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# Advancing Our Understanding of Charcoal Rot in Soybeans

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## Abstract

Charcoal rot [*Macrophomina phaseolina* (Tassi) Goid] of soybean [*Glycine max* (L.) Merr.] is an important but commonly misidentified disease, and very few summary articles exist on this pathosystem. Research conducted over the past 10 yr has improved our understanding of the environment conducive to disease development, host resistance, and improved disease diagnosis and management. This article summarizes the currently available research with an emphasis on disease management.

**Key words**: charcoal rot, *Macrophomina phaseolina*, soybean

## Impact of Charcoal Rot on United States Soybean Production

*Macrophomina phaseolina* (Tassi) Goid (Goidanish 1947) causes charcoal rot in soybeans [*Glycine max* (L.) Merr.], and infects ~500 other plant species including agronomic crops, fruits, legumes, and trees (Dhingra and Sinclair 1977, Dhingra and Sinclair 1978, Kunwar et al. 1986). Charcoal rot in soybean was first observed in the United States in 1949 (Young 1949). Since then, *M. phaseolina* has been observed across the United States (Wyllie and Scott 1988), causing significant yield losses in Kansas and the southern United States (Bowen and Schapaugh 1989, Wrather and Koenning 2009). Although yield losses due to charcoal rot vary among years, charcoal rot has ranked among the most important soybean diseases in the United States over the past 20 yr. In 2003 and 2012, charcoal rot was ranked as the second most important soybean disease in the United States, with an estimated yield loss of ~1.9 million and 2.0 million metric tons, respectively (Wrather et al. 2010, Allen et al unpublished data). From 2010 to 2013, charcoal rot was among the top five yield loss-causing soybean diseases in the United States (Allen et al. unpublished data).

Although charcoal rot incidence has increased in the past 30 yr in the northern United States (Birrenkott et al. 1984, Bradley and Río 2003, ElAraby et al. 2003, Yang and Navi 2005, Cummings and Bergstrom 2013), most of the field-based research on this disease has been conducted in the southern United States due to its wider prevalence and annual importance in this region (Gangopadhyay et al. 1970, Shokes et al. 1977, Short et al. 1978, Bowen and Schapaugh 1989, Mengistu et al. 2007).

## Disease Life Cycle and Pathogen Biology

*Macrophomina phaseolina* survives in the soil as hard, black fungal structures, called microsclerotia (Fig. 1; Crous et al. 2006). Microsclerotia germinate between 20° and 40°C, and infect root tissue. The fungus can infect seedlings and young plants as well as mature plants (Short et al. 1978, Bristow and Wyllie 1986, Collins et al. 1991). *Macrophomina phaseolina* produces enzymes and toxins that degrade root tissue, and once infection has occurred, the root and stem tissue is colonized within 2–3 wk (Islam et al. 2012). The extracellular enzymes include amylase, cellulose, hemicellulose, lipase, and pectinase (Radha 1953, Dhingra et al. 1974, Ahmad et al. 2006, Kaur 2012). Although they have been associated with disruption of the cell and membrane wall, the main activity of these extracellular enzymes, their correlation with fungal growth, and

