS, multi-state field trials have been established in the U.S. However, the data generated from those uniform trials have not been fully explored in a combined framework. Consequently, by combining data across multiple seasons and analyzing it, focusing on not only estimating the means, but also the uncertainty and factors explaining variation among the effects, may provide additional insights into the disease management strategy. Meta-analysis, a statistical technique that combines results from studies following defined criteria, has been used in plant pathology to summarize the effect of treatments, mainly fungicides, on plant disease management (Ascari et al., 2021; Barro et al., 2019; 2021a; 2021b; Dalla Lana et al., 2018; Machado et al., 2017; 2021; Paul et al.,

2008; 2010). Meta-analytic models also provide a way to test and quantify reduced efficacy and yield response over time by including the effect of year as a covariate (Barro et al., 2021b; Dalla Lana et al., 2018; Madden et al., 2016).

In the current research, we gathered FLS severity and soybean yield data from uniform field trials (UFTs). The dataset spanned 10 years (2012 to 2021) of experiments conducted across eight states in the United States. Our objectives were to: i) estimate fungicide efficacy on FLS control and yield response of soybean to different fungicides over time; ii) determine if heterogeneity in treatment effects could be explained by baseline levels of disease and yield; and iii) calculate the profitability of fungicides using the meta-analytic estimates of yield response.

3.2 Materials and Methods

3.2.1 *Data source and criteria for fungicide selection*

Data were obtained from UFTs conducted during ten crop seasons (2012 - 2021). During this period, 66 independent trials were conducted across eight states in the U.S. (Alabama [AL], Arkansas [AR], Illinois [IL], Iowa [IA], Kentucky [KY], Louisiana [LA], Mississippi [MS] and Tennessee [TN]) (Fig. 3.1). The UFTs were conducted using a standardized protocol (same experimental design and a common set of treatments). Each trial also utilized a susceptible cultivar that was regionally adapted for each location. Briefly, plot size varied slightly across the locations but each plot was 4 rows wide (38, 76 or 101 cm inter-row spacing) and at least 6 m long. Plots were arranged in a randomized complete block design, with four replications. Fungicide treatments were applied at the beginning pod developmental stage (R3). Backpack sprayers pressurized by

CO₂ (276 kPa) and calibrated to deliver volumes between 141 and 200 liters/ha, were used to apply the treatments. Fungicide applications included several widely used commercially available fungicide products. To be included in the analysis, a fungicide treatment had to be tested in at least 25 trials conducted in at least four years and compared with a nontreated control treatment in the same trial. Eight treatments met the criteria, including the stand-alone QoI pyraclostrobin (PYRA; 0.2 kg active ingredient [a.i.]/ha; Headline, BASF Corporation, Research Triangle Park, NC), the stand-alone DMI tetraconazole (TTRA; 0.06 kg a.i./ha; Domark, Gowan Company, Yuma, AZ), the stand-alone MBC thiophanate-methyl (TMET; 0.8 kg a.i./ha; Topsin, United Phosphorous Inc., King of Prussia, PA), and mixtures of fungicide products that included numerous active ingredient combinations which include a QoI + DMI as azoxystrobin + difenoconazole (AZOX+DIFE; 0.1 kg a.i./ha of AZOX and 0.05 kg a.i./ha of DIFE; Quadris Top, Syngenta Crop Protection, Inc., Greensboro, NC), and trifloxystrobin + prothioconazole (TFLX+PROT; 0.1 kg a.i./ha of TFLX and 0.09 kg a.i./ha of PROT; Stratego YLD + Proline, Bayer CropScience, St. Louis, MO), a DMI + SDHI as difenoconazole + pydiflumetofen (DIFE+PYDI; 0.1 kg a.i./ha of DIFE and 0.07 kg a.i./ha of PYDI; Miravis Top, Syngenta Crop Protection, Inc.), an MBC + DMI as thiophanate-methyl + tebuconazole (TMET+TEBU; 0.6 kg a.i./ha of TMET and 0.1 kg a.i./ha of TEBU; Topsin XTR, United Phosphorous Inc.), and a QoI + SDHI + DMI as pyraclostrobin + fluxapyroxad + propiconazole (PYRA+FLUX+PROP; 0.09 kg a.i./ha of PYRA, 0.04 kg a.i./ha of FLUX and 0.1 kg a.i./ha of PROP; Priaxor + Tilt, BASF Corporation and Syngenta Crop Protection, Inc., respectively). All locations also included a nontreated set of plots to serve as the control. Fungicide application rates as well as the

total number of locations where the products were applied across the eight states are included in Table 3.1. All nutrient, weed, and insect management practices followed regional suggestions. After treatment selection, FLS severity data were available in 65 trials, and soybean yield data were available in 66 trials.

Disease severity was visually assessed as percent leaf area exhibiting FLS symptoms at the plot level, 4 weeks post-fungicide application (approximately at the full seed developmental stage [R6]) focusing specifically on the upper third of the canopy. The aid of a standard leaf area diagram as a reference was used while rating (Price et al., 2016). Yield was obtained by harvesting the middle two rows of each plot after full maturity (R8 developmental stage) with a small plot combine equipped with a grain gauge that measured total harvested grain weight and seed moisture. Soybean yield was calculated as kilograms/hectare at 13% moisture.

3.2.2 Network meta-analysis estimates and inconsistency

Although the data were available at the plot level for all treatments, including the nontreated check, for each variable of interest (FLS severity and soybean yield), the means at the trial level were used in the meta-analysis (Madden et al., 2016). Given the statistical properties of the data (Fig. S3.1), means of FLS severity were log-transformed, while no transformation was required to obtain the mean absolute difference in yield. Hence, a two-way unconditional linear mixed model was fitted directly to the treatment means (absolute or log-transformed) to further obtain control efficacy and yield response. The model can be written as

$$Y_i \sim N(\boldsymbol{\mu}, \boldsymbol{\Sigma} + \boldsymbol{S}_i) \quad (1)$$

where Y_i is the vector of L (log of the means of FLS severity) or absolute yield for the eight treatments plus the nontreated check for the i th study, $\boldsymbol{\mu}$ is a vector representing the mean of Y_i across all studies, $\boldsymbol{\Sigma}$ is a 9×9 between-study variance-covariance matrix (for the eight treatments plus the nontreated check), and \boldsymbol{S}_i is a within-study variance-covariance matrix for the i th study. N indicates a multivariate normal distribution.

The within-study variability (sampling variance) of L and D was calculated from the mean square error (MSE) obtained from a linear model fitted to raw data at each individual trial, as described in previous studies (Barro et al., 2021b; Machado et al., 2017). The within-study variability is required to weight studies based on the inverse function of the sampling variance (Paul et al., 2008). The *rma.mv* function of *metafor* package (Viechtbauer, 2010) of R (R Core Team, 2020) was used to fit maximum likelihood estimation models to the data.

Estimates of percent control (\bar{C}) were calculated by taking the differences of mean log of the response ratio (\bar{L}_{SEV}) for each fungicide treatment ($\hat{\mu}_{Treat}$) relative to the nontreated check ($\hat{\mu}_{Check}$) estimated as $\bar{L}_{SEV} = \hat{\mu}_{Treat} - \hat{\mu}_{Check}$ (Paul et al., 2008). The \bar{C} values and their 95% confidence intervals (CIs) were obtained by back-transforming \bar{L}_{SEV} and the respective upper and lower limits of their 95% CIs as described in Equation 2.

$$\bar{C} = (1 - \exp(L_{SEV})) \times 100 \quad (2)$$

The yield difference (\bar{D}) was calculated directly after model fitting by subtracting estimated means of fungicide treatment and nontreated control (Barro et al., 2021b; Madden et al., 2016).

To test for network inconsistency, we fitted a factorial-type linear model to determine the significance of the *treatment* x *design* interaction, evaluated based on the Wald test statistic (Higgins et al., 2012). The null hypothesis suggests that the network is consistent (Madden et al., 2016; Piepho, 2014). Sixteen different designs (here design refers to the set of treatments in the trial) were found in the trials reporting FLS severity and 19 designs were found for yield response (Table S3.1).

3.2.3 Analysis of moderator effects

The network model (Equation 1) was expanded to include both categorical or continuous moderator variables that could explain, at least, a portion of the heterogeneity of the effects across trials (Madden et al., 2016). The expanded model (Paul et al., 2010) is given by

$$Y_i \sim N(\mu + \delta_i, \Sigma + S_i) \quad (3)$$

where δ_i is the vector representing the moderator variable effect for the i th study and all other terms are as defined previously.

Categorical variables were included as covariates in the model. First, the FLS severity baseline was divided into two groups, representing low (<14% FLS severity) and high (\geq 14% FLS severity) disease scenarios based on the median severity in the nontreated control. Second, the baseline yield was defined as low (<3,200 kg/ha) or high (\geq 3,200 kg/ha) based on the median yield in the nontreated control.

Additionally, to evaluate fungicide efficacy and yield response over time, we tested year as a continuous moderator variable. For that, only the treatments which were evaluated in at least five out of six consecutive years from 2015 to 2020 across all states were selected. Years 2015 to 2020 were transformed to integers (0 to 6) prior to fitting the model (Barro et al., 2021b; Dalla Lana et al., 2018). Differences in regression intercept and slopes obtained from the relationships between the years and log severity (\bar{L}_{SEV}) and, or absolute yield (\bar{D}) between each fungicide treatment and the nontreated check were used to predict \bar{L}_{SEV} and \bar{D} as well as the upper and lower limits of their 95% CI (Dalla Lana et al., 2018). Predicted percent control (\bar{C}) was obtained by back-transforming \bar{L}_{SEV} and the respective upper and lower limits of their 95% CIs as explained previously (Equation 2).

The moderator variables were included in the model and tested using a Wald-type chi-square test to determine if the moderator variables directly affected the differences in logs of FLS severity and the non-transformed yield values (Paul et al., 2008).

3.2.4 *Economic analysis*

With the estimates of mean yield difference (\bar{D}) between fungicide-treated and nontreated control plots for each class of disease pressure, and the respective between-study variance ($\hat{\tau}$) obtained from the meta-analysis, we calculated the break even probability (P) of the fungicide plus application costs (F_C), as described in Equation 4, used in previous studies (Barro et al., 2019; Machado et al., 2017, 2021; Paul et al., 2008):

$$P = \Phi [\bar{D} - (F_C/S_p)/\sqrt{\hat{\tau}}] \quad (4)$$

where Φ is the cumulative standard-normal function and S_p is the soybean price.

Based on the meta-analysis results, three representative treatments were selected for comparison: the best performing premix (DIFE + PYDI), was compared with an intermediate (TTRA) and the less effective treatment (PYRA). For each fungicide treatment in each disease pressure class, 25 combinations were simulated (five $S_p \times$ five F_C), totaling 125 scenarios. The soybean prices were based on the average prices from 2017 to 2021 (United States Department of Agriculture-National Agricultural Statistics Service, 2022). The averaged costs (fungicide + ground application cost of 21.00 USD/ha [Halich 2022]) for each fungicide treatment were: DIFE + PYDI = \$76.00/ha, TTRA = \$40.00/ha and PYRA = \$40.00/ha. The fungicide prices were obtained by contacting local crop protection retailers and calculating the mean price for each product when price

differences occurred across retailers. Tile plots of the probability classes of breaking even on fungicide costs were produced for each fungicide.

3.3 Results

3.3.1 *FLS severity and yield*

FLS severity in the nontreated control plots was greatest in Mississippi (43%) followed by Illinois (41%), Louisiana (31%), Kentucky (28%), Iowa, (13%), Tennessee (13%), Alabama (12%) and Arkansas (8%) (Fig. 3.1). Considerable variation in FLS severity and yield was recorded in the nontreated plots across crop seasons (Fig. 3.2A-C). Disease severity in the nontreated plots ranged from 0.7 to 86% (median 14%). Median FLS severities were greatest (55%) and lowest (6%) in the 2012 and 2018 crop seasons, respectively (Fig. 3.2A). Average annual yield of the nontreated control ranged from 1,175 to 6,616 kg/ha (median = 3,293 kg/ha) across the trials. The greatest median yield (3,981 kg/ha) was observed in 2019 and the lowest (2,214 kg/ha) in the 2014 crop season (Fig. 3.2C). As expected, decreased FLS severity and increased yield were observed in the fungicide treatments compared with the nontreated control (Fig. 3.2B-D).

3.3.2 *Meta-analytic estimates of control efficacy and yield response*

Overall estimates of percent control efficacy (\bar{C}), obtained from back-transforming differences of the estimates of log of severity (\bar{L}_{SEV}) between the fungicide-treated and nontreated plots, ranged from 11 to 57% across the treatments. Only DIFE + PYDI reduced FLS severity by at least 57% on average. The latter was not significantly

different from TMET + TEBU, AZOX + DIFE and TFLX + PROT, all with percent control greater than 50% and not differing statistically between them ($P > 0.05$). This latter group was followed by TMET (49%), TTRA (44%) and PYRA + FLUX + PROP (40%). All treatments were different from the least effective fungicide PYRA (11%) ($P < 0.0001$) (Table 3.2). The difference in percent control efficacy between the most and least effective fungicide was 46 percentage points. The Wald test determined that network consistency was significantly affected by the study design ($P = 0.0260$).

