Frogeye Leaf Spot Information

General information about symptom development: FLS usually develops during reproductive growth stages (blooming to maturity) but may develop sooner in continuous soybean fields and/or under optimal environmental conditions (warm, humid conditions). In fields where soybean has been planted for consecutive seasons, FLS may develop in the lower canopy initially, but with prolonged adequate moisture infection of new leaves that are fully expanded can develop resulting in symptoms throughout the soybean canopy.

Lesions on leaves are angular or circular spots up to 5 mm in diameter with light grey centers and distinct purple to red-brown margins. The leaf spots can be single or coalesce to form larger lesions with irregular margins. Dark areas within lesions on the underside of leaves are clusters of spores (sporulating lesions). These spores (conidiophores) can be used to test if the pathogen is resistant or sensitive to fungicides.

Detailed review of Frogeye Leaf Spot:

Introduction

Frogeye leaf spot (FLS) caused by *Cercospora sojina* Hara is a common foliar pathogen of soybean in the southern United States and regularly present in parts of the Midwestern United States. Since 1999, increased severity and prevalence of FLS have been reported in some north central areas of the United States (Cruz and Dorrance, 2009; Mengistu et al., 2002; Wrather et al., 2003; Yang et al., 2001). FLS is also present in other soybean production areas in the world including Brazil, Argentina, India, and China (Wrather et al., 2010). In the United States warmer winter temperatures, susceptible soybean germplasm, and conservation tillage practices have been proposed as potential causes for resent outbreaks of FLS (Grau et al., 2004). Yield loss from FLS is mainly the result of reduction in photosynthetic area and premature defoliation (Akem and Dashiell, 1994). Over the past 5 years, FLS has been estimated to cause 16% yield loss in Tennessee soybean production (M. Newman, *observation*) totaling 8.2 million bushels and with an average market price of $11.45 per bushel totals over $93.8 million lost due to FLS.

Symptoms, Disease Cycle, and Epidemiology

FLS primarily occurs on soybean foliage, although infection can also occur on stems, pods, and seeds (Phillips, 1999). Lesions are circular to angular spots with diameters ranging from 1 to 5 mm and initially appear as dark, water-soaked spots and develop into brown and gray spots surrounded by narrow, dark-brown borders (Grau et al., 2004; Phillips, 1999). In optimal conditions lesions can coalesce to from larger irregular spots and eventually cause premature defoliation (Phillips, 1999). *C. sojina*, like other *Cercospora* plant pathogens produces a toxin, cercosporin, which has been shown to play an important role in Cercospora diseases of diverse hosts (Daub and Chung, 2007). Cercosporin is a photosensitizer (photoactivated and lacks toxicity in the dark), its toxicity is due to oxidative damage to lipids, proteins, and nucleic acids of cell membranes and it is a potent producer of singlet oxygen (Valenzeno and Pooler, 1987). Exposure of plant cells and tissues to cercosporin results in peroxidation of the membrane lipids, leading to membrane breakdown and death of the cells (Daub and Briggs, 1983). Daub and Chung (2007) hypothesized that membrane damage cause by cercosporin allows for leakage of nutrients into the leaf intercellular spaces, allowing for fungal growth and sporulation.

C. sojina can survive as mycelium in infected seed and infested soybean residue. With current production practices, where new seed is bought each year, it is very rare that FLS occurs due to infected seed, the primary source of inoculum each year is in the soybean residue that is prevalent with conservation tillage. Cruz and Dorrance (2009) reported greater probability for *C. sojina* survival on soybean residue in Ohio when mean monthly temperatures are greater than -4.2°C during December, January, and February. Although more research is still needed to refine this temperature threshold and to include other forecasting variables to better understand the conditions influencing *C. sojina* survival.

Infection and symptom development is favored by warm (25-30°C) and humid (>90% relative humidity) conditions, where sporulation can occur within 48 h of visible symptoms. Primary and secondary inocula are hyaline conidia of 5 to 7 µm by 39 to 70 µm that form on tips of conidiophores and are pushed aside as the conidiophores continue to grow. Conidia can germinate on a leaf surface within an hour of deposition in the presence of water at 25 to 30° with visible lesions developing 8 to 12 days after inoculation (Phillips, 1999).

Conidia can be carried short distances by air currents and rain splashes, where secondary infections can continue throughout the soybean growing season under favorable conditions (Laviolette et al., 1970). The pathogen has not been confirmed to cause disease on any other crop or weed host (Mian et al., 2008).