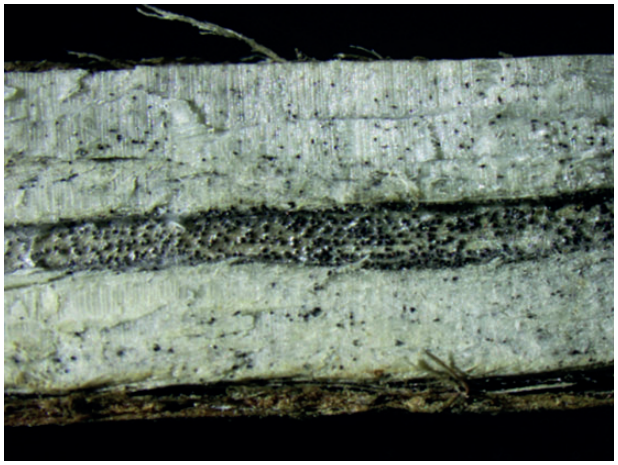

**Fig. 1.** *Macrophomina phaseolina* microsclerotia on stem, pith, and root tissue on soybean stem.

their influence on the infection process are still largely unknown (Schinke and Germani 2012). Two toxins isolated from *M. phaseolina* that are thought to be involved in the infection process are phaseolina and – (-) botryodiplodin. Phaseolina was first identified by Siddiqui et al. (1979), and later described in more detail by Dhar et al. (1982). However, Ramezani et al. (2007) was only able to isolate – (-) botryodiplodin from *M. phaseolina* cultures, but not phaseolina. Further research is needed to confirm which toxins are produced by *M. phaseolina* and how they facilitate infection.

Under conducive environmental conditions, *M. phaseolina* will infect the vascular system, interfering with the normal plant function of transporting water and nutrients to the leaves, causing the common disease symptoms of wilting and premature leaf death (Gupta and Chauhan 2005, Khan 2007). After soybean plants are harvested, microsclerotia in the plant residue return to the soil. It has not been widely studied how long these microsclerotia survive in soil under field conditions. According to Short et al. (1980), *M. phaseolina* survives for almost 2 yr on residue at or below the soil surface; however, this study did not assess *M. phaseolina*'s ability to cause infection after this length of time. Research conducted in Brazil indicated that although microsclerotia could survive for almost 35 mo on infected crop residue, pathogenicity decreased after 6 mo (Fig. 2; Reis et al. 2014).

*Macrophomina phaseolina* is an Ascomycete, and classified in the family Botryosphaeriaceae. The sexual state of *M. phaseolina* has not yet been identified. Pycnidia production has been observed occasionally in different host plants (Dhingra and Sinclair 1978) and in artificial media such as potato dextrose agar (Gaetán et al. 2006) and water-agar-leaf medium (Chidambaram and Mathur 1975, Ma et al. 2010). The role of pycnidia on disease development is not well understood. Conidia are hyaline, ellipsoid to ovoid with dimensions of 14 to 30 × 5 to 10 µm (Crous et al. 2006). Conidia have been shown to infect soybean seedlings under laboratory conditions (Ma et al. 2010), but the role of conidia in the disease cycle in soybean is unknown.

To date there have been no reports of races or specific pathotypes within *M. phaseolina* on soybean. Many studies have been conducted trying to classify variation among isolates; however, only differences in isolate pathogenicity and virulence have been observed (Dhingra and Sinclair 1973, Mihail and Taylor 1995), and recently on regionally adapted cultivars (Sexton et al. 2016). In 1973, Dhingra and Sinclair evaluated the pathogenicity of *M. phaseolina* isolates obtained from stem, petioles, pods, and seed of three different soybean plants, and found that isolates obtained from roots were most pathogenic. Variation among isolates was also observed based on growth rate and colony type. Mihail and Taylor (1995) evaluated the pathogenicity of 114 isolates obtained from bean, spurge (*Euphorbia lathyris*), sunflower, and sorghum under controlled conditions. Although not all isolates were able to infect all crops, all isolates were capable of infection at least one host. In a more recent study, Sexton et al. (2016) evaluated and compared pathogenicity and virulence on 42 isolates collected from soybean in the northern and southern United States. Pathogenicity and virulence were determined on soybean under controlled conditions based on the length of the stem lesion and the lesion growth rate over 9 d after inoculation, respectively. It was observed that northern isolates were more virulent compared to southern isolates on southern cultivars.

Studies on host-specificity of *M. phaseolina* have been inconclusive. Su et al. (2001) did not observe any variation after analyzing *M. phaseolina* isolates obtained from different crops with restriction fragment length polymorphism and random amplified polymorphic DNA markers. Similar findings were reported by Vandemark et al. (2000). They determined that *M. phaseolina* is a heterogeneous species and did not observe any differences in host specificity from isolates collected from different locations and hosts. However, several other studies have shown evidence for morphological differences among isolates from different hosts, including isolates collected from soybean and corn [*Zea mays* (L.)] (Pearson et al. 1987), soybean and sorghum [*Sorghum bicolor* (L.) Moench] (Cloud and Rupe 1991), and soybean and cotton [*Gossypium hirsutum* (L.)] (Jana et al. 2005). Studies by Arias et al. (2011) indicated that from the group of markers identified, the marker associated with pisatin demethylase-P450 had a unique allele for isolates from pumpkin [*Cucurbita pepo* (L.)] and snap bean [*Phaseolus vulgaris* (L.)], and another unique allele for soybean isolates.

