Studies on Wheat Scab in Alabama

by

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Abstract

Wheat scab is one of the more destructive diseases of wheat in the US. In Alabama, scab was not considered a problem in the past; however, during recent years it has been reported in north Alabama. The overall goal of this study was to address the prevailing status and management of wheat scab in Alabama. The specific objectives were: 1) to evaluate the efficacy of fungicides in control of wheat scab and foliar diseases of wheat in Alabama; 2) to evaluate perithecial development by Gibberella zeae on wheat straw; and 3) to determine the distribution of wheat scab incidence across Alabama and collect and characterize Fusarium species associated with the disease. For the first objective, fungicide trials were conducted for two years at each of three locations. Fungicides were evaluated for controlling scab as well as foliar diseases of wheat, which were effective in reducing foliar diseases in the areas with relatively high disease pressure, and had a consistently positive effect in improving yield components. Concentration of the mycotoxin, deoxynivalenol (DON), was assayed for wheat samples in 2012, where only a few samples had detectable levels of DON. To address the second objective, wheat straw were incubated in different soils with varying levels of moisture and temperature. Incubated straw were later inoculated with G zeae and observed for perithecial development. Results showed greater perithecial development on straw incubated in finer textured soils from Tennessee Valley (Decatur silt loam) or Plant Breeding Unit (Independence loamy fine sand) compared to a coarse soil from Headland

(Dothan sandy loam). Fewer perithecia developed on straw incubated for longer durations and at higher soil moistures or at higher temperatures. Results infer that crop residues in soil under warmer conditions with more rainfall, as in south Alabama provide less support for scab inoculum than residues in cooler and drier conditions more common in north Alabama. For the third objective, random wheat fields across the state were inspected for the presence of scab. Greater scab incidence was frequently noted in north Alabama, while a low incidence of scab was noted in central and southwestern Alabama and no disease was found in southeastern AL. Morphological characterization of the isolates showed that the fungal species associated with wheat scab in Alabama is *F. graminearum*.

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I. Introduction and Literature Review

Wheat

Wheat (*Triticum aestivum* L.) is one of the most important cereal crops grown in the world and can be cultivated over a wide range of climates with year-round production (Atwell, 2001). Wheat is well adapted to harsh environments, from severe dry to cold conditions, compared to other major cereal crops, e. g., rice and corn (Schery, 1969). The center of origin of wheat is presumed to be near present-day Turkey. Findings suggest that wheat was grown in the Nile Valley around 500 B.C. and had extended through India, China, and England during the same time (Schery, 1969). In the US, wheat was grown for the first time in 1602, on an island off the Massachusetts coast (Schery, 1969).

Currently, wheat is ranked as the third largest grain crop after corn and rice in terms of world production (653 million MT annually) (FAO, 2012a). China, India, USA, Russia, and France are the leading wheat-producing countries; the US ranks third among these (FAO, 2012b). Wheat is the principle cereal crop grown in the US, ranking third both in terms of quantity (60 million MT), value (\$8.5 billion) and total exports after corn and soybean (FAO, 2012c). The US is the largest exporter of wheat in the world, which exports 50% of its total annual production (FAO, 2012d).

Wheat, in the US, is classified as spring or winter wheat on the basis of its growth season. In addition, wheat is classified by grain hardness, shape and color, such that there are six different classes: hard red winter, hard red spring, soft red winter, durum, hard

white, and soft white wheat. The US is unique in that every wheat class can be cultivated in some part of the country (Atwell, 2001).

Winter wheat is generally planted in autumn and is harvested from late spring to early summer. Winter wheat alone represents 70-80% of the total US wheat production (USDA-ERS, 2013). All winter wheat cultivars need some period of exposure to near-freezing temperatures known as vernalization. Spring and durum wheats do not require vernalization (Atwell, 2001). Generally, three to seven weeks of vernalization is considered sufficient for most winter wheat cultivars (Mahofoozi *et al.*, 2001).

Soft red winter wheat comprises 15 - 20% of total US wheat production (USDA-ERS, 2013) and is cultivated through the Ohio and Mississippi river valleys, and in southeastern states including Alabama (Atwell, 2001). Soft red winter wheat has medium protein content and is primarily used in cakes, cookies, crackers and other batter-based products (Atwell, 2001; Runge, 2012). The flour from this type of wheat cannot be used to produce highly elastic products, which includes bread and pasta products (Atwell, 2001).

Wheat production in Alabama

Alabama has a mild climate with a slightly increasing temperature and rainfall from the north to south parts (USDA-NASS, 2011). In 2011, Alabama produced 387,417 MT (14,235,000 bu) of wheat, valued at \$97,510,000 from 78,914 hectares (195,000 acres). Alabama has a higher yield for winter wheat at 4.9 MT/ha (73 bu/A) than the national yield of 3.11 MT/ha (46.2 bu/A). Alabama ranks as the 28th state in total winter wheat production (MT) among all states (USDA-NASS, 2011).

Wheat in Alabama is grown for grain, grazing (Mask *et al.*, 1997), livestock feed (Runge, 2012), and as a winter cover crop (Bowen *et al.*, 2003). It is the 5th largest crop cultivated in Alabama in terms of total acreage (USDA-NASS, 2011). The recent past five years state-wide yield average (2008 to 2012) has been 4.2 MT/ha (62.6 bu/A) which is greater than the 5 year average yield of the 10 preceding years (2002-1998) by 1.07 MT/ha (16 bu/A) (Runge, 2012). The increasing yield of soft red winter wheat over recent years has positioned this crop as an important crop rotation option for this region (Runge, 2012).

Wheat is successfully grown throughout Alabama. However, wheat cultivation is concentrated in the far northern and southern counties of the state (USDA-NASS, 2009). It is grown only in few counties in central Alabama. Limestone, Madison, Baldwin, and Lauderdale are the leading counties in wheat acreage (USDA-NASS, 2009). Traditionally, wheat is planted earlier (October 15 - November 10) in northern as compared to southern Alabama (November 15 - December 1) (Ortiz, 2012).

Wheat diseases in Alabama

The warm and humid climate of Alabama during spring months supports the development of foliar and seed-borne diseases of wheat (Hagan *et al.*, 1990). Septoria blotch, leaf rust, powdery mildew, loose smut, and barley yellow dwarf (BYD) are major and common diseases of wheat grown in Alabama and are statewide in distribution (Hagan, 2012). Among these, rust diseases (leaf rust and stripe rust) and Septoria blotch have been reported to cause significant yield losses throughout the state (Bowen and Burch, 2007). Other diseases such as stem rust, black chaff, wheat spindle streak mosaic,

and soil-borne wheat mosaic are occasionally found. Some diseases are found in specific locations and are not found elsewhere. For example, stripe rust can be found occasionally in the Tennessee Valley, take-all is common to the Tennessee Valley, and soil borne wheat mosaic can sometimes be found in south Alabama but not in other regions. Wheat scab (Fusarium head blight) is mainly found in northern Alabama, particularly in the Tennessee Valley, and is found at lower levels in southern Alabama (Glass *et al.*, 2012; Hagan, 2012).

Wheat Scab in Alabama

Wheat scab had not been considered a major problem in Alabama wheat production (Gazaway, 1997). Unlike the states in the northern Great Plains and the central US, there are no reports of significant yield losses or mycotoxin contamination in Alabama wheat due to scab. In recent years, however, destructive scab outbreaks have been reported from northern parts of Alabama, especially in the Tennessee Valley (Hagan, 2011). In foliar fungicide trials done in north Alabama, wheat scab has been reported at minimal levels of intensity (Bowen *et al.*, 2011; Burch and Bowen, 2010). Although this disease has not been noted in southern parts of Alabama, Gale *et al.* (2011) collected isolates of the wheat scab pathogen, *Fusarium graminearum*, from Autauga and Baldwin counties in central and south Alabama, respectively. Apparently, wheat scab occurs more often in northern Alabama and can be found less often and at lower intensities then in southern parts of the state (Hagan, 2012).

Introduction and history of wheat scab (Fusarium head blight)

Wheat scab, also known as Fusarium head blight or ear blight, is one of the more destructive diseases of wheat worldwide (Osborne and Stein, 2007). The disease is responsible for reduction in yield and grain quality of wheat due to *Fusarium* incited damage to kernels and contamination of grain with various mycotoxins (McMullen *et al.*, 1997b). Wheat scab was first reported in 1884 in England (Arthur, 1891) and was recognized as a key problem of wheat and barley during the early twentieth century (Stack, 2003). Since then, wheat scab has been reported from various cereal crop growing areas of the world (McMullen *et al.*, 1997b). In the US, it was reported for the first time in Ohio in 1890 (Arthur, 1891).

Wheat scab is regarded as one of the most destructive plant diseases occurring in the US (McMullen *et al.*, 1997b; Windels, 2000). Since 1917, various scab outbreaks reported in the US have caused significant economic losses (Atansoff, 1920; McMullen *et al.*, 1997b; Stack 2003) with the most devastating epidemics recorded between 1991 to 1996 in various winter and spring wheat production regions (McMullen *et al.*, 1997b). During that period, losses attributed to scab in wheat and barley reached an estimated \$3 billion (Windels, 2000). Since then, scab has caused sizable losses around the northern Great Plains and the central US (Nganje *et al.*, 2002). Over time, wheat scab has also appeared as a serious threat in the Mid-Atlantic and the Southeastern US (Cowger and Sutton, 2005).

Causal organisms and geographical distribution. Wheat scab is caused by several species of *Fusarium* and two species of *Microdochium* (Parry *et al.*, 1995; Simpson *et al.*, 2001). The major *Fusarium* species that incite scab include *F*.

graminearum, F. culmorum, F. avenaceum, and F. poae (Edwards et al., 2001; Parry et al., 1995). The genus Microdochium contains two species: M. nivale and M. majus (Glynn et al., 2005). Fusarium species are responsible for producing mycotoxins, however, Microdochium is considered non-toxigenic (Simpson et al., 2001). Despite differences in pathogenicity and mycotoxin production, symptoms of wheat scab incited by any of the above pathogens are indistinguishable (Simpson et al., 2001).

Wheat scab pathogens can be distinguished from each other morphologically on the basis of colony color, size, shape, and septation of macroconidia, presence or absence of microconidia, chlamydospores and perithecia, as well as with the help of molecular tools (Kammoun *et al.*, 2009). Macroconidial structures are usually used to differentiate species as they are easy to distinguish on the basis of size, shape and segmentation (Summerell *et al.*, 2003). For example, *F. graminearum* macroconidia have five to six septae, and are long and narrower than those of *F. culmorum* which have three to four septae, and are thick and bluntly pointed at the apex. Similarly, macroconidia of *F. poae* are falcate to lunate in shape, with three to five septae, and have a curved and tapered apical cell (Leslie and Summerell, 2006).

Morphological methods have commonly been used to identify *Fusarium* species (Leslie and Summerell, 2006). However, because of the simplicity, speed and reliability of DNA sequencing, molecular techniques are preferred for *Fusarium* identification (Geiser *et al.*, 2004; Summerell *et al.*, 2003). Such nucleic acid based techniques include restriction enzyme digests of genomic DNA to the sequence analysis of specific gene or gene segments (Summerell *et al.*, 2003). Genomic sequencing of amplified segments of one or more commonly used genes such as translation elongation factor-1α (TEF), α–

tubulin, β-tubulin, or histone H3 can help in the differentiation of most *Fusarium* species (Chakraborty, *et al.*, 2006; Geiser *et al.*, 2004; O'Donnell *et al.*, 2004). The DNA sequences obtained from the amplification of these genes can be compared with that of related strains in a standard database and are assigned a species name based on evolutionary distance and relationship (Summerell *et al.*, 2003). Identification of known species can be obtained by using gene sequences from TEF region in a query of the publicly available BLAST database, known as FUSARIUM-ID (Geiser *et al.*, 2004). In addition, species specific polymerase chain reaction (PCR) methods are also being widely used to identify the *Fusarium* species (Abedi-Tizaki and Sabbagh, 2012; Demeke et al., 2005; Leslie and Summerell, 2006).

Until 2000, members of the *F. graminearum* species complex (*Fg* complex) causing wheat scab were considered as a single cosmopolitan species – *F. graminearum* (Telomorph = *Gibberella zeae*) (O'Donnell *et al.*, 2000). Recently, however, as many as 13 phylogenetically distinct species have been identified within the *Fg* complex by the use of genealogical concordance phylogenetic species recognition and molecular marker technologies combined with high-throughput multilocus genotyping (O'Donnell *et al.*, 2000; O'Donnell *et al.*, 2004; O'Donnell *et al.*, 2008; Starkey *et al.*, 2007; Yli-Mattila *et al.*, 2009).