Management and Isolate Classification

Best management practices of FLS include planting a resistant cultivar, crop rotation, plowing under crop residues, and application of foliar fungicides (Phillips, 1999). Resistant cultivars greatly reduced FLS incidence in the United States until the late 1950s when the development of new virulent races began to emerge. From the late 1950s to 1978 *C. sojina* races 2, 3, 4, and 5 were discovered in the United States based on the virulence on soybean differentials (Athow et al., 1962; Ross, 1968; Phillips and Boerma, 1981; Mian et al., 2008). Currently there are 3 single genes conditionaing resistance to *C. sojina* that are recognized by the Soybean Genetics Committee: *Rcs1* in 'Lincoln' confers resistance to race 1 (Athow and Probst, 1952), *Rcs2* identified in 'Kent' confers resistance to race 2 (Athow et al., 1962), and *Rcs3* from 'Davis' was found to condition resistance to race 5 and all other known races of *C. sojina* in the United States in the early 1980s (Phillips and Boerma, 1982).

In publications since the early 1990s there has been confusion about how many and how to differentiate races of *C. sojina*. Grau et al. (2004) referred to 12 races of *C. sojina* from various states in the United States and indicated that more are likely present. Cruz and Dorrance (2009) reported 20 different pathotypes (races) resulting from evaluating 50 *C. sojina* isolates, collected from symptomatic soybean leaves in Ohio during 2007, on 12 differential soybean lines. Additionally, 22 races have been reported in Brazil (Yorinori, 1992) and 14 races in China (Ma and Li, 1997). The previous reports have all used different sets of soybean differential cultivars to identify the *C. sojina* races. Recently, to attempt to develop a universally accepted set of soybean differential cultivars for the classification of *C. sojina* isolates into races Mian et al. (2008) proposed a core set of 12 soybean differentials and 11 races of *C. sojina* based on the reactions of 93 *C. sojina* isolates on 38 putative soybean differentials.

While the *Rcs3* gene still confers resistance to all known *C. sojina* isolates tested from the United States, past events of the development of new virulent races of *C. sojina* that have overcome single genes for resistance is a looming threat and emphasizes the importance of maintaining the efficacy of other management strategies such as fungicide applications. Application of fungicides has been another method of managing frogeye leaf spot, the first *C. sojina* isolate with resistance to quinone outside inhibitor (QoI) fungicides was discovered in Tennessee in 2010 (Zhang et al., 2012a). Since that time *C. sojina* isolates with resistance to QoI fungicides have been confirmed in 8 states and across 44 different counties/parishes in the United States (Carl Bradley, personal communication). Zhang et al. (2012b) evaluated the *in vitro* sensitivity of 163 *C. sojina* isolates to QoI fungicides: azoxystrobin, pyraclostrobin, and trifloxystrobin; where 55 isolates were from soybean fields prior to QoI fungicide registration on soybean in the United States and 108 isolates collected in 2007, 2008, and 2009. Zhang et al. (2012b) reported a range of effective concentrations that inhibited 50% of conidial germination (EC_{50}) varied from 19-fold to 52-fold among these isolates, depending on the fungicide. The first reported QoI resistant isolates from Tennessee had EC_{50} values that were approximately 249- to 7,144-fold greater than the EC_{50} values of baseline isolates (Zhang et al., 2012a). QoI fungicides inhibit mitochondrial respiration of fungi by binding to the cytochrome *bc1* enzyme complex (complex III) at the Qo site (Bartlett et al., 2002). Resistance has developed to QoI fungicides in multiple pathogens beginning soon after QoIs were

first introduced in 1996 (Bartlett et al., 2002). The majority of QoI resistant pathogens develop from a point mutation in the mitochondrial cytochrome b (*cyt b*) gene, which has been confirmed to be the mutation in QoI resistant *C. sojina* isolates (Carl Bradley, personal communication). The point mutation at codon 143 leads to an amino acid change from glycine to alanine of the *cyt b* gene, which does not have any negative effects on enzyme activity, is the dominant mutation in the *cyt b* gene that confers resistance to QoI fungicides (Bartlett et al. 2002). Other point mutations have been identified, but seem to have a less important role in QoI resistance.

Population Diversity

While results of the aforementioned assays implied that the variability within *C. sojina* appears high due to a range of virulence on soybean differential lines and sensitivity to QoI fungicides, there has been few genetic investigations to support this assumption. The genetic diversity of a historical collection of 62 *C. sojina* isolates was conducted using amplified fragment length polymorphism (AFLP) markers (Bradley et al. 2012). The average genetic similarity of the isolates was 0.56 on a scale of 0 to 1, indicating a high degree of genetic diversity within the species. Very little separation of isolates based on origin was found and cluster analysis resulted in two major clusters and seven sub-clusters. Andris et al. (2012) developed 8 dimple sequence repeat (SSR) markers from genomic sequence of *C. sojina* and were evaluated on 72 isolates from an Arkansas population. All loci were polymorphic with 3 to 6 alleles per locus; polymorphic information content ranged 0.3774 to 0.6053. Also, Nei's gene diversity (*H*) ranged from 0.4785 to 0.6714 per locus. Although the diversity of *C. sojina* has been measured in the aforementioned ways, little is known about the actual genetic diversity within *C. sojina* and how it relates to overcoming single gene resistance in cultivars and the predisposition to develop resistance to fungicides.

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