The mean estimates of yield difference (\bar{D}) between fungicide-treated and the nontreated plots ranged from 136 to 441 kg/ha among the fungicide treatments. Yield response values as high as above 380 kg/ha were estimated only for DIFE + PYDI (441 kg/ha) and AZOX + DIFE (381 kg/ha), not differing statistically between them ($P = 0.1235$). The latter did not differ statistically from TMET + TEBU (355 kg/ha), TMET (350 kg/ha) and TFLX + PROT (345 kg/ha) ($P > 0.26$). This latter group was followed by TTRA (305 kg/ha) and PYRA+FLUX+PROP (301 kg/ha) which were not statistically different ($P = 0.9011$). The least effective fungicide in protecting yield was PYRA (136 kg/ha), which was statistically different from all other fungicide treatments ($P < 0.0001$) (Table 3.3). The difference between the highest and lowest estimated yield means was 305 kg/ha. The Wald test for the treatment x design interaction showed that the network was inconsistent ($P < 0.001$).

In general, the pattern of the relationship between control efficacy and yield differences was consistent. As shown previously, the most effective in reducing disease severity and leading to the greatest mean yield response was the premix DIFE + PYDI.

Again, the least effective fungicide in reducing disease severity and protecting yield was PYRA (Fig. 3.3).

3.3.3 *Effect of year on control efficacy*

The increase in log response ratio for disease severity (\bar{L}_{SEV}) and consequently reduction in \bar{C} per unit time (characterizing a decline in efficacy) varied among fungicides and the slope was statistically different from zero ($P < 0.0001$) for the single active ingredients PYRA and TTRA, and the premixes AZOX + DIFE and TMET + TEBU (Table 3.4). TTRA showed the greatest relative reduction in percent control (27 percent points [p.p.]) from the first season (2015; 53%) compared to the last season (2020; 26%). For TMET + TEBU, a reduction of 19 p.p. in efficacy was observed between 2015 (58%) and 2020 (39%). AZOX + DIFE and PYRA had a reduction of 18 p.p. each. The other two fungicides showed a relatively stable efficacy over the years, including the single a.i. TMET (52 - 40%) and the triple mixture of PYRA + FLUX + PROP (40 - 30%) (Table 3.4; Fig. 3.4). No significant decline ($P = 0.4810$) in yield response was observed across the six fungicides evaluated during the six consecutive crop seasons (2015 to 2020) (Table 3.4).

3.3.4 *Effect of disease pressure on yield response*

The expanded model including the categorical interaction term (baseline severity) differed statistically from the simpler model based on the Wald test ($P < 0.05$), meaning

that severity in the nontreated check treatment explained at least a portion of the variability in yield response. In general, \bar{D} was greater in the high-disease than in the low disease scenarios, with significant differences ranging from 247 kg/ha (TTRA) to 448 kg/ha (TMET + TEBU) among treatments. The only treatment in which the difference in \bar{D} between disease pressure scenarios was not significant, was the less effective treatment PYRA (90 kg/ha) (Table 3.5; Fig. S3.2). Baseline yield effect was not significant based on the Wald test ($P = 0.8974$).

3.3.5 Economic analysis

In general, the probability of breaking even on the fungicide costs (P) was affected by a range of scenarios of costs and soybean prices, and ranged from 46 to 70%. A higher number of favorable scenarios ($P > 55\%$), were identified for the moderate effective treatment (TTRA); and best scenarios ($P > 65\%$) for the most effective fungicide (DIFE + PYDI), both in high disease pressure ($\geq 14\%$ FLS severity). However, in low disease pressure ($< 14\%$ FLS severity), probabilities were between 45 and 55% in most scenarios, regardless of the fungicide treatment (Fig. 3.5). For PYRA, the less effective fungicide, probabilities were between 45 and 55% in most scenarios, regardless of the disease pressure class (Fig. 3.5).

3.4 Discussion

The present study updates critical information on the management of FLS on soybean with fungicides in the U.S. during 10 growing seasons (2012 - 2021) across eight

soybean-producing states. On average, we observed the best performing fungicide to be the premix DIFE + PYDI, and the poorest performing fungicide to be PYRA. A statistically significant decline in performance was detected for two dual premixes (AZOX + DIFE and TMET + TEBU) and two single active ingredients (PYRA and TTRA). Finally, we observed generally greater yield in trials with conditions favorable for severe epidemics (severity in the nontreated greater than 14%).

Our results agree with a previous study that evaluated FLS control by applying six different fungicides across three growing seasons (2014-2016) in Tennessee (Mengistu et al., 2018). The authors reported an overall greater control efficacy for the treatments AZOX + DIFE (71%) and TMET (73%) compared to PYRA (27%), which was the least effective treatment in controlling FLS. Accordingly, AZOX + DIFE (16%) and TMET (17%) were more effective in protecting yield compared to PYRA (10%) (Mengistu et al., 2018).

The reduced control efficacy and the decline over time reported here for PYRA is likely linked to reports of *C. sojae* populations resistant to QoIs across all states where trials were conducted (Standish et al., 2015; Zeng et al., 2015; Zhang et al., 2012a; 2012b; 2018). Similarly, a previous study, also using a meta-analytic approach, identified a decline for the performance of QoIs applied either as a single a.i. (azoxystrobin) and as a premix amended with cyproconazole against soybean rust (caused by *Phakopsora pachyrhizi*) across several locations in Brazil (Dalla Lana et al., 2018). In this case, the reduction of sensitivity to QoIs has been clearly associated with the occurrence of the

F129L substitution in the *Cytochrome b* gene, which was first reported in *P. pachyrhizi* isolates collected in 2012/13 (Klosowski et al., 2016).

The greatest significant reduction in control efficacy among the treatments from 2015 to 2020 was reported for TTRA (27 p.p.), indicating a possible shift of decreased *C. sojina* sensitivity to DMIs. However, a previous study reported no shift towards reduced sensitivity to TTRA in *C. sojina* isolates collected from 2007 to 2012 (Zhang et al., 2021). Even so, continued monitoring of *C. sojina* population sensitivity to DMIs is warranted after the results reported here. Additionally, resistance to DMI fungicides has been reported in another species of *Cercospora*, *C. beticola*, which causes Cercospora leaf spot in sugar beet (*Beta vulgaris*) (Bolton et al., 2012; Karaoglanidis et al., 2000; Secor et al., 2010). Although no significant reduction in control efficacy was detected for the single application of TMET, a difference of 13 p.p. was calculated between 2015 and 2020. On the other hand, a significant decline was reported for the premix TMET + TEBU, which raises the concern of fungicide resistance development for both DMIs and MBCs. Isolates of both *C. beticola* and *C. kikuchii*, another soybean pathogen, with resistance to MBC fungicides have previously been described in the U.S. (Campbell et al., 1998; Imazaki et al., 2006; Price et al., 2015). Moreover, isolates of *Cercospora* spp. resistant to the MBC group from soybean in Brazil were reported recently (de Mello et al., 2021).

In general, greater yield response was observed for the greater disease pressure scenarios which could be explained by the more evident effect of the fungicides among

the treated plots when compared to the nontreated. The increased levels of yield return during greater severity epidemics conditions agrees with previous studies on the effect of fungicides for additional diseases of soybean and other crops, suggesting a more likely benefit than when disease pressure is low to moderate (Barro et al., 2019, 2021b; Delaney et al., 2018; Molina et al., 2019;). Accordingly, more profitable scenarios (> 55%) were calculated for the most effective (DIFE + PYDI) and moderate (TTRA) fungicides under greater disease pressure. As expected, the less effective treatment PYRA reported the increased risk of not offsetting the costs. Pyraclostrobin is an active ingredient that belongs to the QoI class, which has previously been reported to induce physiological changes within the plant, such as: greater chlorophyll retention, increased water and nitrogen use efficiency, and delayed senescence, hence, increasing yield (Glaab & Kaiser, 1999; Kohle et al., 2002). However, in the current study, the yield responses by the single application of PYRA were not likely worth the fungicide costs which likely occurred as a result of QoI resistant *C. soja* populations present at each location.

Fungicide application timing and coverage are critical for optimal disease management. Akem (1995) evaluated applications of the fungicide benomyl at six different growth stages, starting from V3 (leaf at 4th node is unrolled) to R5 (beginning seed), to determine the effect of the fungicide timing on frogeye leaf spot severity and found that applications at R1 (beginning bloom) and R3 (beginning pod) significantly reduced disease severity. Additionally, achieving adequate fungicide coverage of leaves in a dense soybean canopy during its reproductive stages can present challenges (Bradley

et al., 2007; Viggers et al., 2022). Results from ten field trials conducted from 2017 to 2020 in Iowa by applying fluxapyroxad + pyraclostrobin at the R3 developmental stage using a traditional ground sprayer with an overhead spray boom and a ground sprayer with 360 undercover sprayers showed that the upper canopy received significantly more coverage through traditional spray application than the undercover (Viggers et al., 2022). Conversely, the undercover application sprayed a greater percentage of fungicide in the lower canopy than the traditional application method. However, no statistical difference for fungicide application methods was observed on FLS severity (%) and yield (kg/ha) for the traditional application (2.7%; 3,994 kg/ha) compared to the undercover application (3.2%; 3,896 kg/ha). That can be explained by the low disease severity in the nontreated, which ranged from 3.3% in the upper canopy to 2.7% in the middle canopy over all site-years, and did not differ from the fungicide treated plots (Viggers et al., 2022).

The use of FLS-resistant soybean cultivars can be more widely adopted to improve FLS management and to reduce the potential development of fungicide resistant fungal populations (Mian et al., 2009). However, the sole use of resistant cultivars as a management choice has the same limitations of the fungicide use, which is the potential selection of *C. sojae* races that are virulent against *Rcs* genes (Hollomon, 2015). Another strategy to improve FLS management and to reduce the risk of resistance development is to alternate modes of action and the use of premixes of single-site amended with multi-site fungicides (Fungicide Resistance Action Committee, 2020). However, Mengistu et

al. (2018) reported that the solo use of the multi-site chlorothalonil provided poor FLS control (30%), which can be linked to its nonsystemic action, and the fact that it washes off easily, hence, requiring multiple applications.

In conclusion, the results of our study provide critical information to support decision making procedures whereby the selection of a fungicide is needed when managing FLS in a susceptible cultivar. Decisions must take into account not only technical information such as control efficacy and yield return, but also profitability and strategies to mitigate fungicide resistance issues, seeking to preserve the lifespan of site-specific fungicides. The continuing evaluation of fungicides with multi-site across UFTs is essential and should be encouraged. The results obtained in this study can also guide the selection of fungicides to be tested in future trials.

3.5 Tables

Table 3.1. Fungicide treatments applied for controlling frogeye leaf spot (FLS) in 66 independent trials from 2012 to 2021 across eight states in the United States (AL, AR, IA, IL, KY, LA, MS and TN).

| FRAC ^a | Fungicide | Abbreviation | Trade name | Rate ^b | <i>n</i> ^c |
|-------------------|---|-----------------------|---------------------------|-------------------|-----------------------|
| - | nontreated | CHECK | - | - | - |
| 11 | pyraclostrobin | PYRA | Headline | 0.4 | 55 |
| 3 | tetraconazole | TTRA | Domark | 0.3 | 51 |
| 1 | thiophanate-methyl | TMET | Topsin | 1.5 | 52 |
| 11+3 | azoxystrobin+difenoconazole | AZOX+DIFE | Quadris Top | 0.5 | 47 |
| 11+3 | trifloxystrobin+prothioconazole | TFLX+PROT | Stratego YLD + Proline | 0.3+0.1 | 26 |
| 3+7 | difenoconazole+pydiflumetofen | DIFE+PYDI | Miravis Top | 1.0 | 25 |
| 1+3 | thiophanate-methyl+tebuconazole | TMET+TEBU | Topsin XTR | 1.5 | 48 |
| 11+7+3 | pyraclostrobin+fluxapyroxad+ propiconazole | PYRA+FLUX+PROP | Priaxor + Tilt | 0.3+0.3 | 37 |

^a Fungicide Resistance Action Committee code.

^b Rate (L/ha) for each fungicide.

^c Number of trials in which each fungicide was evaluated.

Table 3.2. Overall means and respective confidence intervals of log response ratio (\bar{L}_{SEV}) and calculated percent control (\bar{C}) of frogeye leaf spot (FLS) relative to nontreated provided by eight fungicides evaluated in 66 independent trials conducted across eight states in the United States (AL, AR, IA, IL, KY, LA, MS and TN) during 10 growing seasons (2012-2021).

| Fungicide ^a | <i>k</i> ^b | Effect Size | | | | | Efficacy (%) | | |
|------------------------|-----------------------|-----------------|-----------------------|------------------------------|------------------------------|----------------|--------------|------------------------------|------------------------------|
| | | \bar{L}_{SEV} | SE(\bar{L}_{SEV}) | CI _L ^c | CI _U ^c | <i>P</i> value | \bar{C} | CI _L ^c | CI _U ^c |
| DIFE + PYDI | 21 | -0.8507 | 0.0712 | -0.9903 | -0.7112 | <0.0001 | 57.28 | 50.89 | 62.85 |
| TMET + TEBU | 48 | -0.7498 | 0.0490 | -0.8459 | -0.6536 | <0.0001 | 52.75 | 47.98 | 57.08 |
| AZOX + DIFE | 48 | -0.7119 | 0.0489 | -0.8077 | -0.6161 | <0.0001 | 50.92 | 45.99 | 55.41 |
| TFLX + PROT | 28 | -0.7078 | 0.0618 | -0.8290 | -0.5867 | <0.0001 | 50.72 | 44.38 | 56.35 |
| TMET | 50 | -0.6896 | 0.0474 | -0.7825 | -0.5967 | <0.0001 | 49.82 | 44.93 | 54.27 |
| TTRA | 48 | -0.5872 | 0.0469 | -0.6790 | -0.4953 | <0.0001 | 44.40 | 39.06 | 49.28 |
| PYRA+FLUX+PROP | 35 | -0.5198 | 0.0530 | -0.6236 | -0.4159 | <0.0001 | 40.53 | 34.02 | 46.39 |
| PYRA | 54 | -0.1173 | 0.0424 | -0.2004 | -0.0342 | 0.0057 | 11.06 | 3.36 | 18.15 |

^a PYRA = pyraclostrobin; TTRA = tetraconazole; TMET = thiophanate-methyl; AZOX+DIFE = azoxystrobin+difenoconazole; TFLX + PROT = trifloxystrobin+prothioconazole; DIFE + PYDI = difenoconazole+pydiflumetofen; TMET + TEBU = thiophanate-methyl+tebuconazole; PYRA+FLUX+PROP = pyraclostrobin+fluxapyroxad+propiconazole.