Fusarium graminearum is the major causal organism, followed by F. poae, associated with wheat scab in North America (Gale et al., 2007; Wilcoxson et al., 1988; Zeller et al., 2004) while F. poae, F. avenaceum, F. sporotrichoides, and M. nivale, along with F. graminearum are associated with scab in wheat and barley in Europe (Brennan et al., 2003; Parry et al., 1995). Among these, only F. graminearum and F. culmorum are

considered to be highly pathogenic (Brennan *et al.*, 2003). Species with lower pathogenicity can still play an economically important role in disease occurrence by aiding in the production of mycotoxins with or without association with the major pathogens (Salas *et al.*, 1999). For example, *F. poae* can aid in disease development by early colonization of the host prior to establishment of *F. culmorum* or *F. graminearum* (Sturz and Johnston, 1983).

Based on the sexual stage, Gibberella zeae (Schwein) Petch (anamorph: Fusarium graminearum Schwabe), the major causal agent of wheat scab can be classified in the Kingdom Fungi, Phylum Ascomycota, Subphylum Pezizomycotina, Class Sordariomycetidae, Hypocreomycetidae, Order Hypocreales, Subclass Family Nectriaeae, and Genus Gibberella (Goswami and Kistler, 2004). F. graminearum, in addition to affecting wheat, can cause scab on barley (Hordeum), rice (Oryza), oats (Avena), and Gibberella stalk and ear rot disease in corn (Zea) (Goswami and Kistler, 2004).

The different members of the fungi causing wheat scab may have different biological and environmental requirements, because of which their occurrence and frequency also differ according to geographic location (Osborne and Stein, 2007). Temperature plays a major role in *Fusarium* distribution (Parry *et al.*, 1995). For example, *F. graminearum* is responsible for scab in warmer regions of the world such as the US, Australia and central Europe, whereas *F. culmorum* predominates in the cooler parts of northwestern Europe (Parry *et al.*, 1995). *F. graminearum* can grow well in temperatures up to 30°C, while *F. poae*, a cool temperature fungus, is active at 20°C. Compared to other species, *F. graminearum* can be found over a wide range of

temperatures and moisture conditions, preferring warmer and wetter weather patterns relative to other species (Osborne and Stein, 2007).

Species prevalence can vary over time because of several factors such as changing climate, crop rotation, cultivar resistance, and interactions among different species (Xu et al., 2005). In Europe, the predominant species varies among F. graminearum, F. poae, F. avenaceum, F. culmorum, M. nivale, and M. majus (Xu et al., 2005). In some parts of Europe, species such as F. culmorum is being displaced by F. graminearum (Waalwijk et al., 2003). In the US, it has been reported that F. graminearum consists of a diverse population with different chemotypes indicating the introduction and movement of distinct genetic populations (Gale, 2003).

In addition to climatic factors, cultural practices can also affect species abundance. Over a four year period in Belgium, Isebaert *et al.* (2009) reported that *F. graminearum* and *F. culmorum* were the species most frequently associated with wheat scab. Their finding suggested that *F. graminearum* predominated in the areas where corn (*Zea mays*) was cultivated, while *F. culmorum* prevailed in areas where small grains were planted.

Fusarium graminearum life cycle and mode of dispersal. G. zeae is a perithecium-producing homothallic ascomycete, able to outcross (Guenther and Trail, 2005). G. zeae produces two types of spores: sexual spores called ascospores and asexual spores called macroconidia (Markell and Francl, 2003). Ascospores are produced in purplish-black sexual fruiting bodies called perithecia while macroconidia are produced in pinkish spore masses called sporodochia (Sutton, 1982). Both ascospores and macroconidia play a role in causing the disease (Markell and Francl, 2003).

Infested crop debris is the primary inoculum source as it supports the saprophytic growth of the fungus (Guenther and Trail, 2005). In a favorable warm and moist environment, development of conidia and perithecia occurs, which later give rise to the production and forced dispersion of ascospores towards the wheat head (Markell and Francl, 2003). Sticky ascospores get discharged from mature perithecia onto crop debris (Trail *et al.*, 2005) and are carried to the wheat head by wind, rain, or by insects (Parry *et al.*, 1995).

Usually, discharge of ascospores occurs 2 to 5 days after a rain event (Inch *et al.*, 2005). It is thought that the ejection distance from the perithecia is very small, only a few millimeters in height, and that ascospore and conidia dispersal occurs with the assistance of air movement (Trail *et al.*, 2005) and rain splash (Paul *et al.*, 2004), respectively. In addition to the discharge of ascospores from crop residue to wheat heads, long distance spore dispersal via air currents has also been reported (Schmale *et al.*, 2006). Frequency of precipitation, humidity level, and temperature play a significant role in numbers of airborne ascospores of *G. zeae* (De Wolf *et al.*, 2003; Gilbert and Tekauz, 2000; Paulitz, 1996).

Weather conditions, particularly temperature and moisture, have a major impact on ascospore release and head infection. If relative humidity (RH) is less than 80%, ascospore release may not occur (Gilbert and Tekauz, 2000). After ascosspore release, and upon landing on a host plant, weather conditions can affect infection. Temperatures between 15 and 30°C coupled with extended periods (48 - 72 hours) of RH greater than 90% are considered the ideal conditions for infection (De Wolf *et al.*, 2003).

Wheat heads are most susceptible to infection during anthesis (Sutton, 1982). Direct entry of scab fungi occurs via stomata and underlying parenchyma, partially or fully exposed anthers, openings between the lemma and palea of the spikelet during dehiscence, and the base of wheat glumes, which have a thin walled epidermis and parenchyma (Bushnell *et al.*, 2003). Hyphae grow outside the florets and glumes and later penetrate the inflorescence through stomata and other infection sites (Bushnell *et al.*, 2003). Anthers, stigmas, and lodicules are easily colonized following floret colonization (Goswami and Kistler, 2004). The vascular bundles in the rachis and rachilla are the medium for fungal spread from floret to floret or between spikelets (Ribichich *et al.*, 2000). Mycelia spread to the exterior surface of the glumes, lemma, and palea during wet weather (Bushnell *et al.*, 2003). Thus, the fungus has a brief biotrophic relationship with the plant before entering the necrotrophic phase, which is characterized by increased vigor of the fungus, death of the host and thorough colonization over the host surface (Goswami and Kistler, 2004).

Signs and symptoms. Wheat heads infected by scab fungi develops brown, dark purple to black necrotic lesions on the exterior surface of the florets and glumes. Just below the inflorescence, the peduncle may also show brown or purple discoloration. The awns can become deformed, twisted, and curved downward (Goswami and Kistler, 2004). Symptoms of wheat scab are generally followed by signs of the causal fungus, which includes purple to black perithecia and/or pink sporodochia on heads, especially on glumes (Osborne and Stein, 2007).

Wheat spikelets, when infected with *F. graminearum*, are water soaked and appear straw colored due to loss of chlorophyll. Florets become sterile and result in

poorly filled grains (Sutton, 1982). Later, if there is warm and humid weather favorable for the fungus, abundant masses of pinkish-red mycelium and conidia develop (Kopke *et al.*, 2007).

Economic and quality losses due to scab

Losses due to scab have been reported from all over the world wherever cereal crops are grown (McMullen *et al.*, 1997b). Wheat scab affects the economy of wheat producers in several ways, among them, the major damages are associated with reduction in yield and mycotoxin contamination of the grains (Goswami and Kistler, 2004; McMullen *et al.*, 1997b). Yield and quality losses occur due to floret sterility along with bleached and partially filled light-weight kernels (Goswami and Kistler, 2004). Reduced yield and kernel quality have been responsible for heavily discounted farm-gate prices for scab-damaged wheat (Windels, 2000) as well as creating problems during the storage, shipping, and milling of these kernels (McMullen *et al.*, 1997b).

Mycotoxin production. Toxic secondary metabolites produced by certain fungi while growing in plants and their residues, are called mycotoxins (Kopke *et al.*, 2007). Apart from yield loss, the effects of wheat scab are magnified due to mycotoxin production by the causal fungi (Osborne and Stein, 2007). Mycotoxins may be utilized by fungi as an aid in the pathogenesis or as defense chemicals against other microorganisms but can be toxic to animals (Schmale and Bergstrom, 2003).

The *Fusarium* species complex that cause wheat scab produce a number of mycotoxins that vary in their toxicity to humans and animals (Leslie and Summerell, 2006; Trail *et al.*, 2005). These are trichothecene mycotoxins such as deoxynivalenol

(DON) with its acetyl derivatives (3-ADON and 15-ADON) and nivalenol, zearalenone (Leslie and Summerell, 2006; Trail *et al.*, 2005), aurofusarin, fusarin, and moniliformin (Trail *et al.*, 2005). However, in the US, DON is the predominant mycotoxin associated with scab (Trail *et al.*, 2005). Most of causal fungi produce mycotoxins in varying degrees except *M. nivale*, which produces only disease symptoms similar to those caused by other *Fusarium* species (Simpson *et al.*, 2001). As the fungi grow in the host they begin to produce mycotoxins which are water soluble and can be translocated between tissues or leached from the source tissue (Osborne and Stein, 2007).

Scabby grains can contain significant levels of trichothecenes and the oestrogenic mycotoxin, zearalenone, which are toxic to humans and animals, and makes the grain unfit for food or feed (McMullen *et al.*, 1997b). Trichothecene toxins such as DON, also known as vomitoxin, and nivalenol (NIV), are sesquiterpenoids that inhibit eukaryotic protein biosynthesis. Animals fed with grains containing toxins exhibit feed refusal, as well as diarrhea, vomiting, alimentary hemorrhaging and contact dermatitis (Bennett and Klich, 2003). *F. graminearum* mycotoxins in humans can cause toxic aleukia and Akakabi toxicosis (Bennett and Klich, 2003). Further, chronic exposure to trichothecene can result in several neurological disorders and immunosuppression (Bennett and Klich, 2003). Zearalenone, a non-steroidal oestrogenic mycotoxin, is reported to cause reproductive disorders in animal systems leading to hyperestrogenism (Goodman *et al.*, 1987).

Environmental and cultural factors affecting wheat scab epidemiology

Epidemics of wheat scab are greatly affected by local and regional weather patterns, host factors such as the physiological stage at infection and genetics, and pathogen factors including adaptation and virulence (Osborne and Stein, 2007). Devastating wheat scab epidemics during 1990s in the US likely occurred due to the combination of favorable conditions during wheat flowering and abundant inoculum following conservation tilled of corn (Windels, 2000).

Crop residue. Crop residues of small grain crops act as the media for the overwintering of the causal fungi of scab. Fungal mycelium colonizes crop residues and develops saprophytically during the fall, winter, and spring (Sutton, 1982). Within crop debris, the scab fungi can produce abundant mycelium, conidia, ascospores, and chlamydospores (Brennan *et al.*, 2003). *F. graminearum* produces perithecia containing ascospores early in the growing season (late May and early June in the Northern Great Plains of the US) (Khonga and Sutton, 1988).

Corn stem nodes, grains, and other cereal grains are the most prolific sources of inoculum (Gilbert and Fernando, 2004; Khonga and Sutton, 1988). Schaafsma *et al.* (2005) reported greater numbers of airborne propagules of *F. graminearum* from wheat fields where corn or wheat had been the previous year's crop than in the fields with other non-host crops.

Temperature and moisture. A warm and humid environment with frequent rainfall or dew is a highly favorable environment for the growth, development, and infection of wheat heads by *F. graminearum* (Osborne and Stein, 2007). In a study conducted by Fakhfakh *et al.* (2011), high disease severity was found when high

precipitation coincided with heading and flowering. Major epidemics of wheat scab during the 1990s in the northern US were associated with increased frequency of rain (McMullen $et\ al.$, 1997a). Temperature and moisture play a significant role in scab occurrence as they have direct effect on the development and release of F. graminearum ascospores. Specifically, the germination rate of ascospores of F. graminearum decreases with increasing temperatures from 15 to 30°C (Gilbert $et\ al.$, 2008), suggesting that higher temperatures would slow an epidemic. Ascospore germination rate is highest at RH \sim 90% indicating that epidemics might progress more rapidly at this RH level.