In 2012, the *M. phaseolina* genome was sequenced and assembled (Islam et al. 2012). Large areas of synteny were observed with another important plant pathogen, *Fusarium oxysporum* Schltdl., which also has a wide host range (Islam et al. 2012). The authors suggested that since both pathogens infect a large number of plant species, these two pathogens may use similar pathways to infect hosts. Further studies on *M. phaseolina* genome may improve our understanding of its wide host range.

## Symptoms and Signs

Seedlings can be infected at emergence through the early vegetative stages, but symptoms are not typically observed until the R5 (beginning seed) to R7 (beginning maturity) growth stages (Fehr et al. 1971, Meyer et al. 1974, Mengistu 2015). Early symptoms include yellow leaves, reduced vigor, brown to red discoloration on roots and stems, and a general wilting appearance (Gupta and Chauhan 2005, Short et al. 1978). Later foliar symptoms include premature senescence, wilting, and premature plant death (Fig. 3). These symptoms are often confused with foliar symptoms of other diseases and disorders such as soybean cyst nematode (*Heterodera glycines* Ichinohe), drought stress, and early senescence. Plants that die prematurely due to charcoal rot will retain leaves attached to the petiole (Mengistu et al. 2007). The name charcoal rot comes from the gray or silver discoloration observed on the lower parts of the stem and the roots due to the formation of microsclerotia (Gupta and Chauhan 2005, Wrather et al. 2008, Gupta et al. 2012). In the field, charcoal rot symptoms are more frequently observed in stressed areas of the field such as field edges, compacted soils, and hillsides.