^b Number of trials in which each fungicide was evaluated.

^c Upper (CI_U) and lower (CI_L) limits of the 95% confidence interval around \bar{L}_{SEV} and \bar{C} .

Table 3.3. Overall means and respective confidence intervals of unstandardized difference in soybean yield (\bar{D}) between fungicide-treated and nontreated plots for eight selected fungicide treatments evaluated in 66 independent trials conducted across eight states in the United States (AL, AR, IA, IL, KY, LA, MS and TN) during 10 growing seasons (2012-2021).

| Fungicide ^a | <i>k</i> ^b | Yield response (kg/ha) | | | | |
|------------------------|-----------------------|------------------------|-----------------|------------------------------|------------------------------|----------------|
| | | \bar{D} | SE(\bar{D}) | CI _L ^c | CI _U ^c | <i>P</i> value |
| DIFE + PYDI | 23 | 441.93 | 52.74 | 338.55 | 545.31 | <0.0001 |
| AZOX + DIFE | 47 | 381.04 | 46.10 | 290.68 | 471.40 | <0.0001 |
| TMET + TEBU | 48 | 355.77 | 53.24 | 251.42 | 460.12 | <0.0001 |
| TMET | 52 | 350.72 | 49.26 | 254.16 | 447.28 | <0.0001 |
| TFLX + PROT | 26 | 345.84 | 52.36 | 243.20 | 448.48 | <0.0001 |
| TTRA | 51 | 305.89 | 36.54 | 234.26 | 377.52 | <0.0001 |
| PYRA+FLUX+PROP | 37 | 301.75 | 44.07 | 215.36 | 388.13 | <0.0001 |
| PYRA | 55 | 136.87 | 27.46 | 83.03 | 190.71 | <0.0001 |

^a PYRA = pyraclostrobin; TTRA = tetraconazole; TMET = thiophanate-methyl; AZOX+DIFE = azoxystrobin+difenoconazole; TFLX + PROT = trifloxystrobin+prothioconazole; DIFE + PYDI = difenoconazole+pydiflumetofen; TMET + TEBU = thiophanate-methyl+tebuconazole; PYRA+FLUX+PROP = pyraclostrobin+fluxapyroxad+propiconazole.

^b Number of trials in which each fungicide was evaluated.

^c Upper (CI_U) and lower (CI_L) limits of the 95% confidence interval around \bar{D} .

Table 3.4. Regression parameters (intercept and slope) for the temporal change in log response ratio for FLS severity (\bar{L}_{SEV}) and absolute yield (\bar{D}) for each fungicide treatment relative to the nontreated from a meta-analytical model with year as a continuous moderator variable ($P < 0.05$).

| Fungicide ^a | Parameter | FLS Severity (log scale) | | | | Yield response (kg/ha) | | | |
|---------------------------|-----------|--------------------------|----------|----------|----------------|------------------------|----------|----------|----------------|
| | | \bar{L}_{SEV} | CI_L^b | CI_U^b | <i>P-value</i> | \bar{D} | CI_L^b | CI_U^b | <i>P-value</i> |
| AZOX + DIFE | Intercept | -0.8120 | -0.9736 | -0.6503 | <0.0001 | 391.13 | 251.55 | 530.71 | <0.0001 |
| | Slope | 0.0689 | 0.0051 | 0.1327 | 0.0342 | -8.36 | -65.17 | 48.45 | 0.7730 |
| PYRA | Intercept | -0.1986 | -0.3405 | -0.0568 | <0.0001 | 172.42 | 89.49 | 255.36 | <0.0001 |
| | Slope | 0.0466 | -0.0066 | 0.0997 | 0.0360 | -13.54 | -45.91 | 18.82 | 0.4122 |
| PYRA + FLUX + PROP | Intercept | -0.5656 | -0.7915 | -0.3397 | <0.0001 | 385.09 | 235.17 | 535.0 | <0.0001 |
| | Slope | 0.0423 | -0.0330 | 0.1176 | 0.2710 | -39.14 | -92.72 | 14.44 | 0.1522 |
| TMET | Intercept | -0.7426 | -0.9038 | -0.5815 | <0.0001 | 359.46 | 205.66 | 513.26 | <0.0001 |
| | Slope | 0.0452 | -0.0147 | 0.1050 | 0.1392 | -10.93 | -69.93 | 48.06 | 0.7164 |
| TMET + TEBU | Intercept | -0.8704 | -1.0331 | -0.7078 | <0.0001 | 391.64 | 235.62 | 547.66 | <0.0001 |
| | Slope | 0.0733 | 0.0088 | 0.1379 | 0.0259 | -26.26 | -88.40 | 35.87 | 0.4075 |
| TTRA | Intercept | -0.7580 | -0.9151 | -0.600 | <0.0001 | 363.77 | 257.86 | 469.68 | <0.0001 |
| | Slope | 0.0899 | 0.0318 | 0.1479 | 0.0024 | -35.02 | -76.04 | 5.98 | 0.0941 |

^a PYRA = pyraclostrobin; TTRA = tetraconazole; TMET = thiophanate-methyl; AZOX+DIFE = azoxystrobin+difenoconazole; TFLX + PROT = trifloxystrobin+prothioconazole; DIFE + PYDI = difenoconazole+pydiflumetofen; TMET + TEBU = thiophanate-methyl+tebuconazole; PYRA+FLUX+PROP = pyraclostrobin+fluxapyroxad+propiconazole.

^b Upper (CI_U) and lower (CI_L) limits of the 95% confidence interval around \bar{L}_{SEV} and \bar{D} .

Table 3.5. Overall means of soybean yield response (\bar{D}) for each fungicide treatment, relative to the nontreated check, conditioned (moderator analysis) to two classes of FLS severity representing a low (< 14% in the nontreated check) or high disease pressure (\geq 14% in the nontreated check).

| Fungicide ^a | Condition | Yield response (kg/ha) | | | | | |
|------------------------|-----------|------------------------|-----------|-------------------------------------|-------------------------------------|-----------------|-----------------------------------|
| | | <i>k</i> ^b | \bar{D} | <i>CI</i> _L ^c | <i>CI</i> _U ^c | <i>P</i> -value | Tau ($\hat{\tau}$) ^d |
| AZOX + DIFE | High | 23 | 547.64 | 428.36 | 666.91 | 0.0003 | 871301.69 |
| | Low | 25 | 245.17 | -36.08 | 526.44 | | |
| DIFE + PYDI | High | 7 | 639.11 | 452.67 | 825.55 | 0.0017 | 906885.92 |
| | Low | 16 | 284.51 | -123.16 | 692.20 | | |
| PYRA | High | 26 | 190.28 | 111.12 | 269.43 | 0.0915 | 932867.79 |
| | Low | 29 | 100.86 | -82.15 | 283.88 | | |
| PYRA + FLUX + PROP | High | 15 | 496.65 | 389.94 | 603.36 | <0.0001 | 801516.58 |
| | Low | 22 | 155.52 | -90.89 | 401.93 | | |
| TFLX + PROT | High | 13 | 542.68 | 411.38 | 673.98 | 0.0001 | 758460.79 |
| | Low | 13 | 190.99 | -121.45 | 503.44 | | |
| TMET | High | 25 | 562.30 | 440.28 | 684.32 | <0.0001 | 818775.99 |
| | Low | 27 | 175.74 | -112.69 | 464.19 | | |
| TMET + TEBU | High | 24 | 602.17 | 481.54 | 722.81 | <0.0001 | 798307.00 |
| | Low | 25 | 154.94 | -130.46 | 440.35 | | |
| TTRA | High | 25 | 444.65 | 352.68 | 536.6 | <0.0001 | 861963.00 |
| | Low | 26 | 197.11 | -19.39 | 413.61 | | |

^a PYRA = pyraclostrobin; TTRA = tetraconazole; TMET = thiophanate-methyl; AZOX+DIFE = azoxystrobin+difenoconazole; TFLX + PROT = trifloxystrobin+prothioconazole; DIFE + PYDI = difenoconazole+pydiflumetofen; TMET + TEBU = thiophanate-methyl+tebuconazole; PYRA+FLUX+PROP = pyraclostrobin+fluxapyroxad+propiconazole.

^b Number of trials in which each fungicide was evaluated.

^c Upper (*CI*_U) and lower (*CI*_L) limits of the 95% confidence interval around \bar{D} .

^d Between-study variance.

3.6 Figures

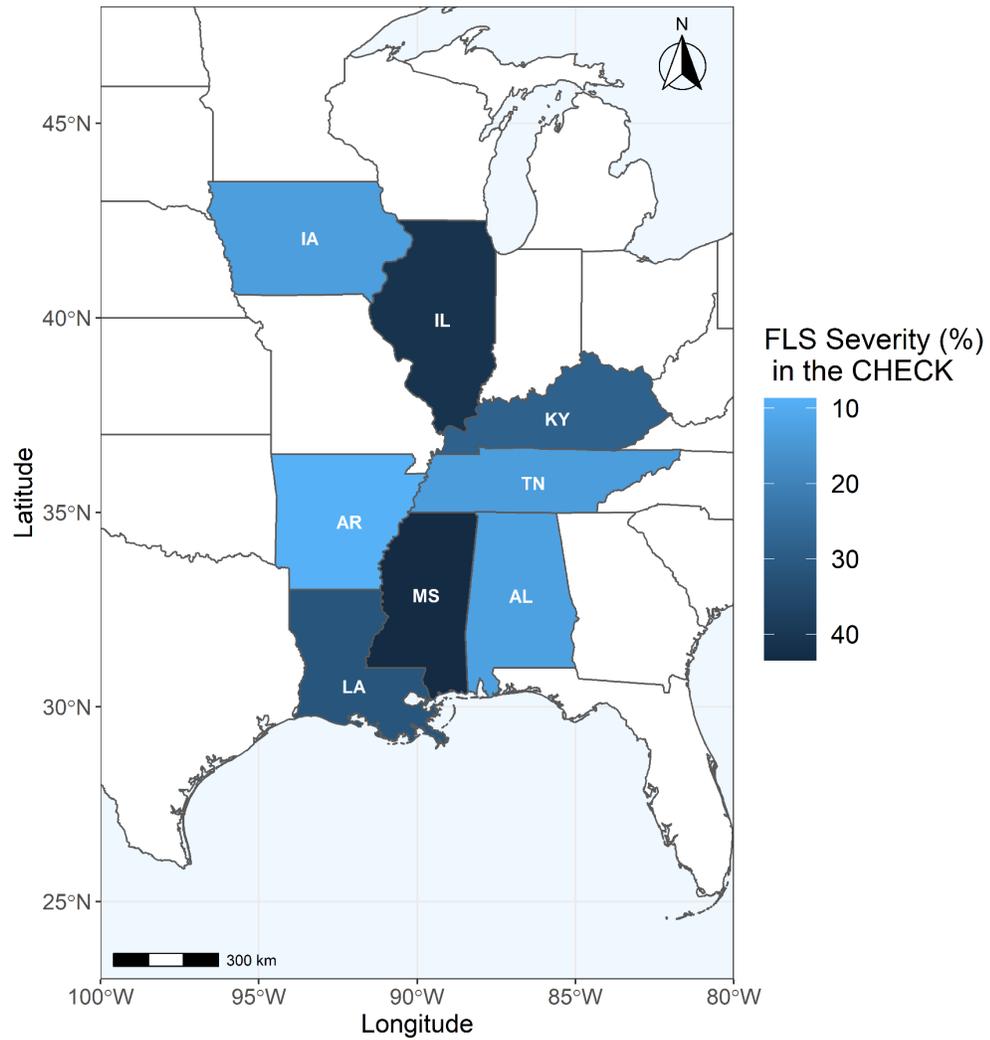


Fig. 3.1. Geolocation of the eight states where 66 fungicide evaluation trials were conducted from 2012 to 2021. States were shaded according to the mean FLS severity in the nontreated (CHECK).