The recovery rate of *F. graminearum* from crop residue in soil is directly related to temperature and moisture conditions. This, ultimately, is due to the decomposition rate of the crop residues and competitive saprophytic ability of *F. graminearum* (Burgess and Griffin, 1968). In a controlled experiment in which inoculated straw were exposed to different levels of soil moistures and temperatures, Burgess and Griffin (1968) found lower recovery of *F. graminearum* from straw subjected to frequently wetted soil or soil exposed to 35°C than to 25°C or 10°C. Higher temperatures and moistures presumably favored high microbial activity which could have resulted in accelerated straw decomposition. In another study conducted by Dufault *et al.* (2006), temperatures from 16-24°C coupled with moisture levels of -0.45 and -1.30 MPa allowed development of abundant perithecia while temperatures of 12 and 28°C, coupled with moisture levels of -2.36 and -4.02 MPa, allowed fewer perithecial development.

Scab management practices

Occurrence of wheat scab is weather dependent. It is impossible to control weather conditions favoring disease development; therefore, wheat scab can only be managed with cultural practices such as conventional tillage, crop rotation, and resistant cultivars, as well as fungicide inputs (McMullen *et al.*, 1997b). Currently, selected commercial wheat cultivars have been released with moderate resistance against wheat scab (Liu *et al.*, 2009; Liu *et al.*, 2012; McMullen *et al.*, 2012). Previously, adoption of conservation tillage (Bai and Shaner, 1994; Wilcoxon *et al.*, 1988) and lack of effective fungicides (McMullen *et al.*, 1997b) were the two major factors contributing to the difficulty in managing this disease. However, in recent years, integration of cultivar resistance along with proper application of fungicides, especially with triazole compounds, has brought substantial success in controlling wheat scab and mycotoxin contamination (Willyerd, *et al.*, 2012).

Fungicides and their effects. Fungicide are effective against wheat scab and DON but with variable results (Wegulo *et al.*, 2011). Variability of fungicide efficacy, to some extent, can be related to application timing and technology, composition of fungicides, and resistance levels of cultivars (Mesterhazy *et al.*, 2003; Mesterhazy *et al.*, 2011; McMullen *et al.*, 1997b). In order to obtain the best result, fungicides should be sprayed at full flowering in sufficient water to cover the entire head (Mesterhazy, 2003). Considerations should be made not only to reduce the disease to an acceptable level, but also to lower the level of possible mycotoxins with the application of the fungicides (Mesterhazy, 2003). Fungicides with different active ingredients could have differential control over different *Fusarium* species and the level of toxin production. In a study

conducted on wheat fields inoculated with different *Fusarium* species, Simpson *et al.* (2001) found that application of tebuconazole selectively controlled *F. culmorum* and *F. avenaceum* populations as well as reduced DON levels but failed to control the nontoxigenic fungus *M. nivale*. On the other hand, application of azoxystrobin showed increased DON production per unit of pathogen (Simpson *et al.*, 2001). Therefore, fungicides containing either single strobilurin product or a mixture of strobilurin + triazole products show a risk of increasing DON levels in wheat (Bradley *et al.*, 2011). In general, triazole fungicides are efficacious for controlling both disease severity and minimizing DON concentration (Bradley, 2011; El-Allaf *et al.*, 2001; Hershman and Draper, 2004).

Scab forecasting model. Scab forecasting models predict outbreaks of this disease and help growers in planning suitable disease management practices. In general, simple prediction can be done on the basis of the resistance level of the cultivar, the previous crop in the field and infection level by *Fusarium* spp., and tillage system (Mesterhazy, 2003). For example, minimum tillage and rainfall during flowering, coupled with temperatures slightly greater than 25°C increases the chance of disease development (Mesterhazy, 2003). Weather-based computer forecasting models have been developed that predict the outbreak of wheat scab and DON contamination in Argentina, Belgium, Canada, Italy, and the US (Prandini *et al.*, 2009). In the US, the risk assessment model for wheat scab is based on an extended period of favorable temperatures between 15 and 30° C and RH $\geq 90\%$ around the time of anthesis of wheat (De Wolf *et al.*, 2003). More recently, in addition to weather variables, the model has been updated to include resistance level of cultivars (De Wolf *et al.*, 2005). This model is available for public use

from the website of National Fusarium Head Blight Prediction Center (www.wheatscab.psu.edu) and now available for the 30 eastern US states including Alabama (De Wolf, personal communication). Universities in some states such as Minnesota, North Dakota, and South Dakota have provided a direct link to the scab forecast that contains information to each state compared to the whole US (Anonymous, 2012).

Varietal resistance. Development of cultivar resistance is the most practical and effective way of managing wheat scab (Rudd *et al.*, 2001) and is becoming a major breeding objective worldwide (Bai and Shaner, 2004). Minimal success has been achieved due to lack of effective resistance genes and poor adaptation of available resistance sources (Anderson, 2007). There are no wheat cultivars developed with complete immunity to wheat scab; however, a few cultivars have been identified with varying levels of susceptibility to disease and DON accumulation (Mesterhazy, 1995; Mesterhazy *et al.*, 2005). For example, the soft winter wheat lines 'Ernie' and 'Freedom' have been identified as moderately resistant to wheat scab (Griffey, 2005). Cultivars with moderate resistance have also resulted in improved fungicidal efficacy in reducing disease and minimizing DON compared to susceptible cultivars (Mesterhazy *et al.*, 2003).

Conventional breeding methods aimed to develop disease resistant cultivars are time consuming and expensive (Buerstmayr *et al.*, 2002). However, it has been found that scab resistance is governed by a few major quantitative trait loci (QTL) and several minor genes. Therefore, marker based selection for these major QTLs could accelerate the process of developing resistant cultivars (Buerstmayr *et al.*, 2002). The resistance gene

from the Chinese cultivar 'Sumai 3' and its Ning derivatives had been used in most resistance breeding programs (Gilbert and Tekauz, 2000). *Fhb*1, the source of resistance from 'Sumai 3', has been deployed in cultivars grown on 40% of the total acreage of spring wheat in the northern US (Anderson *et al.*, 2011). For example, 'Alsen', the first spring wheat cultivar developed with the *Fhb*1 gene, has been commonly cultivated in North Dakota (McMullen *et al.*, 2012). However, *Fhb*1 hasn't been incorporated into soft red winter wheat genotypes as it is associated with undesirable traits responsible for low yield and increased susceptibility to other diseases such as leaf rust, stripe rust, glume blotch, and soilborne wheat mosaic (McMullen *et al.*, 2012). Therefore, current breeding efforts are focusing on reduced use of resistance sources from Asian cultivars and increased use of native resistance sources (Gilbert and Haber, 2013).

Tillage, residue management, and crop rotation. Crop residue in the field is considered the principle substrate supporting the saprophytic growth of *F. graminearum* (Sutton, 1982). Intensive cultivation of cereal crops, particularly corn, wheat and barley, increases the abundance of *F. graminearum* inoculum (Shaner, 2003; Windels, 2000). Decomposition of crop residues reduces the survival and recovery of the causal fungi (Pereyra *et al.*, 2004). Conventional tillage compared to no-till or minimum tillage buries residues and accelerates the decomposition process (Pereyra *et al.*, 2004). Bergstrom *et al.* (2012) suggested that wheat planted in fields following moldboard plowing had lower scab severity and DON levels, in the majority of cases, compared to planting with minimum or no-till wheat. Blandino *et al.* (2010) reported that scab severity and DON contamination were significantly reduced in deep turned as compared to no-tilled fields. Crop rotation with alternate planting of cereal crops following non-cereal crops helps

remove sources of *F. graminearum* inoculum (Bergstrom *et al.*, 2012). Sweets (2012) reported that wheat following soybean rather than corn had reduced scab occurrence and DON even in the years when weather patterns were unfavorable for disease development.

Biological control. Biological control agents such as certain *Bacillus* strains (Bleakley *et al.*, 2012) have been showing some success in suppressing wheat scab. Chen *et al.* (2012) showed that the fungus *Clonostachys rosea* can be used as a biofungicide in combination with chemical fungicides as it acts as an antagonist to *G. zeae* by inhibiting mycelial growth and perithecia formation. In addition, application of some fungal antagonists of *F. graminearum* such as *Alternaria* spp., *Epicoccum* spp., and *Trichoderma* spp., along with chemical fungicides, has provided better control of scab and mycotoxin accumulation than fungicides alone (Musyimi *et al.*, 2012).

Since there is no single method available to completely manage the threat of an epidemic of scab and subsequent mycotoxin contamination, combined implementation of proper cultural practices, fungicide sprays, resistant cultivars and biological control methods are the keys to integrated management of wheat scab (Schmale and Bergstrom, 2003).

Goal of this study

Although scab has not been reported as a major problem of wheat in Alabama, it has been widely observed in wheat in northern parts of the state in recent years. Only a few occurrences of scab have been reported from south Alabama. Even though scab incidence in Alabama is fairly low, the warm and humid environment of the state would favor a future damaging scab outbreak. To date, however, no comprehensive study has

been conducted regarding the actual distribution of wheat scab and its management in Alabama. Therefore, this study addresses the epidemiology and management of wheat scab in Alabama. We tried to determine the prevailing situation of wheat scab in Alabama in terms of both disease severities as well as mycotoxin (DON) contamination in wheat grains with or without visible symptoms of the disease. The specific objectives were: 1) to evaluate the efficacy of fungicides in control of wheat scab and foliar diseases of wheat in Alabama; 2) to evaluate perithecial development by *Gibberella zeae* on wheat straws; and 3) to determine the distribution of wheat scab incidence across Alabama, and collect and characterize *Fusarium* species associated with the disease.

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II. Efficacy of Foliar Fungicides to Control Wheat Scab and Foliar Diseases of Wheat in Alabama.

Abstract

Wheat in Alabama is vulnerable to several diseases such as leaf and glume blotch, leaf rust, powdery mildew, and loose smut. During recent years, wheat scab (Fusarium head blight) has been found in a few locations in the state. Fungicides are commonly applied for controlling foliar diseases of winter wheat in Alabama. However, no studies have been carried out regarding the efficacy of fungicides for the control of wheat scab in Alabama. In this study, in addition to the scab, fungicides were tested for their ability to control common diseases of wheat such as Septoria blotch and rusts. Wheat was planted at three different locations of Alabama in 2011 and 2012. Five fungicides were applied in a completely randomized design with five replicates. The fungicides used in this study were, Absolute 500 SC[®] (tebuconazole + trifloxystrobin), Prosaro 421 SC[®] (prothiconazole + tebuconazole), Stratego YLD[®] (prothiconazole + trifloxystrobin), Caramba[®] (metconazole), Tilt[®] (propiconazole), and Topguard[®] (flutriafol). Wheat was exposed to natural infection by pathogens and showed minimum disease during both years. Results varied among locations and years. Wheat scab was found at trace levels at all locations in both years, but was not significantly affected by fungicide inputs. Among foliar fungal diseases, Septoria blotch was the most commonly occurred disease at all locations followed by leaf rust. In 2012, except in Sand Mountain, wheat was found to

have non-detectable levels of the mycotoxin, deoxynivalenol (DON) in all locations. In general, although the diseases were mostly found in low levels, fungicide treatments showed a positive effect to increase yield, 1000 kernel weight and reduced the disease severities in the locations with relatively higher disease pressure. None of the fungicides were found consistently superior over one another, however, Absolute 500 SC® (tebuconazole+trifloxystrobin) and StrategoYLD® (prothiconazole+trifloxystrobin) showed better response for reducing diseases and improving yield components. Overall, application of foliar fungicides seemed as an advisable option for controlling multiple fungal diseases as well as increasing yield of wheat grown in Alabama.

Introduction

Wheat scab, also known as Fusarium head blight, is one of the more destructive diseases of wheat worldwide. Several species of *Fusarium* have been described as the causal organisms of wheat scab, with *F. graminearum* being the primary one (Parry *et al.*, 1995). Wheat scab causes reduction in yield and grain quality because of *Fusarium*-incited damage to kernels and production of the mycotoxin, deoxynivalenol (DON) (McMullen *et al.*, 1997).

During the 1990s there was an estimated loss of \$3 billion due to scab epidemics in wheat and barley in the US (Windels, 2000). Since then, scab has remained a problem causing economic losses in the northern Great Plains and central US (Nganje *et al.*, 2002). Over time, wheat scab has also appeared as a serious threat in the Mid-Atlantic States and the southeastern US (Cowger and Sutton, 2005). A recent survey revealed the

presence of wheat scab pathogens in many small grain fields of the southern US including Alabama and its neighboring states (Gale *et. al.*, 2011).