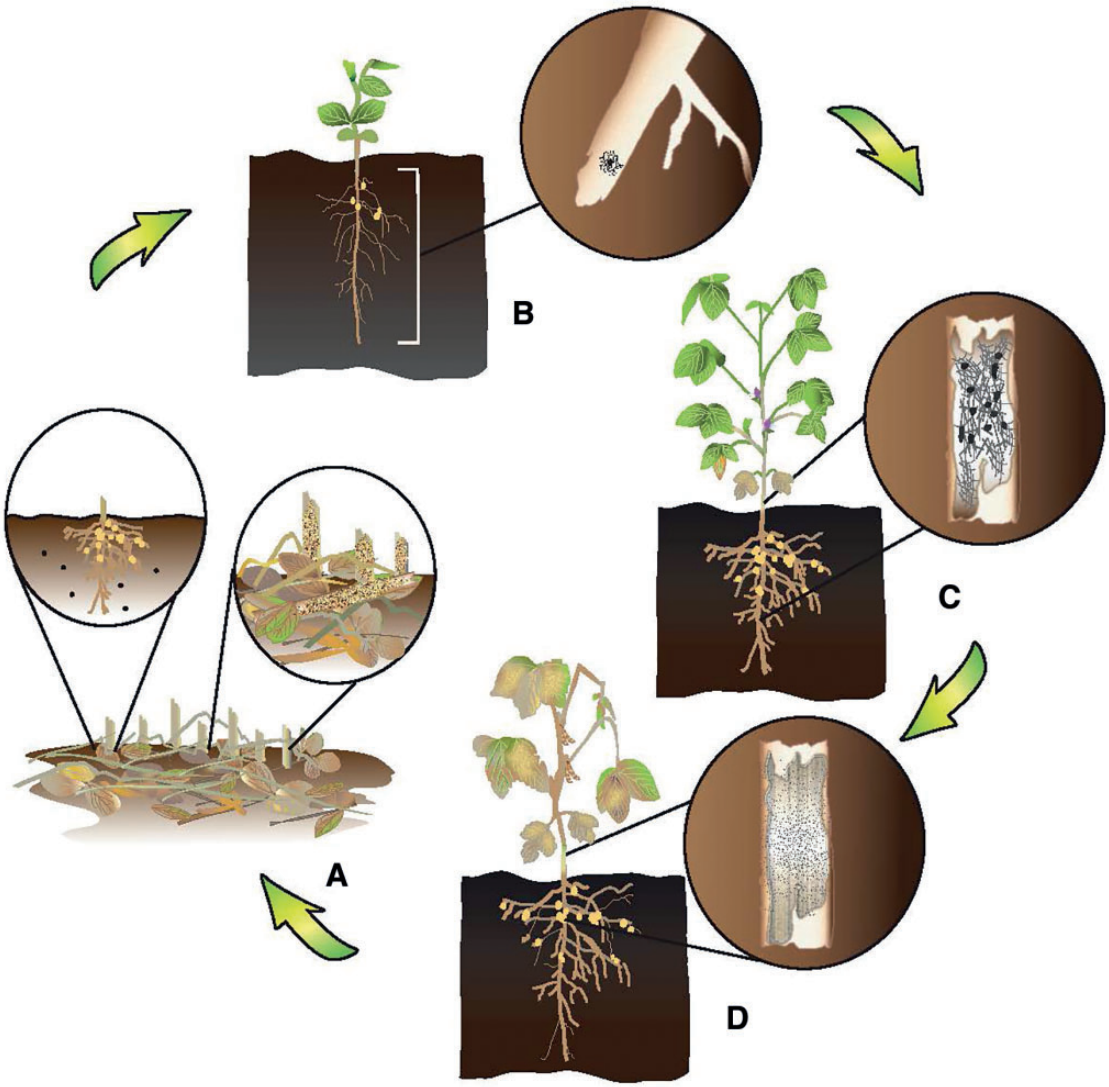


**Fig. 2.** Disease cycle of *Macrophomina phaseolina* (Smith et al. 2014). (**A**) *M. phaseolina* overwintering by microsclerotia in crop residue. (**B**) Infected roots after being in contact or in proximity with microsclerotia. (**C**) Fungus grows within the stem and root affecting and disrupting the vascular system. More microsclerotia are produced. (**D**) Abundant microsclerotia presence on lower stem and taproot tissue gives a charcoal-like appearance. Infected residue will become potential source of inoculum for next planted crop. Image credit: Iowa State University Integrated Pest Management Program.

Symptoms may be more pronounced in years with high temperatures and low soil moisture (Meyer et al. 1974, Mihail 1989). Plant maturity also influences charcoal rot severity (Mueller et al. 1985). Although the mechanism behind this influence of plant maturity on disease severity is not well understood, it is believed that early-maturing plants that flower during cooler temperatures will be less susceptible to high temperature stress that contributes to symptom expression and yield loss (Mihail 1989, Smith and Carvil 1997). Until recently, the association of zone lines (thin black lines in the stem cortex) was considered a symptom of charcoal rot, confusing disease diagnosis (Fig. 4). These lines are actually caused by *Diaporthe longicolla*, and not associated with charcoal rot (Olsen et al. 2015).

## Charcoal Rot Management

Although many studies have been conducted to improve disease management strategies for charcoal rot, farmers currently have limited options to minimize charcoal rot damage.

### Crop Rotation

Based on the wide host range of *M. phaseolina*, crop rotation is not considered as a reliable management practice. Short et al. (1980) found that there was a direct correlation between the population of viable microsclerotia in soil and the number of consecutive years of corn or soybean production, regardless of rotation. However, long-term crop rotation studies observed that even if soybean is rotated with another host of *M. phaseolina*, a long-term rotation will reduce the *M. phaseolina* population compared to continuous soybean planting (Francl et al. 1988, Singh et al. 1990a). It is possible that crop rotation could improve charcoal rot management if long-term rotations including nonhosts are established (Table 1); however, further research is required to confirm the impact of crop rotation on charcoal rot.