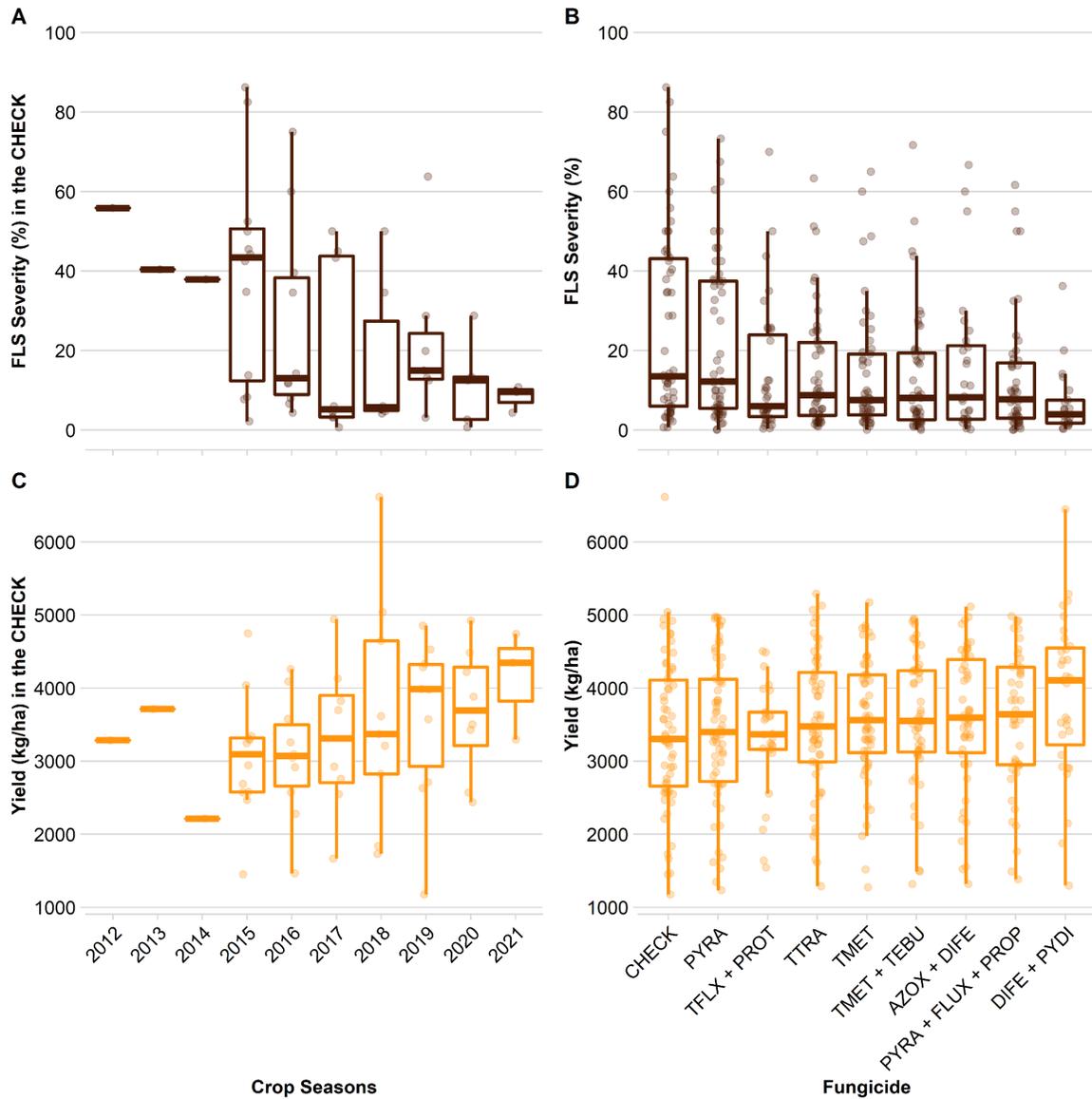


Fig. 3.2. Box plots depicting the means of frogeye leaf spot (FLS) severity (%) and soybean yield (kg/ha) in the nontreated plots within-year (**A,C**); and the means of the same variables in the nontreated and fungicide-treated plots (**B, D**), measured from a set of 66 field trials conducted from 2012 to 2021. The thick horizontal line inside each box plot represents the median, the limits of the box represent the lower and upper quartiles, and the circles represent yearly means of each treatment.

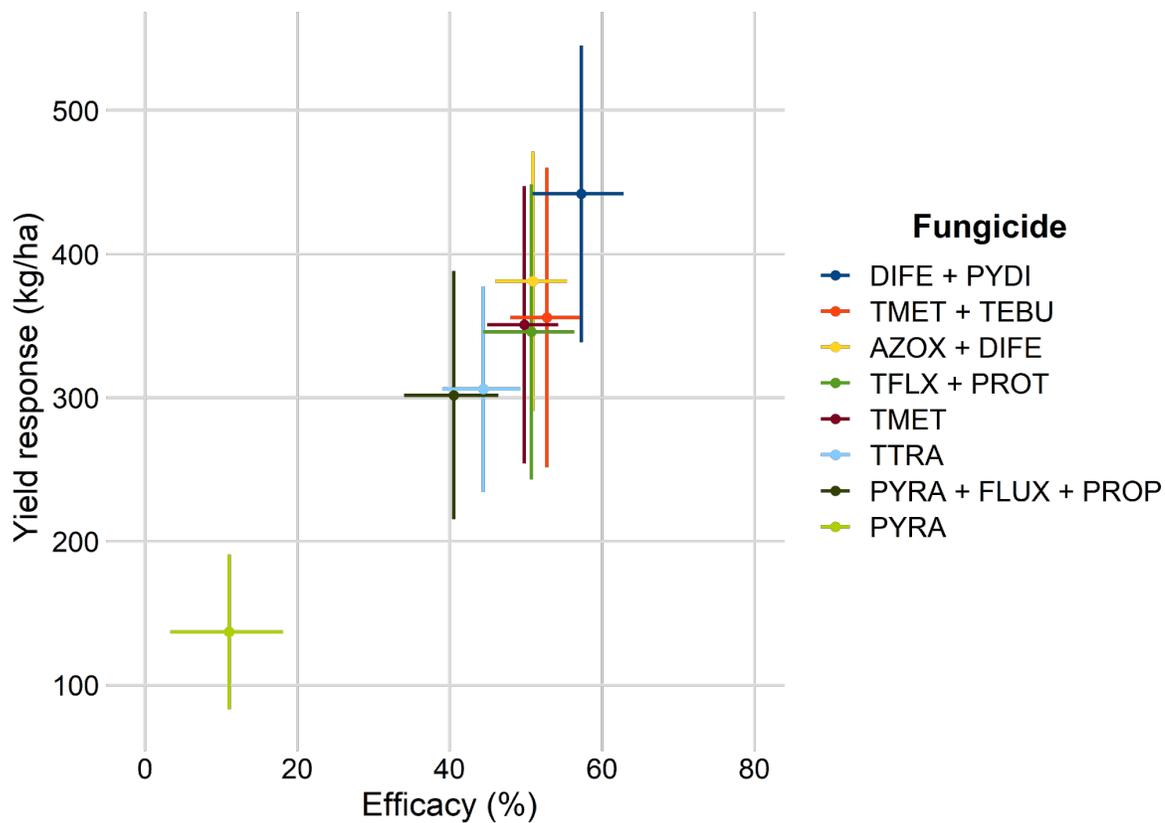


Fig. 3.3. Relationship between percent reduction of frogeye leaf spot (FLS) and yield response relative to nontreated, for eight fungicides evaluated across 66 independent field trials from 2012 to 2021. Bars show the upper and lower limits of 95% confidence intervals around point estimates for both responses.

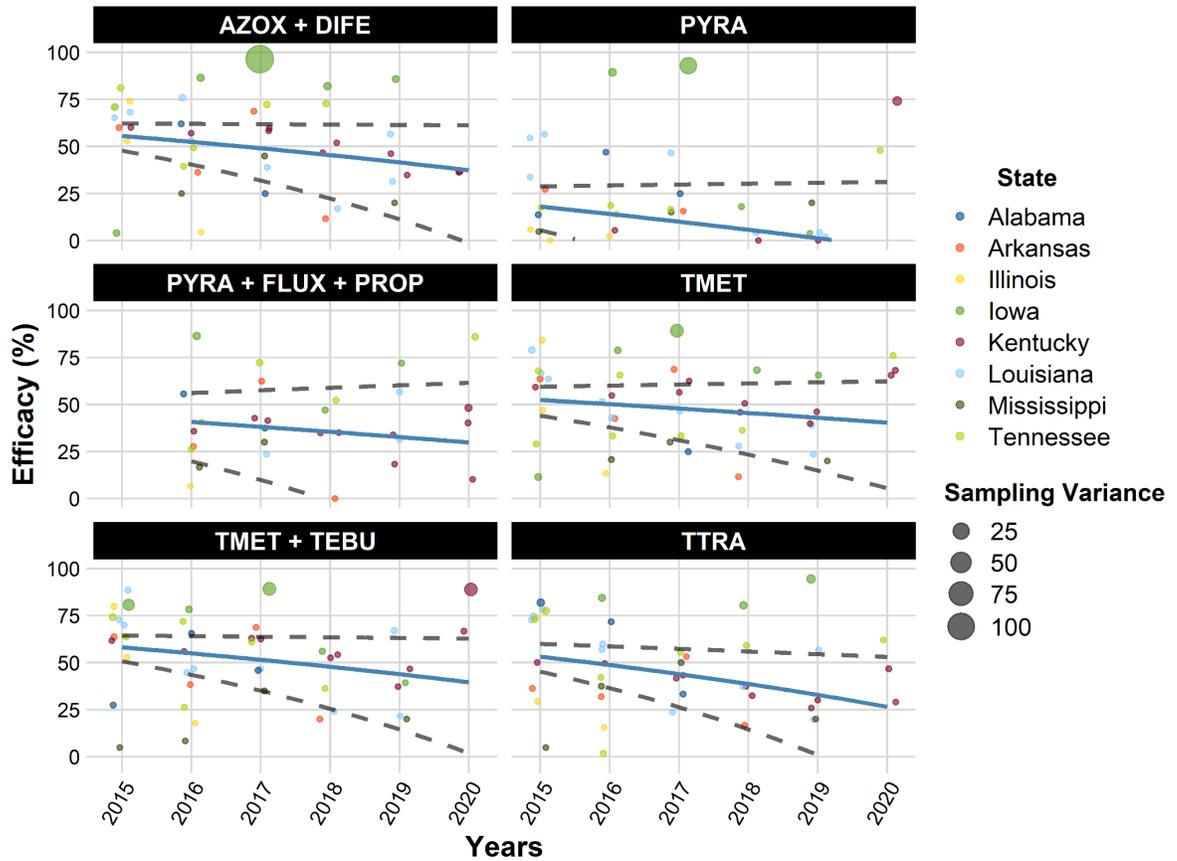


Fig. 3.4. Yearly variation of efficacy (percentage control) for six selected fungicide treatments applied at R3 growth stage during six crop seasons for the control of FLS. Solid (mean) and dashed (95% confidence intervals) lines are the predictions from back-transforming the log response ratio for each year based on the intercepts and slopes of network meta-regression models using year as a continuous covariate. Each dot represents the observed efficacy in an individual trial, colored according to the states where the trials were conducted.

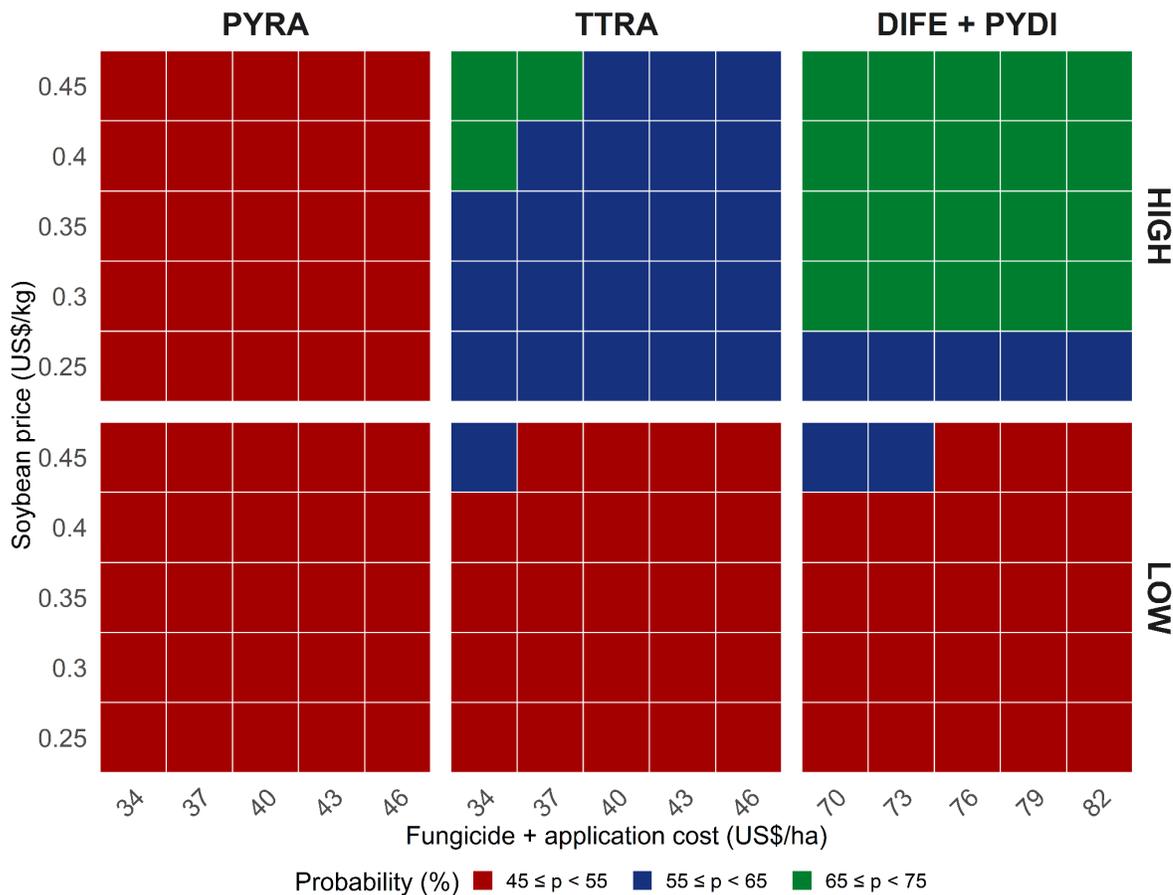


Fig. 3.5. Probability categories of breaking even on fungicide investment for different scenarios of soybean prices and fungicide costs (product price + operational costs [fixed at \$21.00 U.S./ha]) for three representative fungicide treatments applied once (at R3) for FLS control. Probability for each fungicide treatment was calculated using the estimates of the mean difference (\bar{D}) obtained for each disease pressure scenario, and respective between-study variance ($\hat{\tau}$), obtained from meta-analysis of data from 66 studies conducted over 10 years (2012 to 2021) across eight states.