Wheat is an important grain crop in Alabama and ranks 5th in the state among crops in terms of production acreage (USDA-NASS, 2011). Increasing yield of soft red winter wheat over recent years has positioned this crop as an important option in crop rotation for the region (Runge, 2012). While the warm and humid climate of Alabama during spring months supports the development of common foliar and seed borne diseases of wheat (Hagan et al., 1990), wheat scab had not been considered a major problem of wheat production in Alabama (Gazaway, 1997). However, during recent years, some destructive scab outbreaks have been reported in the Tennessee Valley in north Alabama (Hagan, 2011). While presence of a few scabby heads may not be responsible for significant losses in yield, kernel quality and value may be reduced due to mycotoxin contamination. However, if an epidemic occurs, wheat scab alone could reduce yield by 30 to 70% (Bai and Shaner, 1994). Other common diseases associated with wheat in Alabama are glume blotch, leaf rust, powdery mildew, and loose smut, which can be found statewide (Hagan, 2012). Diseases such as leaf blotch, stripe rust, stem rust, take-all and black chaff are not common but can be found occasionally (Hagan, 2012). In order to obtain consistent yields of wheat with standard grain quality, the integrated management of these diseases is important to wheat growers of Alabama.

Various strategies have been used to manage wheat scab and DON contamination such as crop rotation, genetic resistance, chemical, and biological control (Parry *et al.*, 1995). However, conventional methods such as deep tillage and crop rotation have not been successful for managing scab in wheat (Dill-Macky and Jones, 2000). Use of

resistant cultivars (Ali *et al.*, 2008) and fungicides (Cook *et al.*, 1999) are the primary methods for managing diseases of wheat. However, until now, no commercial wheat cultivar has been found to be completely immune to wheat scab (Bai and Shaner 1994, Mesterhazy *et al.*, 2005).

Fungicides have proven beneficial in controlling foliar diseases and increasing yield of wheat in the US. For example, application of foliar fungicides to winter wheat in Nebraska was shown to prevent yield losses of 42% (Wegulo *et al.*, 2009) from tan spot and spot blotch diseases. Similarly, in 5 years fungicide trial conducted in the eastern Great Plains, application of propiconazole had increased wheat yield in 77% of the time by controlling the foliar diseases, powdery mildew, Septoria blotch, and leaf rust (Kelley, 2001). A number of fungicides has been found to be effective for controlling soft red winter wheat diseases such as leaf and glume blotch, rusts, and powdery mildew and protecting wheat yields in Alabama (Bowen and Burch, 2007; Bowen *et al.*, 2011; Hagan, 2012).

Several studies have been conducted evaluating the efficacy of fungicides to control wheat scab and reducing DON contamination (Hollingsworth *et al.*, 2006; Ioos *et al.*, 2005; Jones, 2000; Milus and Parsons, 1994). Fungicides have shown inconsistent effectiveness against wheat scab and minimizing DON (Wegulo *et al.*, 2011b). Such variability, to some extent, could be related to application timing and methods, efficacy of different fungicides, and resistance levels of the cultivars (McMullen *et al.*, 1997; Mesterhazy *et al.*, 2003). Studies have found that fungicides with triazole chemistry have been effective for reducing both scab severity and DON concentrations. Prothiconazole+tebuconazole (Prosaro®) or metconazole (Caramba®) are the most

effective fungicides for controlling both scab severity and minimizing DON contamination (Bradley, 2011). A tebuconazole and prothiconazole mixture was found to be more effective in reducing both scab incidence and DON levels as compared to tebuconazole alone (Paul et al., 2007). Triazoles, strobilurins, or products containing a mixture of these fungicide classes are considered to be effective against most common foliar diseases of wheat; whereas triazoles are considered to be the best option for managing wheat scab (De Wolf et al., 2012). Furthermore, studies have also shown that application of fungicide containing either single strobilurin product or a mixture of strobilurin + triazole products has a risk of increasing DON levels in wheat (Bradley et al., 2011). In Minnesota, benomyl and tebuconazole significantly reduced wheat scab severity and DON concentration; these two fungicides as well as mancozeb significantly reduced the severity of other foliar diseases compared to the non-treated control (Jones, 2000). Increased head weight, test weight, 1000 kernel weight, and yield were obtained with the fungicide treatments as well. Similarly, Ransom and McMullen (2008) reported significantly reduced severity of wheat scab and other foliar diseases as well as reduced DON concentrations and increased yield with fungicides in a year when disease pressures were moderate to high.

Cultivars of soft red winter wheat commonly grown in Alabama have varying levels of resistance to different diseases (Glass *et al.*, 2012). Therefore, routine use of foliar fungicides could be an option to improve wheat yield and quality.

Although fungicide efficacy trials are conducted annually across Alabama, no studies have been done to evaluate the efficacy of fungicides for the control of scab or reducing DON contamination in wheat. Given favorable weather conditions, wheat scab

has the potential to become destructive to Alabama's wheat crop. Therefore, the overall objective of this study was to evaluate the benefits of foliar fungicides for management of wheat scab, foliar diseases such as Septoria blotch and rusts, DON contamination, and improved yield and kernel quality of winter wheat in Alabama.

Materials and Methods

Field sites. Fields were located on the experimental stations of the Alabama Agricultural Experiment Station, Auburn University, located in three different regions: E. V. Smith Research Center, Tallassee, in east-central Alabama; Sand Mountain Research and Extension Center, Crossville, in northeastern Alabama; and Tennessee Valley Research and Extension Center, Belle Mina, in north-central Alabama. Within E. V. Smith, experiments were conducted at Field Crop Unit, and Plant Breeding unit, respectively in 2010 and 2011. The soil types at Field Crop Unit, Plant Breeding Unit, Sand Mountain, and Tennessee Valley were Marvyn loamy sand, Independence (Cahaba) loamy fine sand, Hartsells fine sandy loam, and Decatur silt loam, respectively. At each site, wheat was planted in the autumn of 2010 and 2011 (Table 1). Weather data for each field were obtained from the Alabama Mesonet (AWIS. Inc., Auburn, AL).

Cultural management. Soft red winter wheat cultivars were planted between the first to the second week of November, depending upon the location and weather, in each year (Table 1). The soft red winter wheat variety AGS 2060 was planted in both years at E. V. Smith, and in 2011 at Tennessee Valley and Sand Mountain while, 'Pioneer 26R61' and 'Pioneer 26R15' were planted at Tennessee Valley and Sand Mountain, respectively, in 2010. Among the cultivar used in this study, all three cultivars were

known as moderately resistant to leaf rust, 'AGS 2060' and 'Pioneer 26R15' were moderately resistant to Septoria blotch, 'Pioneer 26R61' was susceptible to both scab and Septoria blotch, whereas 'AGS 2060' and 'Pioneer 26R15' were moderately resistant to wheat scab (Ambrose, 2011; Milus *et al.*, 2013; and Smith, 2013). Cultural practices, including field preparation, tillage, and fertilization, differed slightly among different locations due to available equipment and facilities. However, similar production practices were employed in both years at each location. In general, fields were disk harrowed, field cultivated, leveled and seeds were drilled using a conventional seed drill with 18 cm (7 in.) row spacing at the rate of 101 and 112 Kg/ha (90 and 100 lb/A), respectively, at E. V. Smith and Tennessee Valley in both years. However, at Sand Mountain, seeding rates were 134 Kg/ha (120 lb/A) in 2010 and 101 Kg/ha in 2011. The experimental design was a randomized complete block design of five replications with plots of 9 m length and 3 m width (~ 30 × 10 ft.). Irrigation was not provided at any location and year.

Fungicide applications. Fungicides were chosen based on their broad activity spectrum and systemic mode of action to provide control of leaf spot diseases, rusts, and wheat scab. In 2011, fungicides were applied on two different growth stages: Feekes' stage (FS) 10 (booting) and 10.5 (complete heading) (Large, 1954). In 2012, fungicides were applied at FS 10.5.1 (beginning flowering) as this stage is regarded as the best timing for a fungicide application targeting wheat scab (Jones, 2000; Mesterhazy, 2003). The fungicides used in this study were: Absolute 500 SC® (0.3 L product/ha, 22.6% tebuconazole + 22.6% trifloxystrobin), Prosaro 421 SC® (0.5 L product/ha, 19% prothiconazole + 19% tebuconazole), Stratego YLD® (0.3 L product/ha,10.8% prothiconazole + 32.3% trifloxystrobin) (Bayer CropScience, Research Triangle Park,

NC), Caramba[®] (1 L product/ha, 8.6% metconazole, BASF Corporation, Research Triangle Park, NC), Tilt[®] (0.3 L product/ha, 41.8% propiconazole, Syngenta Crop Protection, LLC, Greensboro, NC), and Topguard[®] (1 L product/ha, 11.8% flutriafol, Cheminova Inc., One Park Drive, NC). Depending on the location, fungicides were applied with either a high clearance sprayer at 45 psi with seven Jet X-10 hollow cone nozzles or a CO₂ pressurized backpack sprayer at 35 psi.

Disease and yield assessment. Disease assessments were conducted at soft dough stage of wheat (FS 11.2) with visual estimation of all diseases in each plot. In addition to Fusarium scab, other diseases assessed were: rusts (leaf, stripe and stem), Septoria blotch, and powdery mildew. Percentage severity was noted for leaf and stripe rust depending upon the area of the flag leaves covered by rust pustules, where 0 indicated the absence of disease and 100% indicated the entire leaf covered by the disease. A disease intensity scale of 0 to 9 was used for rating wheat scab, Septoria blotch, and powdery mildew, where 0 indicated the absence of disease, 5 indicated approximately 50% of flag leaf area or florets affected and 9 was for completely diseased condition or premature senescence due to disease.

Wheat was combined using a 1.5 meter (5 feet) small plot harvester. Plot weights and grain moistures were immediately recorded after harvest and 1000 kernel weights determined. Yield was reported at 13.5% moisture.

DON assessment. Due to lower levels of scab intensity, wheat grains were not accessed for mycotoxin-deoxynivalenol (DON) in 2011. However, scab intensity levels in 2012 were found in similar levels to slightly higher levels than in 2011. Therefore, harvested grain samples were tested for the presence of DON from each 2012 study.

Additional DON assessments were done on samples from two different fungicide trial studies conducted at Prattville Research Center (Prattville, AL) and Gulf Coast Research and Extension Center (Fairhope, AL). A total of 73 samples were assayed, including all treatments from at least one replication from each location. Briefly, this assay used 10 gram of ground wheat, which was blended in 100 ml distilled water. The filtrate was used to assay for DON with the enzyme-linked immunosorbent assay (ELISA) Veratox DON 5/5 kit (Neogen Corporation, Lansing, MI) as specified in the manufacturer's protocol.

Data analysis. Fungicide treatments and cultivars differed slightly among each year and location. Therefore, data were analyzed separately for each location and year. Data for disease intensity or severity, 1000 kernel weight, and yield were analyzed with generalized linear mixed model procedures. Treatment means were separated using Fisher's protected least square mean separation at $P \le 0.05$ level of significance.

Results

Weather. The months over which active vegetative growth and flowering of wheat occurred, in general, were drier and warmer in 2012 compared to 2011 (Table 2). Thus, the weather seemed less conducive for disease development in 2012 than the previous year. The average March-April temperatures in 2011 were 13.5, 14.5, and 17°C, respectively, at Sand Mountain, Tennessee Valley, and E. V. Smith locations. While, the average rainfall amounts during same period and locations were 207, 239 and 94 millimeters, respectively. In 2012, higher temperatures and lower rainfall amounts were recorded in each location compared to the previous year. The average March-April temperature in 2012 was 18°C either at Tennessee Valley or E. V. Smith and was 17.5°C

at Sand Mountain. The average rainfall amounts during the same periods in 2012 were 62.5, 57.0, and 52.5 mm, respectively. Further, only a few events of low rainfall during flowering of wheat at all locations and either year were insufficient to favor the wheat scab development.

E. V. Smith Research Center. In 2011, leaf rust was observed as the predominant disease (Table 3). Fungicide treatments significantly reduced the leaf rust severity (*P*=0.0006) compared to the non-treated control. Stratego YLD® significantly reduced leaf rust levels compared with Topguard®, which had lower leaf rust ratings than the non-treated control. Low levels of Septoria blotch and scab were not impacted by fungicide treatments. Despite significant reductions in disease, fungicides failed to increase 1000 kernel weight and yield as compared with the non-treated control.