### Tillage

Baird et al. (2003) indicated that tillage may reduce charcoal rot severity by accelerating decomposition of microsclerotia or increasing antagonistic interactions. Olanya and Campbell (1988) found that

**Fig. 3.** Foliar symptoms of charcoal rot on soybean seen at the front of the image include premature senescence and plant death while leaves remain attached to the petioles.

**Fig. 4.** Zone lines (top) are a sign of diseases caused by *Diaporthe longicolla* and not associated with charcoal rot, which is characterized by the formation of microsclerotia in root and stem tissue (bottom).

one tillage pass to a depth of 15 cm reduced *M. phaseolina* populations; however, the efficacy of this practice will vary according to the initial quantity of microsclerotia present in the soil. In a later study, Wrather et al. (1998) observed no differences on charcoal rot infection in soybean regardless of whether a conventional tillage or conservation tillage system was implemented, and concluded that tillage did not reduce *M. phaseolina* infection. Most recently, Mengistu et al. (2009) reported that colony-forming units of *M. phaseolina* in soybean tissue were greater under tillage than in no-tillage and suggested that charcoal rot may be better managed in a no-tillage system. Overall, the impact of tillage on charcoal rot has been inconsistent, and it has been difficult to establish tillage as a reliable practice for charcoal rot management.

## Irrigation

Although *M. phaseolina* can infect under a wide range of environmental conditions, yield loss and high disease severity are often linked to areas where soils are dry or drought occurs throughout the reproductive stages of soybean growth (Mengistu et al. 2011a). In these situations, irrigating soybean to reduce drought stress can limit yield losses due to charcoal rot, even though losses may still range from 6 to 30%. In 2000, Kendig et al. observed that although disease still occurs under irrigation, it can be limited by water management. In this study, *M. phaseolina*-infected plants under water stress at the beginning of the season had higher disease severity compared with infected plants that were not water stressed early in the season. More recently, Mengistu et al. (2011a), observed that although infection occurred under both irrigated and nonirrigated

**Table 1.** List of several common agricultural crop hosts of *M. phaseolina*

| Common name | Latin name | Reference |
|---|---|---|
| Corn | *Zea mays* L. | Livingston 1945 |
| Sorghum | *Sorghum bicolor* L. Moench | Livingston 1945 |
| Soybean | *Glycine max* L. Merr | Young 1949 |
| Chickpea | *Cicer arietinum* L. | Dhingra and Sinclair 1977 |
| Cotton | *Gossypium hirsutum* L. | Dhingra and Sinclair 1977 |
| Peanut | *Arachis hypogaea* L. | Dhingra and Sinclair 1977 |
| Sunflower | *Helianthus annuus* L. | Yang and Owen 1982, Khan 2007 |
| Snapbean | *Phaseolus vulgaris* L. | Arias et al. 2011 |

environments, higher yields were reported under irrigated environments. Therefore, limiting water stress through scheduled irrigation throughout the season may reduce yield loss due to charcoal rot.

## Fertility and Plant Nutrition

There are few studies that provide information on the interaction of soil fertility on the severity of charcoal rot on soybeans. Mengistu et al. (2016) studied the effect of phosphorus and potassium on the severity of charcoal rot, and showed that *M. phaseolina* populations were not significantly impacted by phosphorus and potassium applications in no-till soils, and were not sensitive to routine phosphorus and potassium fertilizer application based on soil testing. The study suggested that soybean farmers should still apply phosphorus and potassium based on crop need without regard to potential effects of charcoal rot severity.

## Weed Control

Competition for water and other resources can stress soybean plants. Therefore, implementing recommended weed management programs is key to reducing the number of factors that contribute to stress in the soybean crop (Mengistu et al. 2015). Several studies have been conducted under greenhouse and field conditions to determine the effect of herbicides on stem and root colonization of soybeans by *M. phaseolina* (Edgar Filho and Dhingra 1980, Cerkauskas et al. 1982, Canaday et al. 1986). Some herbicides affect colonization of soybean plants by *M. phaseolina*; however, glyphosate did not affect *M. phaseolina* colonization (Canaday et al. 1986). Mengistu et al. (2013a) observed that glyphosate inhibited *M. phaseolina* growth in vitro, but under field conditions disease severity was only reduced when single glyphosate applications were performed on soybeans at early to mid-vegetative stages (V3 and V6) in a tilled environment.