3.7 SUPPLEMENTARY MATERIAL

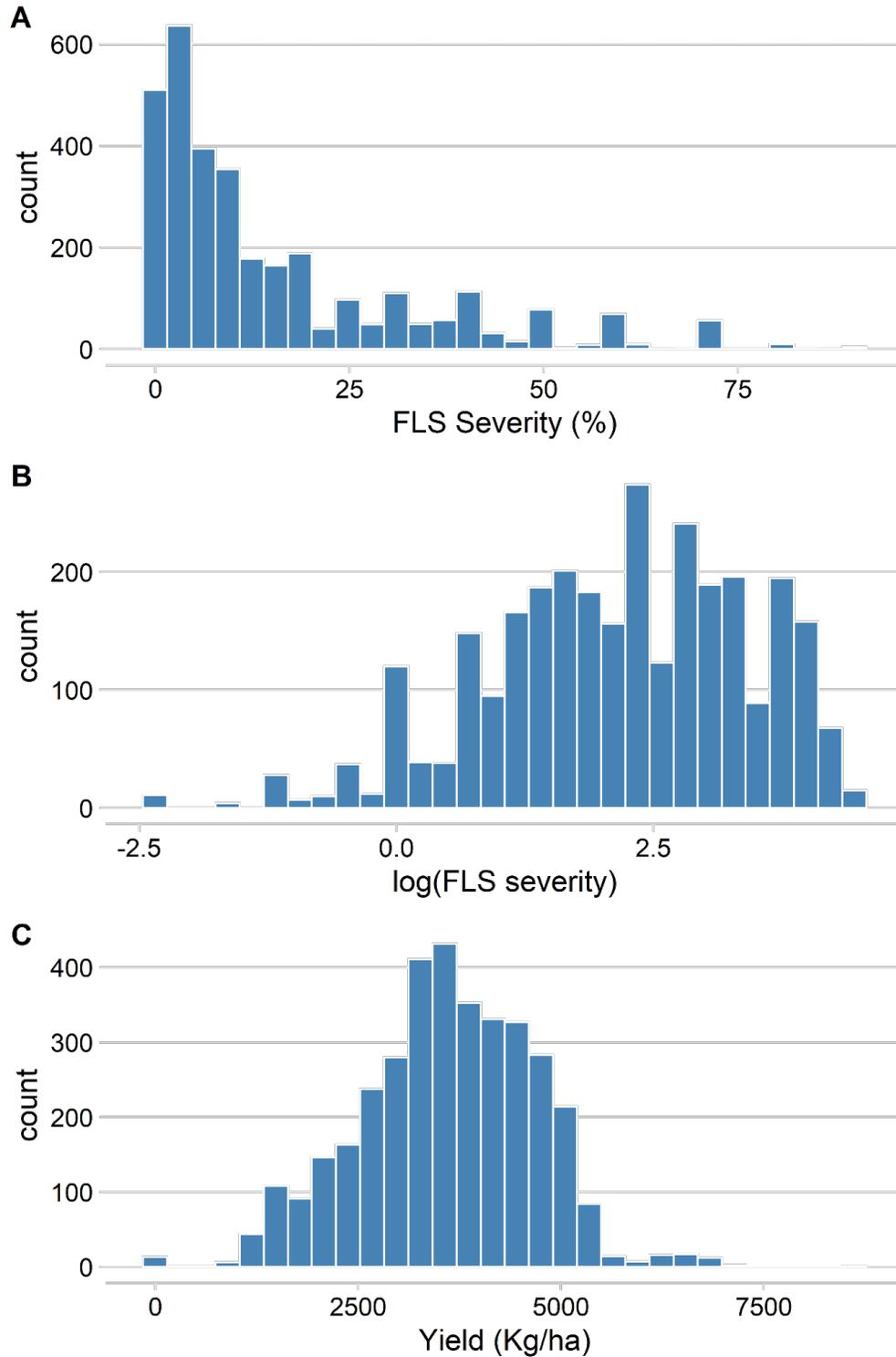


Fig. S3.1. Histograms for the distribution of FLS severity (**A**) and soybean yield (**C**) to check normality; **B**: log-transformed FLS severity data for normalizing the distribution and use in the meta-analysis.

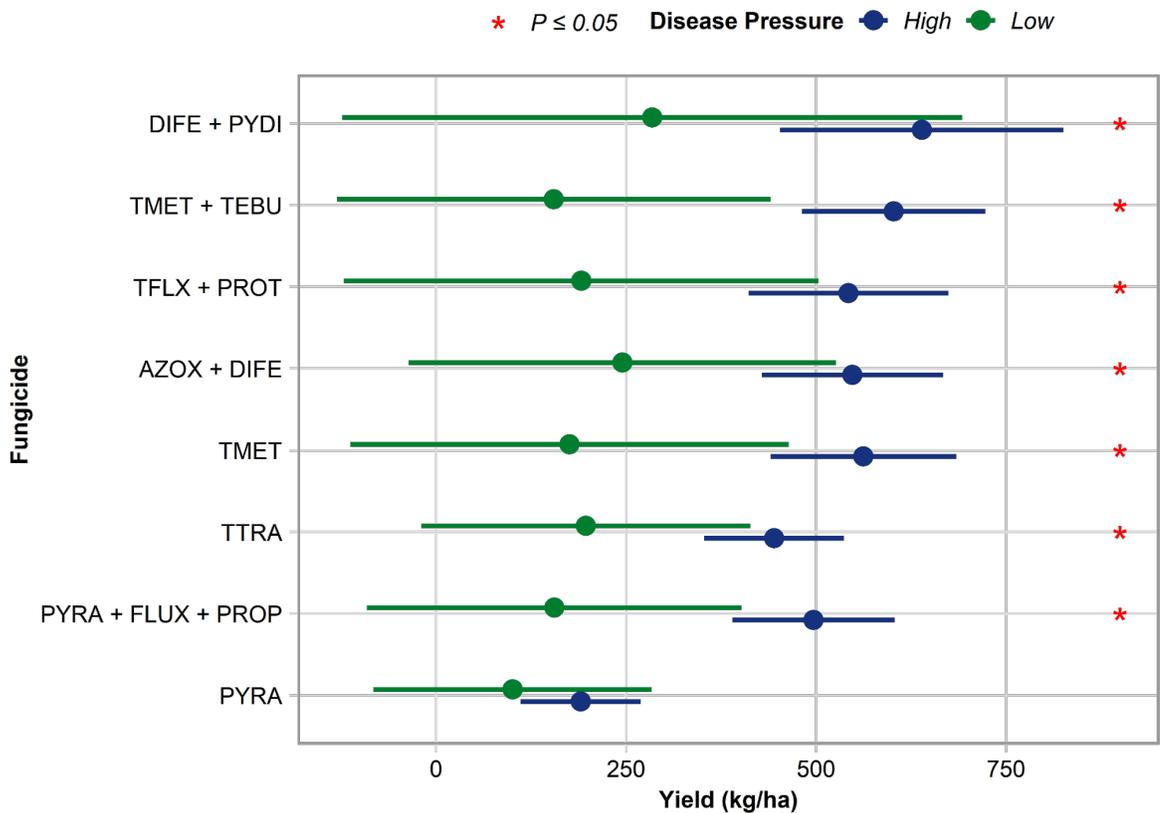


Fig. S3.2. Means and respective 95% confidence intervals (error bars) for soybean yield response (kg/ha) provided by fungicide treatments evaluated over years 2012 to 2021 and grouped into two classes of FLS severity representing a low (< 14% in the nontreated check). The means were calculated using a network meta-analytic model where disease pressure was included as covariate.

Table S3.1. Designs (set of treatments evaluated in the same trial) identified in 66 independent trials conducted across eight states in the United States (AL, AR, IA, IL, KY, LA, MS and TN) during 10 growing seasons (2012-2021) for controlling FLS.

| Designs ^a | FLS severity ^b | Yield ^b |
|--|---------------------------|--------------------|
| CHECK; TTRA; PYRA + FLUX + PROP; DIFE + PYDI; TMET; TMET + TEBU; PYRA; AZOX + DIFE | 14 | 15 |
| CHECK; TTRA; PYRA + FLUX + PROP; TMET; TMET + TEBU; PYRA; AZOX + DIFE; TFLX + PROT | 13 | 11 |
| CHECK; TTRA; TMET; TMET + TEBU; PYRA; AZOX + DIFE; TFLX + PROT | 11 | 10 |
| CHECK; TTRA; PYRA + FLUX + PROP; TMET; TMET + TEBU; PYRA; AZOX + DIFE | 1 | 3 |
| CHECK; TTRA; PYRA + FLUX + PROP; DIFE + PYDI; TMET; PYRA | 3 | 6 |
| CHECK; TMET; TMET + TEBU; PYRA; AZOX + DIFE; TFLX + PROT | 2 | 2 |
| CHECK; PYRA + FLUX + PROP; DIFE + PYDI; TMET + TEBU; PYRA | 2 | 2 |
| CHECK; TTRA; PYRA + FLUX + PROP; DIFE + PYDI; TMET + TEBU; PYRA | - | 2 |
| CHECK; TTRA; TMET; TMET + TEBU; AZOX + DIFE; TFLX + PROT | 1 | 2 |
| CHECK; PYRA + FLUX + PROP; DIFE + PYDI; TMET + TEBU; PYRA; AZOX + DIFE | 1 | - |
| CHECK; TMET; PYRA | 1 | 1 |
| CHECK; TMET; PYRA; AZOX + DIFE | 1 | 1 |
| CHECK; TTRA; DIFE + PYDI; TMET; PYRA; AZOX + DIFE | 1 | 1 |
| CHECK; TTRA; PYRA + FLUX + PROP; TMET + TEBU; PYRA; AZOX + DIFE; TFLX + PROT | 1 | 1 |
| CHECK; TTRA; TMET + TEBU; PYRA | 1 | 1 |
| CHECK; TTRA; TMET; PYRA; AZOX + DIFE | 1 | - |
| CHECK; TTRA; TMET; TMET + TEBU; PYRA; AZOX + DIFE | 1 | 1 |
| CHECK; AZOX + DIFE | - | 1 |
| CHECK; DIFE + PYDI | - | 1 |
| CHECK; TTRA; PYRA + FLUX + PROP; DIFE + PYDI; TMET; PYRA; | - | 1 |

AZOX + DIFE

CHECK; TTRA; PYRA + FLUX + PROP; DIFE + PYDI; TMET; TMET + TEBU; PYRA - 1

^a PYRA = pyraclostrobin; TTRA = tetraconazole; TMET = thiophanate-methyl; AZOX+DIFE = azoxystrobin+difenoconazole; TFLX + PROT = trifloxystrobin+prothioconazole; DIFE + PYDI = difenoconazole+pydiflumetofen; TMET + TEBU = thiophanate-methyl+tebuconazole; PYRA+FLUX+PROP = pyraclostrobin+fluxapyroxad+propiconazole.

^b Number of trials that each design of treatments was identified for both FLS severity and yield.

CHAPTER 4. PROFITABILITY OF FUNGICIDE USE ON FROGEYE LEAF SPOT MANAGEMENT IN LOW-DISEASE ENVIRONMENTS

Abstract

Double-crop soybean production involves planting soybean (*Glycine max*) directly following winter wheat (*Triticum aestivum*) harvest. Frogeye leaf spot (FLS), caused by *Cercospora sojina*, is an important late-season foliar disease affecting soybean fields in the United States. In some instances, foliar fungicides have been used in double-crop soybean production with little to no FLS present, raising questions on the profitability of these applications. This study analyzed yield data from 22 fungicide trials conducted under low FLS pressure, from 2008 to 2021, on double-crop soybean. Fungicide classes evaluated in the trials included quinone outside inhibitors (QoIs), demethylation inhibitors (DMIs) and methyl benzimidazole carbamates (MBC) applied alone, and mixtures of chemistry classes that included DMI+SDHI (succinate dehydrogenase inhibitors), MBC+DMI, QoI+DMI, and QoI+DMI+SDHI. A network meta-analytic model estimated yield differences between fungicide-treated and nontreated plots, which ranged from -16 to 106 kg/ha among the fungicide treatments. Negative yield response values were estimated for the single fungicide classes MBC (-5.5 kg/ha) and QoI (-16.6 kg/ha). Yield difference as high as above 100 kg/ha was estimated only for DMI+SDHI. Economic analyses indicated that, due to the lower yield responses, probabilities of breaking even were lower than 50% for all the single fungicide classes, regardless of the fungicide cost or soybean price values. Therefore, the low yield responses associated with foliar fungicides in low-disease environments linked to the higher risk of not offsetting the costs, suggest that growers should consider disease risk prior to making the fungicide application.