Unlike in 2011, leaf rust was not seen at E. V. Smith in 2012 (Table 3). However, Septoria blotch intensity was higher in 2012 than in 2011. Fungicide treatments significantly reduced the Septoria blotch severity (P=0.0061) compared to the non-treated control. Septoria blotch severity did not differ significantly among fungicide treatments. Wheat scab incidence was low and was not affected by fungicide treatment. Fungicide treatments significantly affected 1000 kernel weights (P=0.02). Significantly greater 1000 kernel weights were obtained for the Absolute-treated wheat when compared to the non-treated control and most other fungicides except for Stratego YLD[®]. Similar yields were recorded for all fungicide treatments and non-treated control.

Tennessee Valley Research and Extension Center. Leaf rust and Septoria blotch were the predominant diseases at the Tennessee Valley location in 2011 (Table 4). Leaf rust severity did not differ significantly among fungicide treatments. All fungicide

treatments proved equally effective in reducing Septoria blotch severity when compared with the non-treated control (P=0.0034). Wheat scab was low with no significant differences due to fungicides. Fungicides treatments had a significant effect on 1000 kernel weight (P=0.035). Absolute 500 SC[®] and Stratego YLD[®] applied at FS 10 significantly increased 1000 kernel weights as compared to the non-treated controls and other fungicide treatments except for Stratego YLD[®] applied at FS 10.51. However, there were no significant differences in disease levels or yield components between Stratego YLD[®] treated plots at two different growth stages: FS 10 or FS 10.5. Yield for the fungicides and non-treated control did not significantly differ.

In 2012, leaf rust was not observed at Tennessee Valley (Table 4). Septoria blotch and scab were found at minimal levels and neither was significantly affected by fungicides treatment, nor was 1000 kernel weight and yield.

Sand Mountain Research and Extension Center. Wheat in 2011 was free from disease except for a low level of Septoria blotch and fungicide treatments had no effect on disease severity (Table 5). However, 1000 kernel weights were significantly higher (*P*=0.009) for wheat treated with Absolute 500 SC® at FS 10 as compared to the nontreated control and most other fungicide treatments except for Prosaro 421 SC® and Stratego YLD® applied at FS 10. The latter fungicide treatments had significantly higher 1000 kernel weight compared to with Caramba® and Prosaro 421 SC® at FS 10.51. Yield was also significantly affected with the application of fungicides (*P*=0.023). Absolute 500 SC®, Prosaro 421 SC®, and Stratego YLD® applied at FS 10 had higher yield than those receiving Caramba® and Prosaro 421 SC® at FS 10.51.

Similarly to 2011, leaf rust was not observed at Sand Mountain in 2012 (Table 5). Septoria blotch and scab were found at slightly higher intensity compared to 2011 but the severity of either disease, nor 1000 kernel weight and yield was impacted by fungicide treatment.

DON assessment. Harvested grains in 2012 were tested for the presence of the trichothecene mycotoxin, deoxynivalenol (DON). Only five samples showed DON content between 0.5 to 1 ppm and none of the samples showed more than 1 ppm (data not shown). All five samples with > 0.5 ppm were from the Sand Mountain study in 2012. This low concentration of DON falls within the acceptable limit for human consumption (<1 ppm) (Anonymous, 2010); however, the results confirmed that wheat in Alabama is not free from DON contamination, even when disease severity is low.

Discussion

This study was initiated with the goal of evaluating the efficacy of foliar fungicides for controlling multiple foliar diseases of wheat as well as wheat scab in natural conditions. Three wheat cultivars commonly grown in Alabama were evaluated for the intensity of scab and other foliar diseases as well as the level of DON contamination in grain. Although disease intensity levels were found at minimal levels, results showed slightly variable disease occurrences over the three study sites over time.

The epidemics of foliar fungal diseases of wheat are affected by a combination of different factors such as long periods of light rain coupled with moderate temperatures, inoculum availability, infection time and maturity stage of the crop, and cultivars susceptibility (Eversmeyer, 2000). The three study sites differed by temperature, rainfall,

and soil type with temperature and rainfall patterns varying between study years. In general, weather patterns during growing season were dryer and warmer in 2012 than in 2011. This indicates that the weather was less conducive for the development of foliar diseases in 2012 compared to 2011. However, rainfall levels even in 2011 seemed insufficient to consistently increase infection of wheat by foliar fungal pathogens. For example, average March-April rainfall was highest at Tennessee Valley in 2011, which is in accordance to the higher levels of leaf rust, Septoria blotch, and scab compared to disease levels at same location in 2012. In contrast, despite the lower average March-April rainfall at E. V. Smith in 2011 compared to Tennessee Valley, leaf rust severity was higher at the former than latter site. The warmer and dryer weather in 2012 at E. V. Smith compared to 2011 might have been responsible for absence of leaf rust, however, Septoria blotch was found at higher levels than in 2011. Similarly, at Sand Mountain, higher disease levels were found in 2012, which was dryer than 2011. These results suggest that besides the weather conditions, disease variability might have been affected by variability in local source of inoculum at different locations.

Among foliar diseases, Septoria blotch was found as the most common disease across years and locations. Fungicides significantly reduced Septoria blotch severity only at E. V. Smith in 2012 and at Tennessee Valley in 2011. Leaf rust was found to be the predominant disease in 2011 at E. V. Smith and was significantly reduced with application of selected fungicides, where Stratego YLD® (prothiconazole+trifloxystrobin) gave the best control. In the remainder of locations and years, leaf rust did not occur at substantial levels and was not affected by fungicide treatments.

We observed that wheat was under low disease pressure in each experimental year and location. In such conditions, the benefits of using foliar fungicides to reduce disease levels and improve yield could be overlooked. Guy *et al.* (1989) found that fungicide treatments gave higher return in cool and moist conditions that favor disease development (leaf rust, Septoria blotch) as compared to warmer and drier conditions. Therefore, fungicides would be expected to provide better protection against disease and improve yield attributes whenever there is higher disease pressure. Similarly, fungicides are often more efficacious when used on a susceptible as compared with a resistant cultivar (Ransom and McMullen, 2008).

In spite of the low disease pressure in all the fields, higher 1000 kernel weights and yield were often obtained with fungicide treatments. When compared with the nontreated control, Absolute 500 SC® (tebuconazole + trifloxystrobin) treated wheat had significantly higher 1000 kernel weights in three of six studies (E. V. Smith in 2012, Tennessee Valley in 2011, and Sand Mountain in 2011). Increased 1000 kernel weight illustrates the value of fungicides for improving grain quality (Kelley, 2001). Significant yield gains due to fungicide inputs were obtained only at Sand Mountain in 2011. These results are similar to those of Wegulo *et al.* (2011b) in Nebraska, where foliar fungicide treatments were beneficial in increasing net returns by improving yield and grain quality in the years with moderate to high disease pressures.

Weather patterns during the study period over locations and years were not conducive for wheat scab development. The few rainfall events that occurred during wheat flowering periods (Mid-March to Mid-April) were insufficient to promote head (floret) infection by *Fusarium*. Therefore, we were not able to find differences in disease

intensity between fungicide treated wheat and non-treated controls or among fungicide treatments. Previous studies have noted inconsistent results on efficacy of fungicides against wheat scab over different locations and years (Blandino *et al.*, 2006; Ioos *et al.*, 2005; Jones, 2000; Mesterhazy, 2003). Significant variations in the inoculum levels as well as unfavorable weather conditions during a critical stage for disease infection were likely responsible. Also, inconsistent field studies results might be related to the interactions among fungicides, *Fusarium* species, and other ear colonizing fungi such as *Alternaria, Septoria, Cladosporium*, and *Botrytis cinerea* (Pirgozliev *et al.*, 2003).

In a recent study with 40 fungicide trials in 12 US states, Willyerd *et al.* (2012) concluded that application of prothiconazole + tebuconazole (Prosaro 421 SC[®]) significantly controlled wheat scab and limited DON concentrations. However, the magnitude of control varied among different environments. In a similar Kansas study, Wegulo *et al.* (2011a) found that the fungicide, Prosaro 421 SC[®], reduced scab, *Fusarium* damaged kernels (FDK), DON levels, and increased yield. However, these results were inconsistent over three study years. Meta-analysis showed that triazole fungicides, propiconazole, prothiconazole, tebuconzole, metconazole, and prothiconazole + tebuconazole, significantly increased the mean yield and test weight compared to the non-treated controls over 12 years in 14 US states (Paul *et al.*, 2010).

In this study, we were not able to determine that fungicides affected DON contamination in wheat which is mostly likely due to trace levels of wheat scab in the field. DON concentrations detected in only a few samples were less than 1 ppm, which fall within the acceptable limit of DON concentration for human consumption (Anonymous, 2010). To our knowledge, this study was the first to determine the status of

DON contamination in Alabama's wheat crop. The presence of detectable levels of DON from only a few samples, however, indicates that wheat in Alabama is not absolutely free from DON contamination. Thus, precautions should continue to manage possible epidemics of wheat scab and mycotoxin contamination in this region.

The economic benefits of fungicide application can vary among years depending upon the weather and disease activity. Ransom and McMullen (2008) found foliar fungicides effective for controlling both foliar diseases and wheat scab in hard winter wheat and recommended routine use of fungicides for susceptible cultivars even in the years with low disease pressure. Similarly, Kelley (2001) recommended foliar fungicides use with favorable disease conditions in order to obtain increased yield and test weight on both hard and soft winter wheat. Fungicide application timing to control the foliar diseases of wheat is ideal during emergence of flag leaf (FS 10) (Cook et al., 1999; Osborne and Stein, 2009); however, for controlling wheat scab, the best application timing is during anthesis (FS 10.5.1) (Mesterhazy 2003; Paul et al., 2007). Economically, a single application at anthesis could be an appropriate timing for controlling both foliar diseases and scab. Therefore, in the second year of our experiment, all the fungicides were applied at beginning of the anthesis. This approach is further supported Wiersma and Motteberg (2005) who found that delaying application of foliar fungicides until initiation of flowering did not decrease foliar disease control when compared to early application, but did have a positive impact on scab control and grain yield.

In conclusion, fungicides in our study often showed positive effects relative to increasing yield and 1000 kernel weights. In addition, fungicides significantly reduced

the severity of leaf rust and Septoria blotch under relatively high disease pressure. Among fungicides, Absolute 500 SC® (tebuconazole+trifloxystrobin) and StrategoYLD® (prothiconazole+trifloxystrobin) had better response for reducing diseases and increasing yields. Though selected fungicides worked well when disease has appeared, little gain in yield was achieved even when disease occurrence was low. Inconsistent disease control and yield improvements with the use of foliar fungicide could be due to the various factors such as yield potential of the cultivar, disease level in the field, level of genetic resistance of the crop, and the weather conditions. Further, in order to insure food safety issues with respect to DON contamination in wheat products, regular studies are recommended to evaluate wheat scab incidence and severity in fields along with the level of DON concentration in harvested grains across the state. In addition, further studies are recommended towards economic analysis of benefits derived through application of fungicides in yield and grain quality along with integrated control of wheat diseases.

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Table 1. Information on year of plantation, cultivars used, previous crop, planting date, fungicide application date, disease rating date, and harvest date in different sites and years.

Year (Planted)	Location	Cultivar	Previous crop in field	Planting date	Fungicide application	Disease rating	Harvest date
2010	E. V. Smith	AGS 2060	Corn	11/8/2010	4/13/2011	5/2/2011	5/19/2011
	Sand Mountain	Pioneer 26R15	Corn	11/2/2010	4/18/2011	5/4/2011	6/8/2011
					4/22/2011		
	Tennessee Valley	Pioneer 26R61	Wheat	11/1/2010	4/13/2011	5/10/2011	7/12/2011
					4/19/2011		
2011	E. V. Smith	AGS 2060	Corn	11/3/2011	3/22/2012	4/24/2012	5/17/2012
	Sand Mountain	AGS 2060	Soybean	11/17/2011	4/3/2012	4/20/2012	5/31/2012
	Tennessee Valley	AGS 2060	Corn	10/27/2011	4/9/2012	4/19/2012	5/31/2012

Table 2. Monthly mean temperature and total rainfall for four critical months of disease development during growing season.

		Mean temperature (°C)				Total rainfall (mm)			
Year	Location	February	March	April	May	February	March	April	May
2011	E. V. Smith	10	15	19	22	100	139	49	56
	Sand Mountain	6	11	16	21	54	246	168	54
	Tennessee Valley	7	12	17	20	42	218	260	64
2012	E. V. Smith	11	18	18	23	101	81	24	176
	Sand Mountain	9	17	18	22	63	110	15	38
	Tennessee Valley	9	18	18	23	67	76	38	72

Table 3. Effect of fungicide application on different diseases and yield components at E. V. Smith.