## Soybean Cyst Nematode Management

The role of *H. glycines*, the cause of soybean cyst nematode, on charcoal rot development is variable. One study indicated that *H. glycines* increases root colonization by *M. phaseolina* (Todd et al. 1987), but others observed no interaction under field conditions when both organisms were present (Francl et al. 1988). Smith and Carvil (1997) did not observe an increase in either disease on soybean cyst nematode-resistant or susceptible genotypes when screened in the presence of *M. phaseolina*. In many areas of the United States, it is important to manage both diseases by selecting cultivars resistant to both soybean cyst nematode and charcoal rot.

## Fungicides

Early research on the efficacy of fungicides for charcoal rot management have focused on the use of soil fungicides that reduced microsclerotia under field conditions (Watanabe et al. 1970, Ilyas et al. 1976, Kittle and Gray 1982); however, some older chemicals induced a phytotoxic reaction in plants (Ilyas et al. 1975). Studies on fungicide efficacy and disease management have observed a reduction on *M. phaseolina* infection on roots and seedlings when soybean seed received a treatment with captan, thiram, carbendazim, thiram + carbendazim, or thiram + carboxin (Vir et al. 1972, Singh et al. 1990b, Kumar and Singh 2000, Gupta and Chauhan 2005); however, these fungicides are not commonly used in modern commercial seed treatments. Further studies are needed to test more recent fungicide active ingredients in seed treatments against charcoal rot.

## Biocontrol

Several past studies have studied the impact of antagonistic organisms on charcoal rot. Seed treated with *Rhizobacterium* strain FPT721, a plant growth-promoting rhizobacteria, reduced disease incidence under controlled conditions (Choudhary 2011). Senthilkumar et al. (2009) observed inhibition and disease reduction on *M. phaseolina* when two soybean bacterial endophytes, *Bacillus* (strain: HKA-121) and *Paenibacillus* (strain: HKA-15), were tested against *M. phaseolina* under in vitro and vivo conditions, respectively. However, these biocontrol strains are not commercially available for use in soybean production.

## Cultivar Selection

With the limited options available for farmers to manage charcoal rot, most research has focused on the development of genetic resistance to charcoal rot. These studies include the development of efficient and reliable methods to characterize resistance, as well as the development of resistant cultivars. Although only moderately resistant cultivars are currently commercially available, significant progress has been made to better understand the genetic resistance behind charcoal rot. In recent years, many studies have been conducted under controlled and field conditions to determine charcoal rot-resistant soybean cultivars. Over 865 soybean genotypes have been screened for charcoal rot resistance, and of these genotypes, 23 were identified as having moderate resistance against charcoal rot (Mengistu et al. 2007, 2011b, 2013b; Pawlowski et al. 2015). Currently, charcoal rot resistance is available in a few publicly available soybean cultivars adapted primarily to southern maturity groups (Table 2).

## Screening Methods

Early screening methods focused on identifying resistance by measuring *M. phaseolina* colonization in the entire root system (Pearson et al 1984). Later, Smith and Carvil (1997) developed a colony-forming unit assay, where microsclerotia are quantified from stem and root. This method consisted of collecting the lower stem and taproot of arbitrarily selected plants, and plating ground, dried samples on selective media. Although these screening methods provided valuable information, results from these assays were variable and there was inconsistency among studies. In 2007, Mengistu et al. studied five different assessment methods in order to establish a reliable and consistent field method for charcoal rot screening evaluations, and proposed the use of a colony-forming unit index (CFUI) as a standard method for resistance screening. This method consisted on collecting lower stem and roots of soybean plants and