Keywords: *Cercospora sojina*, chemical control, yield, economic risk

4.1 Introduction

Double-crop soybean production involves planting soybean (*Glycine max*) directly after winter wheat (*Triticum aestivum*) harvest, which allows for a larger land use efficiency ratio and the potential for higher net economic returns compared with full season soybean (Caviglia et al., 2011; Rod et al., 2021). Reports of soybean producers applying a single fungicide spray as a prophylactic application at the R3 developmental stage (beginning pod development; Fehr et al., 1971) have been increasing regardless of economic thresholds (Rod et al., 2021). This increase in fungicide use is due to an increased awareness of soybean diseases as well as the availability of fungicides for use on soybean (Kandel et al., 2016; Mueller et al., 2013). The prophylactic application usually results in a positive yield response; however, the break-even probability on fungicide costs is variable depending on application cost, product cost, and soybean sale price (Henry et al., 2011; Kandel et al., 2016, 2021; Mahoney et al., 2015).

One of the most important foliar fungal diseases that annually limit soybean yield is frogeye leaf spot (FLS), caused by *Cercospora sojina* (Athow & Probst, 1952; Bradley et al., 2021). Yield losses, which can reach up to 60%, are mainly due to reduced photosynthetic leaf area, premature defoliation, and reduced seed weight (Dashiehl & Akem, 1994). The disease is best managed by integrating multiple tactics such as planting resistant cultivars, applying foliar fungicides, and rotating to non-host crops (Wise & Newman, 2015). Currently in the U.S., fungicide products available for managing FLS may contain one or more active ingredients from different fungicide classes, which include demethylation inhibitors (DMI), methyl benzimidazole carbamate (MBC), quinone outside inhibitors (QoI), and succinate dehydrogenase inhibitors (SDHI) (Crop Protection Network, 2022).

The QoI class of fungicides, also known as strobilurins, have been commercially available in the United States since the mid-to late 1990s but were not registered for use on soybean until the 2000s (Bartlett et al., 2002). This class of fungicides disrupts electron transport in the complex III in the mitochondria and decrease adenosine triphosphate (ATP) production, which effectively inhibits spore germination and inhibits mycelial growth (Bartlett et al., 2002). This mode of action is also known to cause some physiological changes within the plant, such as: greater chlorophyll retention, increased water and nitrogen use efficiency, and delayed senescence, which may result into a positive yield response in the absence of disease (Amaro et al., 2020; Glaab & Kaiser, 1999; Kohle et al., 2002; Mahoney et al., 2015). In essence, QoI-treated plants may display prolonged “greening”, which presumably allows for greater dry matter accumulation through the seed-filling period and greater yields as defoliation is decreased and more photosynthetically efficient leaves are retained longer compared to a non-treated control (Kyveryga et al., 2013; Mahoney et al., 2015).

Another group of fungicides that may result in physiological effects on plants, is the SDHI group, also known as carboxamides (Amaro et al., 2020). SDHIs inhibit the electron transport in mitochondria at complex II by inhibiting the enzyme succinate dehydrogenase (SDH), disrupting the respiratory process, blocking the energy supply of fungal cells, leading to their death (Avenot & Michailides, 2010). Regarding physiological effects, wheat plants had a higher net CO₂ assimilation rate when treated with the SDHI bixafen compared to a non-treated control (Berdugo et al., 2012). Both QoIs and SDHIs are systemic fungicides that operate best when applied preventatively and have little curative disease control effects (Amaro et al., 2020). Previous studies have

demonstrated, mainly for QoIs, that induced physiological effects in soybean led to positive yield responses or to an increase in some yield components compared to a nontreated control (Fagan et al., 2010; Henry et al., 2011). However, some research results indicated that those physiological effects on soybean were inconsistent, or did not always result in a significant positive yield response (Dorrance et al., 2010; Nelson et al., 2010; Swoboda & Pedersen, 2009).

Prophylactic use of fungicides may also confer risks beyond economic losses. QoI fungicides are classified by the Fungicide Resistance Action Committee (FRAC) as high-risk for resistance development (Fungicide Resistance Action Committee, 2020). In fact, *C. sojae* isolates with resistance to QoIs, due to the presence of the G143A mutation in the *Cytochrome b* gene, have been detected across more than 20 soybean-producing states in the U.S. (Harrelson et al., 2021; Mathew et al., 2019; Neves et al., 2020, 2021, 2022; Standish et al., 2015; Zeng et al., 2015; Zhang et al., 2012a, 2012b, 2018; Zhou & Mehl, 2020).

In the last five years, U.S. soybean prices varied greatly, from \$0.34/kg in 2017 to \$0.47/kg in 2021 (United States Department of Agriculture-National Agricultural Statistics Service, 2022). In times of fluctuating prices and market uncertainty, growers need to minimize costs and maximize profitability. To better understand the impact of fungicide applications on soybean yield and profitability in low-disease environments, such as double-crop soybean, data from fungicide trials conducted between 2008 and 2021 were analyzed, using a meta-analytic approach, across five states: Illinois, Indiana, Kentucky, Missouri, and Tennessee. The yield response for each application scenario was

used to calculate the probability of breaking even on fungicide costs and determine if those applications can be profitable for soybean farmers.

4.2 Material and Methods

4.2.1 *Data source and criteria for fungicide selection*

Data were obtained from fungicide trials in double-crop soybean conducted during ten crop seasons between 2008 and 2021. During this period, 22 independent trials were conducted across five states in the U.S. (Illinois [IL], Indiana [IN], Kentucky [KY], Missouri [MO] and Tennessee [TN]) (Fig. 4.1; Table 4.1). In most trials, plot size varied slightly across locations, but each plot had 4 rows and at least was 6 m long, except for the plots in Missouri, which were larger strip trials conducted in farmers' fields. The inter-row spacing varied from 19 to 76 cm in the trials. Plots were arranged in a randomized complete block design, with four replications. Fungicide treatments were applied when soybean plants reached the R3 developmental stage. Application of fungicides in small plots were completed with backpack sprayers pressurized by CO₂ (276 kPa), which were calibrated for spray volumes that ranged between 140 and 200 liters/ha. Application of fungicides to the larger strip trials in Missouri were conducted with tractor-mounted and self-propelled sprayers that applied spray volumes ranging from 140 to 187 liters/ha. All nutrient, weed, and insect management practices followed regional recommendations.

Disease severity was visually assessed as percent leaf area exhibiting FLS symptoms at the plot level, 2 and 4 weeks after fungicide applications (approximately at the R6 developmental stage; full seed), focusing specifically on the upper third of the

canopy. Scouting also was performed to identify other foliar diseases. For small plot trials, yield was obtained by harvesting the 2 middle rows of each plot after full maturity (R8) with a small plot combine equipped with a grain gauge that measured harvest weight and grain moisture. For large strip plots in Missouri, yield information was obtained from combine yield monitors. Soybean yield was calculated as kilograms/hectare at 13% grain moisture.

To be included in the analysis, a fungicide treatment had to be tested in at least 5 trials and compared with a nontreated check treatment in the same trial. Seven treatments met the criteria, including: DMI, QoI, MBC applied alone, and mixtures of DMI+SDHI, MBC+DMI, QoI+DMI and QoI+DMI+SDHI (Table 4.2).

4.2.2 *Network meta-analysis*

Although the data were available at the plot level for all treatments, including the nontreated check, the means were used in the meta-analysis (Madden et al., 2016). Given the statistical properties of the data, non-transformed means of yield were used to obtain the mean absolute difference. Hence, a two-way unconditional linear mixed model was fitted directly to the treatment means to further obtain yield response. The model can be written as Equation 1:

$$Y_i \sim N(\mu, \Sigma + S_i) \quad (1)$$

where Y_i is the vector of absolute yield for the eight treatments plus the nontreated check for the i th study, μ is a vector representing the mean of Y_i across all studies, Σ is a 8 x 8 between-study variance-covariance matrix (for the seven treatments plus the nontreated check), and S_i is a within-study variance-covariance matrix for the i th study. N indicates a multivariate normal distribution.

The yield difference (\bar{D}) was calculated directly after model fitting by subtracting estimated means of fungicide treatment and nontreated check as described in Equation 2 (Madden et al., 2016).

$$\bar{D} = D_{Treated} - D_{Nontreated} \quad (2)$$

The within-study variability (sampling variance) of D was calculated from the mean square error (MSE) obtained after fitting a linear model to raw data at each individual trial, as described in previous studies (Barro et al., 2021; Machado et al., 2017). The within-study variability is required to weight studies based on the inverse function of the sampling variance (Paul et al., 2008). The *rma.mv* function of *metafor* package (Viechtbauer, 2010) of R (R Core Team, 2020) was used to fit maximum likelihood estimation models to the data.

The network model (Equation 1) was expanded to include two categorical moderator variables that could explain, at least, a portion of the heterogeneity of the effects across trials (Madden et al., 2016). The expanded model (Paul et al., 2010) is given by Equation 3:

$$Y_i \sim N(\boldsymbol{\mu} + \boldsymbol{\delta}_i, \boldsymbol{\Sigma} + \boldsymbol{S}_i) \quad (3)$$

where $\boldsymbol{\delta}_i$ is the vector representing the moderator variable effect for the i th study and all other terms are as defined previously.

We created two categorical variables to be included as covariates in the model. First, the baseline severity was divided into two groups, representing healthy (no disease symptoms or FLS severity = 0%) and diseased (FLS severity > 0%) (**Table 4.1**). Second, the baseline yield was defined as low (<3,256 kg/ha) or high (\geq 3,256 kg/ha) based on the median yield in the nontreated check. The moderator variables were included in the model and tested using a Wald-type chi-square test to determine if the moderator variables directly affected the differences in yield values (Paul et al., 2008).

4.2.3 *Economic risk and profitability of fungicides*

With the estimates of mean yield difference (\bar{D}) between fungicide-treated and nontreated control plots for each fungicide, and the respective between-study variance ($\hat{\tau}$) obtained from the meta-analysis, we calculated the break even probability (P) of the fungicide costs (F_C), following Equation 4 used in previous studies (Barro et al., 2019; Machado et al., 2017).

$$P = \Phi [\bar{D} - (F_C/S_P)/\sqrt{\hat{\tau}}] \quad (4)$$

where Φ is the cumulative standard-normal function and S_p is the soybean price.

Using the equation 4, response surfaces of P were obtained using a function of 200 equally spaced values of F_C varying from 40 to 60 (USD/ha) for the chemical classes applied alone (DMI, MBC and QoI) and from 60 to 80 (USD/ha) for the premixes (DMI+SDHI, MBC+DMI, QoI+DMI, QoI+DMI+SDHI) (Halich, 2022). Soybean price (S_p) was also used as a function of 200 equally spaced values of 0.2 to 0.7 (USD/kg). The soybean prices were based on the average prices from 2017 to 2021 (United States Department of Agriculture-National Agricultural Statistics Service, 2022).

4.3 Results

4.3.1 FLS severity and yield

Among the states, Indiana reported the highest average yield (4,093 kg/ha) followed by Kentucky (3,652 kg/ha), Missouri (3,472 kg/ha), Tennessee (2,984 kg/ha) and Illinois (2,798 kg/ha) (Fig. 4.1). *Septoria* brown spot (caused by *Septoria glycines*) was reported in MO trials at low severity (0-10%). FLS severity in the nontreated plots was present in 10 out of the 22 trials and was the greatest in Kentucky in 2018 (26%) and Illinois in 2010 (14%). All the other eight trials had FLS severity below 7% (Table 4.1). Considerable variation in FLS severity and mainly in yield was recorded in the nontreated plots across crop seasons (Fig.4.2A-C). Disease severity in the nontreated plots ranged from 0 to 26% (median 0.14%) (Fig. 4.2A). On the other hand, baseline yield ranged from 2,107 to 4,094 kg/ha (median = 3,256 kg/ha) across the trials (Fig. 4.2C). As expected, disease pressure was very low and FLS severity medians observed in the fungicide treatments were very similar to the nontreated plots (Fig. 4.2B).

Interestingly, soybean yield medians were higher for mixtures of fungicide classes, while the median values for single fungicide classes were similar to the nontreated check (Fig. 4.2D).

4.3.2 *Meta-analytic estimates*

The mean estimates of yield difference (\bar{D}) between fungicide-treated and the nontreated plots ranged from -16 to 106 kg/ha among the fungicide treatments (Table 4.3). Although there was no statistical difference ($P < 0.05$) between all fungicide treatments and the nontreated check, yield response values were the lowest for the single fungicide classes MBC (-5.5 kg/ha) and QoI (-16.6 kg/ha). Yield difference as high as above 100 kg/ha was estimated only for DMI+SDHI. The other premixes also provide higher yield estimates compared to the single fungicide classes including QoI+DMI+SDHI (96.6 kg/ha), MBC+DMI (64.9 kg/ha) and QoI+DMI (57.3 kg/ha) (Table 4.3). Neither baseline yield ($P = 0.1116$) or baseline severity ($P = 0.4750$) affected the yield estimates based on the Wald test.

4.3.3 *Probability of breaking even on fungicide cost*

In general, the probability of breaking even (P) on the fungicide costs was affected by a range of scenarios of costs and soybean prices, and ranged from 26 to 51% (Fig. 4.3). As expected, due to low yield responses, probabilities of breaking even were less than 50% for all the single fungicide classes, regardless of the fungicide cost or soybean price values. Values of P above 50% were only estimated for the premixes in scenarios of

lower fungicide costs ($60 < F_C < 65$ USD/ha) and higher soybean prices ($S_P > 0.6$ USD/kg) (Fig. 4.3).