Year ^t	Fungicide ^u , rate	Application	Leaf	Septoria	•	1000	Yield
		${f timing}^{ ext{v}}$	rust ^w	Blotch ^y	Scab ^y	kernel wt (g)	(kg/ha)
2011	Non-treated control		11.4 a ^x	1.3 a	0.9 a	33.7 a	3881 a
	Absolute 500 SC, 0.3 L/ha	FS 10	1.5 bc	0.4 a	0.7 a	37.0 a	4196 a
	Caramba, 1 L/ha	FS 10.5	0.7 bc	0.4 a	1.1 a	34.5 a	4077 a
	Prosaro 421 SC, 0.5 L/ha	FS 10.5	0.5 bc	0.1 a	0.9 a	34.4 a	4175 a
	Stratego YLD 4.2 SC, 0.3 L/ha	FS 10.5	0.1 c	0.3 a	1.3 a	35.8 a	3947 a
	Topguard, 1 L/ha	FS 10.5	5.2 b	1.2 a	0.7 a	35.3 a	4109 a
	P=		0.0006	0.11	0.74	0.08	0.72
2012	Non-treated control		ND ^z	4.0 a ^x	1.4 a	40.3 b ^x	4236 a
	Absolute 500 SC, 0.4 L/ha	FS 10.51	ND	3.0 b	1.2 a	42.2 a	4533 a
	Caramba, 1 L/ha	FS 10.51	ND	2.6 b	0.6 a	40.2 b	4062 a
	Prosaro 421 SC, 0.5 L/ha	FS 10.51	ND	3.0 b	1.0 a	41.0 b	4370 a
	Stratego YLD, 0.3 L/ha	FS 10.51	ND	2.8 b	1.2 a	41.3 ab	4304 a
	Tilt, 0.3 L/ha	FS 10.51	ND	2.2 b	0.8 a	40.8 b	4330 a
	P=			0.0061	0.15	0.023	0.67

^tCultivar planted: AGS 2060.

^uPlus 0.125% non-ionic surfactant (Induce).

^vApplied at Feekes' growth stage: FS 10 = booting, FS 10.5 = complete heading, FS 10.51 = initiation of flowering.

^w0 to 100% disease severity on flag leaf: 0 = no disease, 100 = entire leaf affected by disease.

^xMeans within column followed by the same letter are not significantly different at P=0.05.

 $^{^{}y}0$ to 9 scale disease intensity: 0 = no disease; 9 = severe disease.

^zNot detected.

Table 4. Effect of fungicide application on different diseases and yield components at Tennessee Valley.

Year ^t	Fungicide ^u , rate	Application	Leaf	Septoria		1000 kernel	Yield
		Timing	rust ^w	Blotch ^x	Scab ^x	wt (g)	(kg/ha)
2011	Non-treated control		3.8 a ^y	2.0 a	1.4 a	34.0 b	4901 a
	Absolute 500 SC, 0.3 L/ha	FS 10	0.6 a	1.0 b	1.0 a	37.0 a	5600 a
	Caramba, 1 L/ha	FS 10.51	0.0 a	0.6 b	1.6 a	34.0 b	5411 a
	Prosaro 421 SC, 0.3 L/ha	FS 10.51	0.1 a	0.6 b	1.0 a	34.0 b	5479 a
	Stratego YLD 4.2 SC, 0.3 L/ha	FS 10.51	0.1 a	0.8 b	1.0 a	36.0 ab	6024 a
	Stratego YLD 4.2 SC, 0.3 L/ha	FS 10	1.8 a	1.0 b	1.4 a	37.0 a	5220 a
	P=		0.06	0.0034	0.35	0.035	0.1
2012	Non-treated control		ND ^z	1.0 a	0.8 a	37.3 a	4887 a
	Absolute 500 SC, 0.4 L/ha	FS 10.51	ND	1.2 a	1.2 a	37.3 a	4897 a
	Caramba, 1 L/ha	FS 10.51	ND	1.4 a	1.0 a	36.5 a	4901 a
	Prosaro 421 SC, 0.5 L/ha	FS 10.51	ND	1.2 a	0.2 a	37.1 a	4870 a
	Stratego YLD 4.2 SC, 0.4 L/ha	FS 10.51	ND	1.0 a	1.2 a	37.4 a	5065 a
	Tilt, 0.3 L/ha	FS 10.51	ND	1.6 a	1.6 a	37.5 a	5004 a
	P=			0.40	0.13	0.21	0.50

^tCultivars planted: Pioneer 26R61 (2010); AGS 2060 (2011).

^uPlus 0.125% non-ionic surfactant (Induce).

^vApplied at Feekes' growth stage: FS 10 = booting, FS 10.5 = complete heading, FS 10.51 = initiation of flowering.

wo to 100% disease severity on flag leaf: 0 = no disease, 100 = entire leaf affected by disease.

 $^{^{}x}0$ to 9 scale disease intensity: 0 = no disease; 9 = severe disease.

^yMeans within column followed by the same letter are not significantly different at P=0.05.

^zNot detected.

Table 5. Effect of fungicide application on Septoria blotch, wheat scab and yield components at Sand Mountain.

Year ^u	Fungicide ^v , rate	Application Timing ^w	Leaf rust	Septoria Blotch ^y	Scab ^y	1000 kernel wt (g)	Yield (kg/ha)
2011	Non-treated control		ND ^x	1.3 a ^z	ND	24.2 bc	5030 ab
	Absolute 500 SC, 0.3 L/ha	FS 10	ND	1.3 a	ND	28.6 a	5812 a
	Caramba, 1 L/ha	FS 10.51	ND	1.8 a	ND	23.1 c	4583 b
	Prosaro 421 SC, 0.5 L/ha	FS 10	ND	1.3 a	ND	26.7 ab	5715 a
	Prosaro 421 SC, 0.5 L/ha	FS 10.51	ND	1.5 a	ND	22.9 c	4528 b
	Stratego YLD 4.2 SC, 0.3 L/ha	FS 10	ND	0.8 a	ND	26.6 ab	6017 a
	P=			0.20		0.0098	0.023
2012	Non-treated control		ND ^x	2.4 a	1.8 a	32.5 a	3526 a
	Absolute 500 SC, 0.4 L/ha	FS 10.51	ND	1.4 a	0.4 a	36.0 a	3997 a
	Caramba, 1 L/ha	FS 10.51	ND	1.4 a	0.6 a	35.9 a	3777 a
	Prosaro 421 SC, 0.5 L/ha	FS 10.51	ND	1.6 a	1.0 a	34.8 a	3400 a
	Stratego YLD 4.2 SC, 0.3 L/ha	FS 10.51	ND	1.4 a	1.0 a	34.9 a	3680 a
	Tilt, 0.3 L/ha	FS 10.51	ND	1.6 a	0.8 a	33.5 a	3647 a
	P=			0.20	0.16	0.20	0.62

^uCultivars planted: Pioneer 26R15 (2010); AGS 2060 (2011).

^vPlus 0.125% non-ionic surfactant (Induce).

^wApplied at Feekes' growth stage: FS 10 = booting, FS 10.5 = complete heading, FS 10.51 = initiation of flowering.

^xNot detected.

 $^{^{}y}0$ to 9 scale disease intensity: 0 = no disease; 9 = entire leaf affected by disease.

^zMeans within column followed by the same letter are not significantly different at P=0.05.

III. Evaluation of perithecial development by *Gibberella zeae* on wheat straw exposed to different soils, temperatures and moisture conditions.

Abstract

Wheat scab is one of the more destructive diseases of wheat in the US, capable of causing sizable economic loss. In recent years, scab has been commonly recorded in the wheat fields at north Alabama, and to a lesser extent, in south Alabama. Crop residues in the field are considered the principle source of inoculum for Gibberella zeae (anamorph = Fusarium graminearum), the primary causal agent of wheat scab. Decomposition of crop residues in the field negatively impacts the saprophytic growth and development of F. graminearum and reduces the chances of scab development in wheat. We hypothesize that due to higher annual rainfall and temperatures, residue degradation is greater and fungal survival lower, in southern than northern fields of the state. Thus, we sought to evaluate the ability of degraded wheat straw to support G. zeae perithecial development. Two experiments were conducted in which, uniform straw pieces were buried in different combinations of soils, soil moistures, and temperatures. In experiment I, straw were incubated in a 3×2×3 factorial arrangement of treatments with three levels of temperature, two types of soil, and three levels of soil moisture. While in experiment II, treatments with non-sterile soil comprised a 3×2×4 factorial with three levels of temperature, two types of soil, and four levels of soil moisture. In experiment II, additional treatments were arranged with two types of sterile soil comprising two levels

of each moisture and temperature. Incubated straw were removed from soil at one, two, and three month intervals and inoculated with a macroconidial suspension of *F. graminearum*. Inoculated wheat straws were incubated in growth chambers at 26°C with a diurnal light cycle. Perithecia were counted on straw up to 90 days after inoculation. In both of the experiments, perithecial numbers on straw were significantly impacted by soil type and moisture. Perithecial numbers were higher on straw incubated in finer textured compared to coarse textured soil. Reduced perithecial development was noted on straw incubated at higher soil moistures. Temperature had a significant effect in perithecia production only in experiment I, where straw incubated at lower temperature tended to support greater numbers of perithecia. In experiment II, no significant effect of soil sterilization was found in perithecia production. These results confirm higher prevalence of wheat scab in north than in south Alabama. Further, the findings of this study could be useful for understanding the occurrence of wheat scab based upon the status of prevailing crop residues in the fields with varying soils, temperatures and rainfall patterns as in Alabama.

Introduction

Wheat scab, also known as Fusarium head blight, is one of the more destructive diseases of wheat worldwide (Osborne and Stein, 2007). This disease is responsible for several harmful effects to wheat (Goswami and Kistler, 2004) including yield reduction and mycotoxin contamination of the grains (McMullen *et al.*, 1997). Reduced yield and quality of wheat due to scab epidemics have been responsible for discounted wheat prices for several years in the US (Windels, 2000). Devastating scab epidemics of the 1990s in

the US caused an estimated loss of \$3 billion in wheat and barley (Windels, 2000). Since then, scab has caused economic losses around the northern Great Plains and the central US (Nganje *et al.*, 2002). Wheat scab has also appeared as a serious threat in the Mid-Atlantic States to the southeastern US (Cowger and Sutton, 2005).

Fusarium graminearum is the primary pathogen causing wheat scab in North America and in the US (Gale et al., 2007; Wilcoxson et al., 1988; Zeller et al., 2004). Infected crop debris in the field acts as the primary source of inoculum as it supports the saprophytic growth of F. graminearum (Guenther and Trail, 2005; Sutton, 1982). In a favorable environment of warm and moist conditions, development of conidia and perithecia occurs from mycelia of this fungus, and, later, ascospores are formed and dispersed towards the wheat head (Markell and Francl, 2003). The important wheat scab epidemics during the 1990s in the US are considered to have occurred due to the combined effects of favorable weather conditions during wheat flowering and abundant inoculum, resulting from conservation tillage (Windels, 2000).

Intensive cultivation of cereal crops on the same piece of land with shorter duration between crops enhanced the abundance of *F. graminearum* inoculum in fields (Champeil *et al.*, 2004; Windels, 2000). Almost all cultivated plants in the *Gramineae* are a host of a *Fusarium* species (Parry *et al.*, 1995). In addition to wheat, *F. graminearum* can cause ear rot and stalk rot in corn (Goswami and Kistler, 2004; Osborne and Stein, 2007; Parry *et al.*, 1995), and head blight of barley, rice, and oats (Goswami and Kistler, 2004). Wheat and barley straw is the favored substrate for perithecia formation, and subsequently ascospores, by *F. graminearum* (Pereyra and Dill-Macky, 2008). A wheat-corn rotation augments inoculum levels in the field (Osborne and Stein, 2007; Schaafsma

et al., 2005; Seaman, 1982). Therefore, incidence of wheat scab partially depends upon the frequency of cropping susceptible hosts (McMullen et al., 1997) and abundance and type of residues present in the field (Dill-Macky and Jones, 2000).

Fusarium graminearum is the predominant species causing wheat scab in warmer parts of the world including the US, where it can grow well at temperatures up to 30°C (Osborne and Stein, 2007). Ideal conditions for infection of wheat by *F. graminearum* are temperatures from 15 to 30°C and RH levels greater than 90% (De Wolf *et al.*, 2003). Further, warmer and wetter conditions are highly favorable for macroconidia production and dispersal (Osborne and Stein, 2007); whereas cooler conditions (20 to 24°C) favor perithecial development and ascospore release (Dufault *et al.*, 2006).