**Table 2.** Publicly available soybean lines rated as moderately resistant to charcoal rot, caused by *M. phaseolina*

| Genotype | Maturity Group | Reference |
|---|---|---|
| DG3905 | III | Mengistu et al. 2011b |
| Manokin | IV | Mengistu et al. 2011b |
| DT97-4290 | IV | Paris et al. 2006 |
| DT99-16864 | V | Mengistu et al. 2007<br>Mengistu et al. 2011b<br>Gillen et al. 2016 |
| DT99-17483 | V | Mengistu et al. 2007<br>Mengistu et al. 2011b |
| DT98-7553 | V | Mengistu et al. 2007<br>Mengistu et al. 2011b |
| DT99-17554 | V | Mengistu et al. 2007<br>Mengistu et al. 2011b |

plating ground, dried samples on sterilized selective media containing PDA, rifampicin, and tergitol, and incubated for 3 d at 30 °C. After 3 d, colonies are counted and converted to CFU per gram of root and stem tissue. The CFUI is later obtained by dividing the CFU for each genotype by the CFU for the genotype that has the highest CFU in the current study. According to Mengistu et al. (2014) this method allows researchers to consistently determine reactions to *M. phaseolina* across different cultivars and environments under field conditions. In 2012, Twizeyimana et al. developed a cut-stem inoculation technique where soybean cultivars could be evaluated under greenhouse conditions in a consistent and reliable manner. Disease severity is measured by the extent of necrosis that a plant presents from the inoculation point. This technique offers the advantage of quantifying the amount of inoculum in each plant tested as well as providing a precise and less time consuming measurement of disease severity, and so far it has successfully distinguished susceptible and resistant cultivars for charcoal rot in multiple studies (Mengistu et al. 2015, Sexton 2016) under greenhouse environments.

In 2016, Sexton et al. screened northern and southern *M. phaseolina* isolates on regionally adapted cultivars under greenhouse conditions. They observed regional differences between *M. phaseolina* populations and concluded that soybean cultivars should be screened under local conditions with local *M. phaseolina* isolates (Sexton et al. 2016). Ma et al. (2010) developed a *M. phaseolina* conidia production method which consisted of soaking filter paper disks in peanut butter extract supernatant. The pretreated filter papers are placed on the surface of a soynut butter extract agar sterile plate. This conidia production method and the previous inoculation technique is proposed for evaluating resistance of soybean cultivars to different isolates. This method could facilitate cultivar screening against local *M. phaseolina* isolates that was previously suggested by Sexton et al. (2015). In 2011, a quantitative DNA assay was developed to detect *M. phaseolina* from infected tissues and soil using either TaqMan or SYBER Green (Babu et al. 2011). These assays provide a reliable and accurate method for quantifying the level of pathogen in soil or plant tissue, which is likely to aid in screening assays for genetic resistance.

The soybean genome was sequenced in 2010 (Schmutz et al. 2010). Although no information on quantitative trait loci studies or genome-wide association mapping is currently available for charcoal rot in soybean, research on common bean (Hernandez-Delgado et al. 2009) has identified three novel QTL and one QTL for charcoal rot resistance, respectively. Further research will provide needed information for breeders to develop soybean cultivars with improved levels of charcoal rot resistance by identifying resistance alleles that could be combined in moderately resistant cultivars. Improving drought tolerance through genetic resistance could also provide new possibilities for controlling charcoal rot. The combination of these studies with the traditional screening methods will likely improve resistance to charcoal rot.

## Overall Recommendations for Disease Management and Future Research Needs

Effective charcoal rot management requires an integrated approach. Farmers who have fields with a history of charcoal rot, or are in areas prone to charcoal rot development should select cultivars with the best available commercial resistance. Cultural management practices that minimize plant stress during the reproductive growth stages will also help limit damage from charcoal rot. Planting at recommended plant populations, using adequate fertility and tillage practices, controlling weeds, and irrigating where possible can help mitigate stress in soybeans, and consequently reduce the impact of charcoal rot.

Research on charcoal rot management will improve in the coming years, as there are have been advances in understanding the role of resistance genes in expression of cultivar resistance, molecular interactions between the host and the pathogen, and a better understanding of the role of environment in disease development. This information will improve our ability to screen for resistant genotypes in maturity groups adapted to each region of the United States. By advancing our knowledge of this disease, we can minimize the impact of charcoal rot on soybean yield.

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