4.4 Discussion

The present study provides critical information on the profitability of foliar fungicides applied in double-crop soybean with low levels or the absence of FLS. Our results were obtained after summarizing a dataset of 22 trials conducted between 2008 and 2021 across five soybean-producing states in the U.S. On average, we found no significant difference in yield response between the fungicide treatments and the nontreated check. However, negative yield response values were estimated for the single fungicide classes MBC (-5.5 kg/ha) and QoI (-16.6 kg/ha). The higher yield response was estimated for the premix DMI+SDHI (106 kg/ha). Finally, economic analyses indicated that, due to the lower yield responses, probabilities of breaking even were inferior to 50% for all the single fungicide classes, regardless of the fungicide cost or soybean price values.

Positive effects in soybean yield or yield components after prophylactic QoI applications, which may be attributed to a phenomenon called the “greening effect”, have been observed in previous studies (Amaro et al., 2020; Fagan et al., 2010; Henry et al., 2011; Kandel et al., 2021; Nelson et al., 2010). For instance, an increase of 100 kg/ha in soybean yield was reported after an application of pyraclostrobin at the R4 developmental stage across three locations in Indiana during the 2009 and 2010 crop seasons (Henry et al., 2011). Similarly, a yield difference of 96 kg/ha was reported for a QoI application at beginning pod (R3) stage compared to the nontreated check in a previous meta-analysis, which used data obtained from 240 field trials conducted between 2005 and 2018 across

nine U.S. states and Ontario, Canada (Kandel et al., 2021). A higher yield response (1,080 kg/ha) was obtained from two applications of pyraclostrobin in a single field trial in southern Brazil during the 2005/06 crop season (Fagan et al., 2010). Additionally, an R4 application of QoI fungicides (pyraclostrobin, azoxystrobin) increased yields from 230 to 360 kg/ha across experiments conducted in northeastern Missouri in 2006 and 2007 compared to the nontreated check (Nelson et al., 2010). However, in the latter study, no yield increase due to QoIs was observed in the southeastern states compared to the nontreated check (Nelson et al., 2010). Inconsistencies or nonexistent soybean yield responses due to QoI applications have been reported. Swoboda & Pedersen (2009) found no physiological effect or associated yield improvement by applying a QoI (pyraclostrobin) compared to the nontreated check in the absence of foliar diseases at soybean developmental stages R1, R3, and R5 in Iowa at one location in 2005 and two locations in 2006. Moreover, non-significant soybean yield responses between pyraclostrobin compared to the nontreated check were found in all the three locations with absence of foliar diseases in Ohio during the 2006 crop season, and in three out of four locations during the 2007 crop season (Dorrance et al., 2010).

In this study, an average yield response obtained from the application of a single DMI was below 20 kg/ha. Kandel et al. (2021) reported a yield difference of 66 kg/ha as a result from the application of DMI at R3 in low foliar disease environments. Greater yields (600 kg/ha) were reported after the application of a single DMI (tebuconazole) in a single field trial in southern Brazil (Fagan et al., 2010). Conversely, Swoboda & Pedersen (2009) reported no yield improvement by applying the same DMI in Iowa. Similarly, the single application of the DMI, tebuconazole, or in a mixture including the QoI,

pyraclostrobin, resulted in non-significant soybean yield responses compared to the nontreated check in three different locations in Ohio during the 2006 growing season (Dorrance et al., 2010). In the same study, the authors found no significant yield improvements by applying another DMI (tetraconazole) in three out of four locations during the 2007 crop season (Dorrance et al., 2010).

Negative values of yield responses were observed for the single application of QoI and MBC fungicides. Regarding QoIs, negative results for the physiological effects of this chemical group in soybean were described by Nason et al., (2007) due to reduced stomatal conductance, transpiration rate and net CO₂ assimilation rate. Both fungicide classes are classified as a high risk of fungicide resistance development (Fungicide Resistance Action Committee, 2020). *C. sojae* populations resistant to QoIs were already reported across all the five states in which the trials were conducted (Zhang et al., 2012a, 2018). Although no shift towards reduced sensitivity of *C. sojae* isolates to MBC has been reported so far (Zhang et al., 2021), *C. beticola* and *C. kikuchii* isolates with resistance to MBC fungicides have been described already in United States (Campbell et al., 1998; Imazaki et al., 2006; Price et al., 2015), as well as resistant isolates of *Cercospora* spp. to MBC were reported recently from soybean in Brazil (de Mello et al., 2021). Caution must be taken when applying single active ingredients, such as DMI or MBC, because additional pressure on the pathogen population exists, mainly when resistance to QoI is already present (Mengistu et al., 2018; Phillips et al., 2021).

Furthermore, although there was no significant difference between fungicide treatments and the nontreated check, premixes including the SDHIs reported higher yield response values. Kandel et al. (2021) also reported higher yield responses (139 kg/ha)

from mixtures including QoI+SDHI and QoI+DMI+SDHI. Similarly to our results, no differences in yield were reported between mixtures of QoI+SDHI and DMI+SDHI with the nontreated control, under very low FLS pressure (<5% severity), at the same location (Kanawha), in Iowa during the 2018 and 2019 crop seasons (Phillips et al., 2021). Additionally, Kandel et al. (2016) analyzed data collected over multiple years (2008 to 2014) and 14 locations across four states (Illinois, Indiana, Iowa, and Nebraska) and found no significant effect on yield responses from the application of QoI+SDHI, QoI, DMI and DMI+QoI in seven out of 14 total site-years.

Research has indicated that, despite yield increases being possible using below threshold applications of fungicides, growers may not benefit from such applications if input costs are higher than the economic returns of the increased yield (Henry et al., 2011; Kandel et al., 2016; Mahoney et al., 2015). Our results showed a higher risk of not offsetting the fungicide costs as probabilities of breaking even were inferior to 50% for all the single fungicide classes, regardless of the fungicide cost or soybean prices. Kandel et al. (2016) found that fungicide applications can increase soybean yield in the absence of disease, however, application of solo fungicide active ingredients were profitable only 14% of the time (Kandel et al., 2016). Additionally, although an application of pyraclostrobin at R3 contributed to a yield increase of 4.1%, Mahoney et al. (2015) found no significant effect on profit margins under conditions of low to moderate levels of *Septoria* brown spot severity. In other words, the increased revenue from the increased yield associated with the use of pyraclostrobin were offset by fungicide and application costs (Mahoney et al., 2015). Kandel et al. (2021) also reported that probability of offsetting fungicide cost was very low (<50%) in most grain price–application cost

combinations, mainly for single fungicide groups. Finally, several studies have indicated lower levels of yield return and lower probabilities of breaking even during lower disease severity conditions on the effect of fungicides for other diseases of soybean, such as white mold (caused by *Sclerotinia sclerotiorum*) and soybean rust (caused by *Phakopsora pachyrhizi*) (Barro et al., 2019, 2021; Delaney et al., 2018).

Therefore, given the marginal agronomic and economic benefits, and potential risks associated with pathogen fungicide resistance development, caution would be advised before advocating a widespread adoption of prophylactic fungicide applications in double-crop soybean. However, if environmental conditions are conducive for disease, such as warm (25–30°C) and humid conditions (>90% relative humidity) for FLS (Wise & Newman, 2015), fungicide applications could be warranted and more consistently profitable.

4.5 Tables

Table 4.1. List of the 22 trials used in the analysis with respective frogeye leaf spot severity (%) and soybean yield (kg/ha) in the nontreated check.

| Study | Year | State | Severity (%) | Yield (kg/ha) |
|-------|------|-----------|--------------|---------------|
| 1 | 2018 | Kentucky | 26 | - |
| 2 | 2019 | Kentucky | 7 | 4,087 |
| 3 | 2008 | Illinois | 0 | 2,880 |
| 4 | 2008 | Illinois | 0 | 2,804 |
| 5 | 2009 | Illinois | 0 | 3,093 |
| 6 | 2009 | Illinois | 0 | 4,062 |
| 7 | 2010 | Illinois | 14 | 2,307 |
| 8 | 2013 | Illinois | 1 | 2,125 |
| 9 | 2014 | Illinois | 3 | 2,971 |
| 10 | 2017 | Tennessee | 3 | 3,560 |
| 11 | 2018 | Tennessee | 4 | - |
| 12 | 2020 | Tennessee | 3 | 2,348 |
| 13 | 2020 | Kentucky | 0 | 3,540 |
| 14 | 2020 | Kentucky | 0 | 3,976 |
| 15 | 2020 | Kentucky | 0 | 3,589 |
| 16 | 2021 | Kentucky | 2 | - |
| 17 | 2019 | Kentucky | 5 | 2,994 |
| 18 | 2020 | Indiana | 0 | 4,094 |
| 19 | 2019 | Tennessee | 0 | 2,106 |
| 20 | 2020 | Missouri | 0 | 2,893 |
| 21 | 2020 | Missouri | 0 | 3,796 |
| 22 | 2020 | Missouri | 0 | 3,680 |

- No yield recorded.

Table 4.2. Fungicide treatments applied for controlling Frogeye leaf spot in 22 independent trials conducted from 2008 to 2021 across five states in the United States (Illinois, Indiana, Kentucky, Missouri and Tennessee).

| Active ingredient(s) | Abbreviation | FRAC ^a | <i>n</i> ^b |
|---|---------------------|-------------------|-----------------------|
| Nontreated | CHECK | - | 22 |
| pyraclostrobin azoxystrobin fluoxastrobin | QoI | 11 | 14 |
| tetraconazole propiconazole prothioconazole cyproconazole tebuconazole flutriafol | DMI | 3 | 15 |
| thiophanate-methyl | MBC | 1 | 11 |
| difenoconazole+pydiflumetofen flutriafol+bixafen | DMI+SDHI | 3+7 | 7 |
| thiophanate-methyl+tetraconazole thiophanate-methyl+tebuconazole thiophanate-methyl+propiconazole | MBC+DMI | 1+3 | 10 |
| azoxystrobin+difenoconazole trifloxystrobin+prothioconazole azoxystrobin+propiconazole trifloxystrobin+propiconazole azoxystrobin+tebuconazole picoxystrobin+cyproconazole | QoI+DMI | 11+3 | 17 |
| pyraclostrobin+propiconazole+fluxapyroxad azoxystrobin+propiconazole+benzovindiflupyr pyraclostrobin+tetraconazole+fluxapyroxad pyraclostrobin+mefentrifluconazole+fluxapyroxad trifloxystrobin+prothioconazole+fluopyram | QoI+DMI+SDHI | 11+3+7 | 11 |

^a Fungicide Resistance Action Committee code.

^b Number of trials that each treatment was evaluated.

Table 4.3. Overall means and respective confidence intervals of difference in soybean yield (\bar{D}) between fungicide-treated and nontreated plots for seven selected fungicide treatments evaluated in 22 independent trials conducted across five states in the United States (Illinois, Indiana, Kentucky, Missouri and Tennessee).

| Fungicide ^a | <i>k</i> ^b | Yield response (kg ha ⁻¹) | | | | |
|------------------------|-----------------------|---------------------------------------|-----------------|------------------------------|------------------------------|----------------------|
| | | \bar{D} | SE(\bar{D}) | CI _L ^c | CI _U ^c | Tau ($\hat{\tau}$) |
| DMI+SDHI | 7 | 106.31 | 66.67 | -24.36 | 236.99 | 331,500.3 |
| QoI+DMI+SDHI | 11 | 96.65 | 46.11 | 6.27 | 187.04 | 291,985.7 |
| MBC+DMI | 10 | 64.97 | 49.64 | -32.32 | 162.26 | 370,823.2 |
| QoI+DMI | 17 | 57.33 | 36.57 | -14.34 | 129.02 | 450,084.2 |
| DMI | 15 | 15.85 | 44.88 | -72.11 | 103.81 | 402,081.3 |
| MBC | 11 | -5.50 | 69.01 | -140.77 | 129.76 | 413,531.1 |
| QoI | 14 | -16.69 | 55.32 | -125.12 | 91.73 | 467,989.6 |

^aSee Table 2 for complete information of the evaluated fungicides.

^bNumber of trials in which each fungicide was evaluated.

^cUpper (CI_U) and lower (CI_L) limits of the 95% confidence interval around \bar{D} .

4.6 Figures

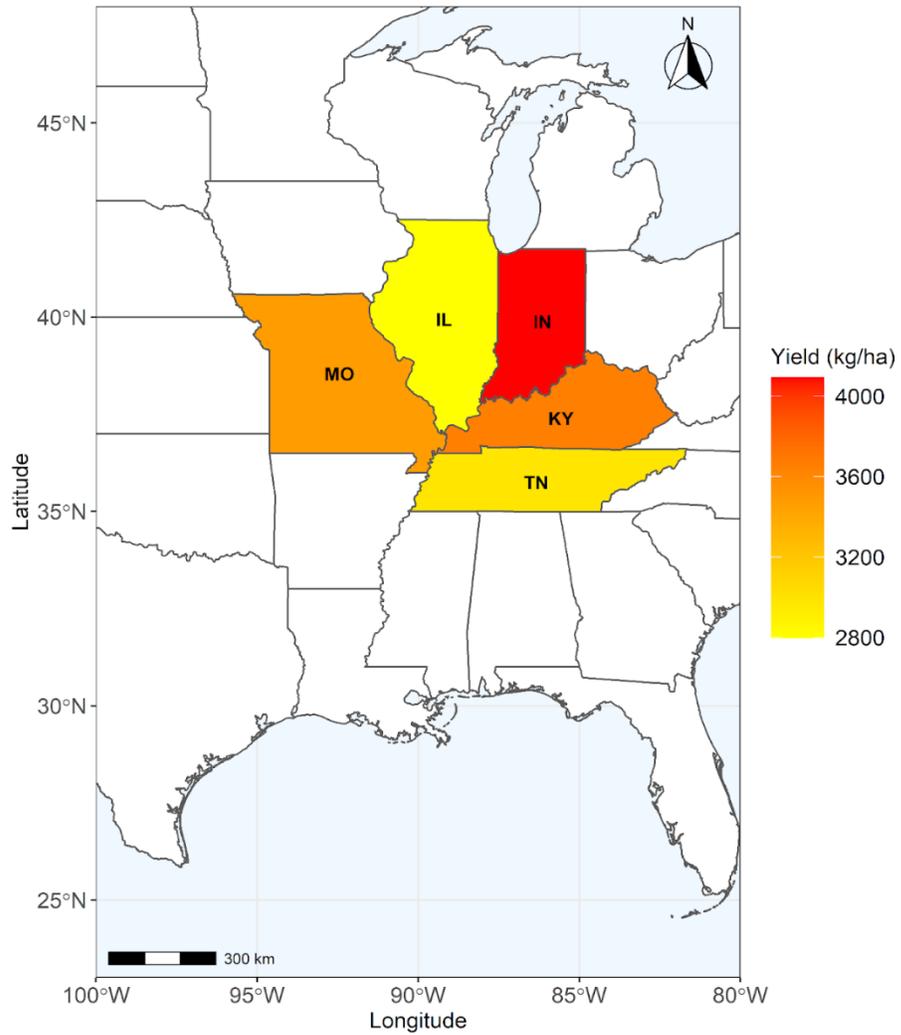


Fig. 4.1. Geolocation of the five states where 22 fungicide evaluation trials were conducted on double crop soybean between 2008 to 2021. States were shaded according to the mean soybean yield in the nontreated check.