In addition to ascospores, conidia, chlamydospores, or hyphal fragments of *Fusarium* spp. can also serve as inoculum for wheat scab. However, for *Gibberella zeae* (the teleomorph of *F. graminearum*), ascospores are considered the most important type of inoculum (Parry *et al.*, 1995). Crop residues of wheat, barley, corn and other grasses support perithecial development of *G. zeae* even after 2 years on the soil surface (Pereyra and Dill-Macky, 2008). Further, residues that do not degrade easily, such as stem nodes, act as a long term substrate for the pathogen (Sutton, 1982). However, ascospore production from crop residues in fields decreases over time (Pereyra and Dill-Macky, 2008).

Residue decomposition reduces the survival and recovery of *G zeae* (Pereyra *et al.*, 2004). Thus, significant reductions in scab incidence can be achieved where residue is removed or plowed under (Teich and Hamilton, 1985; Teich and Nelson, 1984; Wilcoxson *et al.*, 1988). Enhanced survival of *G zeae* and scab risk increased in

minimum till wheat (Bergstrom *et al.*, 2012; Khonga and Sutton, 1988; Parry *et al.*, 1995; Pereyra and Dill-Macky, 2008; Pereyra *et al.*, 2004).

Soil moisture and temperature are important environmental factors that affect residue decomposition (Stott *et al.*, 1990; Stroo *et al.*, 1989). Stott *et al.* (1986) found that the rate of decomposition increases with increasing water potential from -5.0 Mpa to 0 Mpa and increasing temperatures from 0°C to 30°C. Although soil moisture and temperature both play an important role in crop residue decomposition, the majority of studies have focused on the impact of soil moisture (Quemada and Cabrera, 1997). The rate of crop residue decomposition is directly proportional to the soil water content (Adiku *et al.*, 2010), which is rapid at field capacity, and decreases gradually declining soil moisture (Vigil and Sparks, 1995). In the field, increased soil moisture during winter has a negative effect on pathogen survival as it accelerates residue decomposition (Sutton, 1982). Burgess and Griffin (1968) found lower *F. graminearum* recovery from inoculated wheat straw in frequently wetted soil at 35°C as compared to 25°C or 10°C. Higher levels of moisture and temperature might have enhanced microbial activity and increased the rate of straw decomposition.

Wheat can be successfully grown throughout Alabama. However, wheat cultivation is concentrated in the far northern and southern counties of the state (USDA-NASS, 2009). Although wheat scab had not been reported as a major problem Alabama wheat (Gazaway, 1997), some destructive scab outbreaks have been reported in recent years from the northern Alabama, especially in the Tennessee Valley region (Hagan, 2011). Scab has been found at lower levels at a few locations in south Alabama (Glass *et al.*, 2012).

The reasons behind such differences in wheat scab occurrence between northern and southern Alabama are not clear. In general, Alabama has a mild climate with a pattern of slightly increasing temperature and rainfall from north to south (USDA-NASS, 2011). Variation in soil type, temperatures, and rainfall patterns in northern versus southern Alabama (USDA-NASS, 2011) could have influence the rate of crop residue degradation and ultimately on the saprophytic growth and sporulation of *F. graminearum*. Therefore, a possible reason for differences in scab occurrence across Alabama could be the variability of in-field sources of inoculum resulting from the differential rate of crop residue degradation.

Several studies have been conducted regarding recovery of *F. graminearum* from crop residues buried in the soil under controlled laboratory and field conditions (Burgess and Griffin, 1968; Khonga and Sutton, 1988; Summerell and Burgess, 1988). Findings of these studies showed a similar trend in which growth and survival of the fungus was negatively correlated with residue decomposition. It is well understood that crop residues colonized by *F. graminearum* are the major inoculum source for wheat scab (Guenther and Trail, 2005; Sutton, 1982). Further, residues of other crops, irrespective of their host status, could also serve as the substrate for saprophytic growth of scab pathogens. Therefore, we sought to determine the ability of wheat residue to support growth and development of *G. zeae* after incubation in soil. Specifically, our objective was to evaluate perithecial development by *G. zeae* on wheat straw as influenced by soil type, temperature, and moisture in the laboratory. The findings could be useful for understanding wheat scab occurrences in Alabama based on crop residues in fields and varying soils, temperatures, and rainfall patterns.

Materials and Methods

Experiment I. Wheat straw were collected during harvest from the Field Crops Unit of E. V. Smith Research Center, Tallassee, AL. Straw were cut into uniform pieces of 2.5 cm (1 in.) in length and were without nodes and outer sheaths, and sterilized by autoclaving for 45 minutes on each of 2 consecutive days.

The treatments for incubation of straw were arranged in a split-split plot design with incubation duration as the main plot, temperature as the sub plot, and soil type and moisture as the sub-sub plots. In each of incubation duration, treatments comprised a 3×2×3 factorial with three levels of temperature, two types of soil, and three levels of soil moisture. Soil samples were collected from two locations: the Wiregrass Research Center in Headland (HL) (southeastern Alabama) and the Plant Breeding Unit (PBU) of E. V. Smith Research Center near Tallassee (east-central Alabama). The soil from HL was a Dothan sandy loam, while the soil from PBU was an Independence (Cahaba) loamy fine sand. The A horizon Independence (Cahaba) loamy fine sand is composed of 62.3% sand, 23.3% silt, and 14.4% clay particles, whereas the Dothan sandy loam is composed of 82.1% sand, 9.0% silt, and 8.9% clay particles (USDA-NCSS, 2013). Soil samples were air dried at room temperature for 2 months then sieved through a 2 mm mesh screen and put into an incubator at 27°C for 3 days before use. Sixty grams of soil from each site was put into each of 54 petri dishes (2 cm deep); 18 samples were adjusted to each of three moisture levels (5, 10, and 15% wt/wt) by adding sterilized water. Petri dishes containing each soil moisture level were divided into three groups for each of three temperature regime. Four pieces of sterilized wheat straw were buried in the

soil in each petri dish, placed into airtight plastic boxes, and incubated in growth chambers at 20, 26, and 32°C.

One straw piece was removed from each petri dish at 1, 2, and 3 month intervals, rinsed with distilled water; air dried and sterilized by autoclaving for 45 minutes on each of 2 consecutive days. Sterilized straw pieces were then put onto water agar in separate plates. A macroconidial suspension was prepared from two week old cultures of *F. graminearum* on quarter strength potato dextrose agar (PDA). One hundred µl of macroconidial suspension (5×10⁵ macroconidia/ml) was applied over each straw piece. Plates were sealed with parafilm and incubated in a growth chamber at 26°C with diurnal light cycle (12 hr light/12 hr dark) to promote the perithecial formation. After inoculation, straw were inspected for fungal development and perithecial formation. Perithecial counts were recorded only from 14 to 77 days after inoculation (DAI) at 7 day intervals with the help of a binocular microscope.

Experiment II. The treatments were arranged in split-split plot design as in experiment I. However, the treatments comprised a 3×2×4 factorial with three levels temperature, two types of soil, and four levels of soil moisture. Soils were taken from Tennessee Valley Research and Extension Center (TV) (north Alabama) and Headland (HL) (southeastern Alabama) that were previously cropped to wheat and were dried as in experiment I. Soils from TV and HL were Decatur silt loam and Dothan sandy loam, respectively. The A horizon Decatur silt loam soil is composed of 38.9% sand, 38.8% silt, and 22.3% clay particles (USDA-NCSS, 2013). In order to find the differences in perithecia production on the straw incubated in non-sterilized compared to sterilized soil, a portion of each soil type was autoclaved twice for 60 minutes at 24 hour intervals.

Treatment combination with autoclaved soil comprised a 2×2×2 factorial with two levels temperature, two types of soil, and two levels of soil moisture. Autoclaved soils were adjusted to two levels of moisture (4 and 12%, wt./wt.) while non-sterilized soils were adjusted to four levels of moisture (0, 4, 8, and 12%, wt./wt.) with sterile water. Petri dishes (7.5 cm deep) containing each soil moisture level were divided into three groups for each of three temperatures (20, 26, and 32°C) for non-sterile soil, whereas only two levels of temperatures were used with autoclaved soil. Uniform straw pieces were prepared as in experiment I. Straw were dried by incubating at 60°C for 48 hours and dry weights of 15 straw pieces were recorded. A set of 15 straw pieces was buried in the soil in each petri-dish, which were put into airtight plastic boxes and incubated in growth chambers.

Three straw pieces were removed at 1, 2, and 3 month intervals, and dried as in experiment I. Dry weight of each set of straw was recorded and compared with average weights of the same set of straw before incubation. Sterilization of wheat straw, inoculation, incubation, and assessment of fungal development were carried out as in experiment I. Perithecial counts were recorded at 7 day intervals up to 91 DAI.

Data analysis. The total numbers of perithecia on each straw at 77 DAI were used for the data analysis. Data were checked for normality and outliers were removed. However, data were not normalized after logarithm or square root transformation, therefore, rank transformations were used for data analysis. Generalized linear mixed model analyses were used to determine the effect of the factors on perithecia production. Initially, only effect of incubation duration on perithecia production was analyzed. Later, the effects of soil type, moisture, and temperature were analyzed over incubation

duration. Treatment means were separated using Fisher's protected least square mean separation at $P \le 0.05$. In the second experiment, cubic regression analysis was done in order to find the effect of incubating soil moisture on perithecia formation. All data analyses were done using SAS 9.2 (SAS Institute, Inc., Cary, NC).

Results

Experiment I. Mycelial growth on inoculated straw was observed 5 days after inoculation. Perithecial development began at 7 to 14 DAI and perithecial numbers increased until 70 DAI when perithecial formation largely ceased. Therefore, total perithecia numbers on each straw at 77 DAI were used for data analysis. Perithecial counts were significantly higher on straw incubated for 1 as compared with 2 or 3 months (P<0.0001), where average perithecial counts after 1, 2, and 3 months were 7.0, 2.3, and 2.4, respectively.

Effects of the different factors were consistent over all incubation durations; therefore, final analysis was conducted using incubation duration as a random variable. Soil type, soil moisture, temperature, and the interaction of soil type and moisture as well as the interaction of all of the three factors had a significant effect on perithecia production by *G. zeae* (*P*<0.05) (Table 1). Straw incubated in the Independence loamy fine sand soil (PBU) generally supported higher perithecia numbers compared to those incubated in the Dothan sandy loam (HL), where the average perithecia numbers in each soil were 6.8 and 1.0, respectively. Fewer perithecia formed on straw incubated at higher temperatures, where average perithecia numbers formed on 20, 26, and 32°C temperature were 5.7, 3.3, and 2.8, respectively. In Independence loamy fine sand soil (PBU), higher

perithecial numbers were noted at lower soil moisture and temperature (Fig. 1), where average numbers of perithecia on 20°C with 5% moisture, 26°C with 10% moisture, and 32°C with 15% moisture were recorded as 14.8, 11.3, and 9.8, respectively.

Experiment II. Average straw dry weight after 1, 2, and 3 months incubation showed 10, 23, and 24 % loss, respectively when compared to initial weights. For the *G. zeae* inoculated straw, as in first experiment, mycelial growth was noticed at 5 DAI and perithecial formation, which was seen at 7 to 14 DAI, continued up through 77 DAI and then further perithecial formation was ceased (Fig. 2). Therefore, the perithecial counts at 77 DAI were used for the data analysis. As seen with experiment I, more perithecia formed on straw incubated for 1 month than for longer durations (Fig. 2). Accordingly, average numbers of perithecia formed on 1, 2, and 3 months incubated straw were 30.6, 7.8, and 12.4, respectively. A similar trend of perithecial formation was noted on the straw incubated in sterilized soil (P<0.0001), where average perithecial counts after 1, 2, and 3 months incubation were 20.7, 9.8, and 3.0, respectively.

The effect of soil type, soil moisture, and the interaction of these two factors significantly impacted perithecia production by G. zeae (P<0.05) (Table 1). Average perithecia numbers (22.4) on straw incubated in Decatur silt loam soil (TV) were higher than the average numbers of perithecia (11.5) formed on straw incubated in Dothan sandy loam (HL). Regression analysis showed that perithecia formation generally decreased with increasing soil moistures except for perithecia on straw from TV soil, where 4% soil moisture yielded similar counts to 0% (Fig. 3). The interaction of soil moisture and temperature also had a significant effect on perithecia production (P=0.05), where higher perithecia counts were often observed on straw incubated at lower soil moisture and

lower temperature (Fig. 4). The average numbers of perithecia formed on straw at 20°C with 0% moisture, 26°C with 4% moisture, 32°C with 8% moisture, and 32°C with 12% moisture were 35.7, 23.7, 9.0 and 5.7, respectively. Temperature as an autonomous factor did not have any effect on perithecia production.