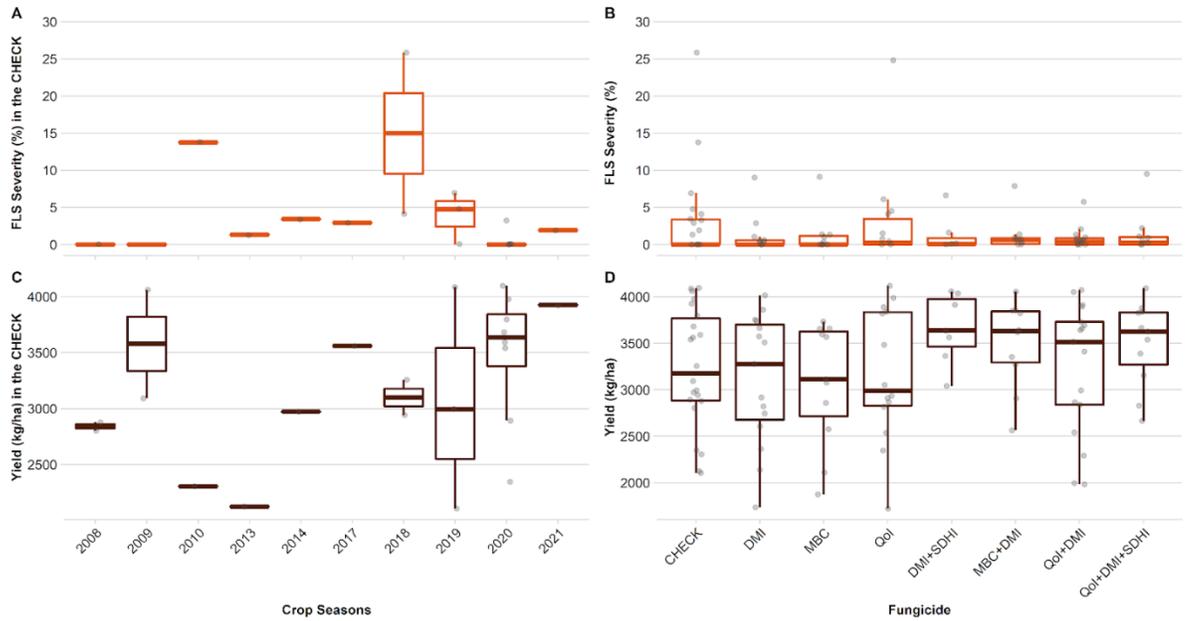


Fig. 4.2. Box plots depicting the means of frogeye leaf spot (FLS) severity (%) and soybean yield (kg/ha) in the nontreated plots within-year (**A,C**); and the means of the same variables in the nontreated and fungicide-treated plots (**B, D**), measured from a set of 22 field trials conducted from 2008 to 2021. The thick horizontal line inside the box represents the median, the limits of the box represent the lower and upper quartiles, and the circles represent yearly means of each treatment. See Table 2 for information on the fungicide treatments.

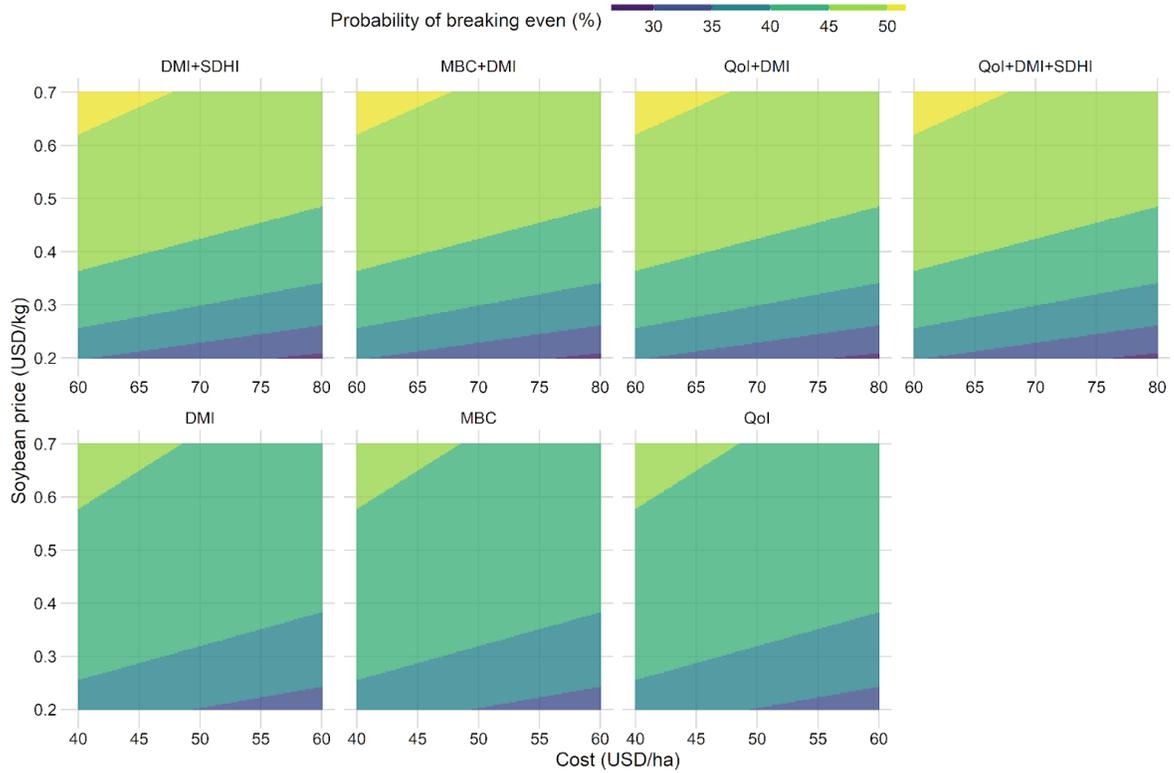


Fig. 4.3. Probabilities of breaking even on fungicide investment for different scenarios of soybean prices and fungicide costs for seven fungicide treatments applied once (at R3 developmental stage) in a double-crop soybean system. Probability for each fungicide treatment was calculated using the estimates of the mean difference (\bar{D}), and respective between-study variance ($\hat{\tau}$), obtained from meta-analysis of data from 22 studies conducted across five states in the United States (Illinois, Indiana, Kentucky, Missouri and Tennessee).

CHAPTER 5. CONCLUDING REMARKS

In this dissertation I report my work as a Dual Degree Doctoral/PhD student at the Universidade Federal de Viçosa and the University of Kentucky. In this work, a meta-analytic approach was used to summarize a large data set of 66 uniform fungicide trials designed to evaluate fungicide efficacy against frogeye leaf spot (FLS; caused by *Cercospora sojina*) on soybean (*Glycine max*). The dataset spanned 10 years (2012 to 2021) of experiments conducted collaboratively with 13 researchers across eight soybean-producing states in the U.S. I started with the hypothesis that foliar symptoms of FLS and soybean yield were linearly and negatively associated. The results of Chapter 2 supported my hypothesis by showing that a significant slope obtained through random effects meta-analytic models confirmed the negative linear relationship between FLS severity and soybean yield. Additionally, I calculated the overall relative damage coefficient to be 0.51%, indicating that a percent increase in FLS severity would result in a 0.51% yield reduction. I also obtained economic damage thresholds, by using the calculated damage coefficient, for a range of soybean prices and control costs taking into account three different fungicide efficacies representing lower (25%), moderate (50%) and higher (75%) disease control. As expected, the threshold values increased as the control efficacy also increased and were affected by different crop prices and fungicide simulated costs. The economic damage thresholds should never be exceeded throughout the crop season and growers should pay attention to the potential grain yield, cost of fungicide application and soybean price.

In my study, after I identified the potential yield losses caused by FLS, I investigated the best fungicide options to control the disease, the most used strategy to manage FLS. Despite several options available for farmers, the decision of which fungicide to incorporate in the spray programs should take into account current information on their efficacy and yield response. The results presented in Chapter 3 suggest that fungicide efficacy against FLS differs among active ingredients and is decreasing over time, possibly linked to reports of fungicide resistant in the *C. sojina* populations (mainly to QoIs). The best performing fungicide reported in this study was the mixture difenoconazole+pydiflumetofen (DMI+SDHI), and the poorest performing fungicide was pyraclostrobin (QoI). A statistically significant decline in performance was detected for two fungicide mixtures (azoxystrobin+difenoconazole and thiophanate-methyl+tebuconazole) and two single active ingredient fungicides (pyraclostrobin and tetraconazole). These results indicate a possible shift of reduced *C. sojina* sensitivity to DMIs and MBCs, hence, continued monitoring of *C. sojina* population sensitivity to those fungicide classes is critical. I also found greater yield in trials with conditions more favorable for severe epidemics, which could be explained by the more pronounced effect of the fungicides among the treated plots when compared to the nontreated control. Accordingly, the most effective treatments were more likely to be profitable under higher disease pressure and, as expected, the less effective treatment reported the higher risk of not-offsetting the costs.

Furthermore, the use of fungicide applications in low-disease environments does not only increase the risk of fungicide resistance development due to selection pressure, but also might be not profitable. In Chapter 4, I investigated the profitability of applying

fungicides in the absence or under very low levels of FLS in double-crop soybean by using a data set of 22 fungicide trials conducted between 2008 and 2021 across five soybean-producing states in the U.S. Our results showed no significant difference in yield response between the fungicide treatments and the nontreated control. Economic analyses indicated that, due to the lower yield responses, probabilities of breaking even were lower than 50% for all the single fungicide classes, or up to 51% for premixes depending on fungicide cost and soybean price values.

Overall, my research findings provide useful information for regional risk assessment of potential yield loss caused by FLS, and for planning fungicide programs to control this important foliar disease in the United States. Decisions on fungicide planning must take into account not only technical information such as control efficacy and yield return, but also profitability and strategies to mitigate fungicide resistance issues, seeking to preserve the lifespan of site-specific fungicides. A valid strategy to improve FLS management and to reduce the risk of resistance development is to alternate modes of action and to use mixtures of single-site fungicides with multi-site fungicides. Application of fungicides in the absence of disease or under very low-disease environments may not be warranted given the marginal agronomic and economic benefits, and potential risks associated with pathogen fungicide resistance development. Growers should be encouraged to evaluate disease risk prior to making fungicide applications. Finally, the damage functions generated in this study can be incorporated in interactive web apps for risk assessment by simulating different scenarios of disease pressure, potential yields and fungicide efficacies.

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VITA

JHONATAN PAULO BARRO

EDUCATION

- 2020-present** ***PhD in Plant Pathology**
University of Kentucky, Lexington-KY, USA
- 2018- present** ***Doctor of Science in Plant Pathology**
Universidade Federal de Viçosa, Viçosa-MG, Brazil
- 2017-2018** **Master of Science in Plant Pathology**
Universidade Federal de Viçosa, Viçosa-MG, Brazil
- 2010-2016** **Bachelor of Science in Agronomy**
Universidade Federal Fronteira Sul, Erechim-RS, Brazil

* Dual Degree Program

PUBLICATIONS

*Machado FJ, **Barro JP**, Godoy CV, Dias AR, Forcelini CA, Utiamada CM, et al. (2022). Is tank mixing site-specific premixes and multi-site fungicides effective and economic for managing soybean rust? a meta-analysis. **Crop Protection** 105839. <https://doi.org/10.1016/j.cropro.2021.105839>

*Ascari JP, **Barro JP**, Santana FM, Pádua JM, Maciel JLN, [...], Alves KS, Del Ponte EM (2021) Sequential post-heading applications for controlling wheat blast: a nine-year summary of fungicide performance in Brazil. **Plant Disease**, 105: 4051-4059. <https://doi.org/10.1094/PDIS-06-21-1183-RE>.

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* Shared first authorship

AWARDS

- **2022**: APS North Central Division Meeting, Lincoln-NE, USA.
 - 2nd place at the Student Oral Presentation Competition.
- **2021**: 49th Southern Soybean Disease Workers (SSDW) Meeting, online.
 - 2nd place at the Student Oral Presentation Competition.