There were no effects on perithecia production due to soil sterilization (P=0.095). However, soil type, soil moisture, and interaction of these factors had a significant effect on perithecia production (P<0.0001) (Table 2), where straw incubated in Decatur silt loam soil (TV) at 4% soil moisture supported the highest numbers of perithecia compared to all other combinations of soil type and moisture regimes.

Discussion

The goal of this study was to determine whether soil type as well as soil temperature and moisture can differentially affect decomposing wheat straw for supporting the saprophytic growth and development of *G. zeae*. Results indicate that growth and development of *G. zeae* varies among residues according to their exposure to different soil types and soil moistures.

Mycelium developed in inoculated straw during first few days after inoculation, whereas perithecial development started 7 to 14 days after inoculation. Dufault *et al.* (2006) similarly observed perithecial development on *G. zeae* inoculated cornstalks at 10 DAI. In our study, perithecial numbers increased up to 70 to 77 DAI after which perithecia formation ceased. Asexual development of *F. graminearum* in crop residue is favored when the residues are fresh with readily available carbohydrates (Khonga and Sutton, 1988; Leplat *et al.*, 2013), while sexual development initiates as substrate nutrient

content is nearly exhausted (Khonga and Sutton, 1988). Continual nutrient depletion in the straw might be responsible for the cessation perithecia formation after 77 DAI.

Perithecial numbers were higher on straw incubated for 1 month than for longer durations. This may be because straw incubated for 1 month had lost lesser amount of soluble and simple carbon compounds compared to straw incubated for longer periods and provided the better support for the growth and development of G. zeae. The sequential events during crop residue decomposition was described by Summerell and Burgess (1989) which involved an initial loss of simple and soluble carbon compounds followed by breakdown and leaching of complex carbohydrates. Reductions in the amounts of water soluble C and N in the first few weeks from decomposing wheat residues has also been reported by Marshner et al. (2011). In addition, Marshner et al. (2011) found that, in decomposing residue, fungi dominated bacteria after day 15 and were able to breakdown even the non-soluble C-components of the residue. Correspondingly in our study, the fungal community in soil might have increasingly degraded wheat straw such that, with longer incubation, their ability to support perithecial development by G. zeae was reduced. This result supports the findings by Burgess and Griffin (1968), who noted reduced recovery of G. zeae over time from buried wheat straw. Similarly, Summerell and Burgess (1988) and Pereyra and Dill-Macky (2008) also suggested that the recovery rate of G. zeae from crop residue decreased over time and was negatively affected by residue decomposition.

Significant variation in perithecial development was found between different soil types. Interestingly, in both experiments, straw incubated in finer textured soils from the northern sites of Alabama (PBU and TV) supported significantly higher numbers of

perithecia compared to those incubated in the coarser Dothan sandy loam soil from south Alabama (HL). In a study in which of *G. zeae*-colonized wheat straws were buried in different soils in laboratory conditions, Burgess and Griffin (1968) found no significant effect of soil type on *G. zeae* recovery. However, in field conditions, they found greater recovery of *G. zeae* from straw buried in comparatively finer textured and clay-particle-containing soils compared to soils containing less clay and more sand. Singh *et al.* (2009) found that residue decomposition rate was slower in silt loam soil compared to sandy loam. They suggested that an association of organic materials of the residue with clay particles in comparatively finer textured soil could slow the degradation rate. Smith *et al.* (2012) noted that turnover of soil organic carbon was faster in coarser as compared higher clay content soils. Therefore, quicker loss of soluble carbon compounds might account for the lower perithecial counts on straw incubated in Dothan sandy loam (HL) as compared to the Independence fine loamy sand (PBU) or the Decatur silt loam soil (TV).

The degradation rate of crop residues either from the perspective of microbial decomposition or from physical degradation might depend on the residue quality and type, presence of organic and inorganic elements in the soil, and composition of the soil microbial community (Henriksen and Breland, 1999; Schomberg *et al.*, 1999). We did not categorize the microbial communities in the soils used in our study, which could have influenced study results. Studies have shown that microbial activity is higher in fine textured than coarser soils (Hamarashid *et al.*, 2010; Scott and Robert, 2006). Thus, greater straw degradation, as evidenced by lower perithecia numbers for HL as compared with PBU or TV soils, could be due to differences in soil microbial community

composition. Since we used uniform straw pieces, straw quality and type did not impact perithecia formation on wheat residue. In order to confirm the effect of soil types on degradation of crop residues and development of *G. zeae* inoculum in the field, future studies might be conducted on the recovery of *G. zeae* from *G. zeae* inoculated buried wheat straw at different locations with different soil types across Alabama under natural conditions.

The effect of soil sterilization had no effect on perithecia formation on straw. However, higher perithecia numbers tended to form on straw incubated in non-sterilized as compared to sterilized soil. This result was not expected because microbial populations in the non-sterile soil should have caused greater degradation of residues resulting in lower perithecial numbers than residues incubated in sterile soil. However, in non-sterilized soil, the microbial populations could have begun to break down the complex carbon compounds into simpler compounds, which later might have been used by *G. zeae* for its accelerated growth and development. On the other hand, drying soil at 27°C prior to incubation could also have affected the microbial population.

Straw incubated with lower soil moisture resulted in elevated perithecial numbers compared to straw incubated in higher soil moistures. This suggests that moist conditions in the field reduce the ability of crop residues to support the production of *G. zeae* inoculum. Reduced perithecial formation on straw could be due to more rapid loss of simple carbon compounds at higher soil moisture levels. Franzluebbers *et al.* (1994) reported increased mineralization of C and N from plant residues incubated in moist as compared drier soils. Further, increased crop residue decomposition with rising soil water content levels have been reported by Adiku *et al.* (2010), Stott *et al.* (1986) and

Summerell and Burgess (1989). Additionally, Burgess and Griffin (1968) had noted reduced recovery of *G zeae* from straw buried in wet than dry soils.

Higher incubation temperatures decreased the ability of straw to support perithecial formation. This could be related to more rapid straw degradation at higher soil incubation temperatures. A decline in carbon compound loss rate at reduced temperature and moisture were also described by Stott *et al.* (1986), Summerell and Burgess (1989) and Quemeda and Cabrera (1997).

Crop residues are considered the primary source of inoculum for the wheat scab, thus adequate knowledge of the development and survival of inoculum on crop residues can provide insight for the management of this disease. However, in addition to discharge of inoculum from crop residues to wheat heads, long distance dispersal of spores through air can also aid in development of scab as described by Schmale *et al.* (2006). Therefore, future studies should consider exploring if there is any aerial spore dispersal mechanism involved in variable occurrence of wheat scab in Alabama. Our results suggest that crop residues in soils exposed to higher moisture and temperature levels, as in Alabama from north to south, and for longer periods of time, will suppress *G. zeae* perithecia production. The findings could also be useful for refining the scab risk models based upon soil types as well as temperature and moisture conditions in the field.

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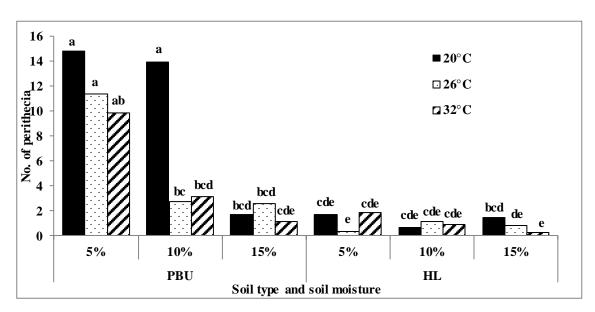


Figure 1. Perithecia counts on straw after incubation in two soil types from PBU and HL, at varying moistures and temperatures. Straw were inoculated with *Gibberella zeae* after incubation. Counts are averages from straw incubated for 1, 2, and 3 months in soil and 77 days after inoculation. Different letters above bar represent significant differences among treatments at P=0.05.

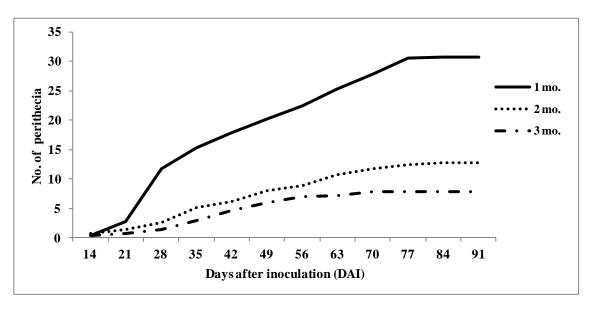


Figure 2. Perithecia counts on straw after inoculation with *Gibberella zeae* (experiment II). Different lines represent perithecia counts on straw incubated for 1, 2, and 3 months (mo.).

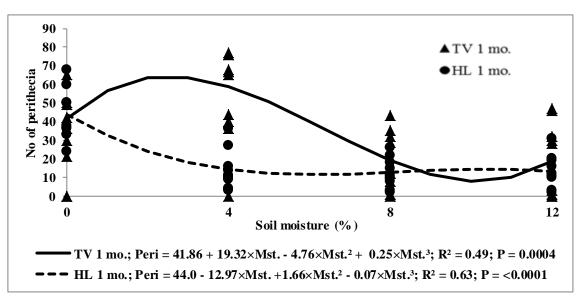


Figure 3. Relationship of soil moisture on perithecia production on *Gibberella zeae* inoculated straw (one month incubated straw, experiment II). Cubic regression was conducted using perithecia counts (Peri) at 77 DAI as the dependent variable and soil moisture (Mst) as the independent variable. Prior to inoculation, straw had incubated in two soil types (TV and HL), at varying moistures.

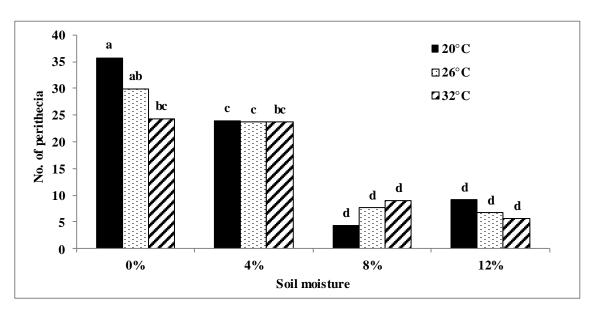


Figure 4. Effects of the interaction of soil moisture and temperature on perithecia production (experiment II). Different letters above bar represent significant differences among treatments at P=0.05.

Table 1. Table of significance for the effect of soil type, soil moisture, and temperature on perithecia production by *G. zeae* (experiment I and experiment II (non-sterile soil)).

	Factors	DF	F statistic	Pr > F
Experiment I	Soil (S)	1	53.85	< 0.0001
	Moisture (M)	2	4.87	0.009
	Temperature (T)	2	5.96	0.0033
	$S \times M$	2	6.86	0.0014
	$S \times T$	2	0.48	0.6192
	$M \times T$	4	0.21	0.9326
	$S \times M \times T$	4	2.71	0.0325
Experiment II	S	1	25.57	< 0.0001
	M	3	66.94	< 0.0001
	T	2	0.49	0.6452
	$S \times M$	3	15.24	< 0.0001
	$S \times T$	2	0.52	0.5955
	$M \times T$	6	2.14	0.0508
	$S \times M \times T$	6	1.17	0.3219

Table 2. Table of significance for the effect of soil type, soil sterilization, soil moisture, and temperature on perithecia production by *G. zeae* (experiment II).

Factors	DF	F statistic	Pr > F
Sterilization (St.)	1	2.84	0.095
Moisture (M)	1	83.01	< 0.0001
Temperature (T)	1	0.64	0.507
Soil (S)	1	45.91	< 0.0001
$S \times M$	1	62.72	< 0.0001
St.×M	1	0.03	0.866
$St.\times S$	1	0.28	0.601
St.×T	1	0.08	0.78
$M \times T$	1	1.65	0.201
$S \times T$	1	1.78	0.185
$St.\times S\times M$	1	0.39	0.532
$St.\times M\times T$	1	0.45	0.501
$St.\times S\times T$	1	0.76	0.385
$S \times M \times T$	1	0.37	0.545
$St.\times S\times M\times T$	1	0.28	0.595

IV. Distribution of wheat scab and its incidence across Alabama, and collection and characterization of *Fusarium* isolates.

Abstract

Wheat scab (Fusarium head blight) is an increasingly important disease of wheat in Alabama. Scab was not considered as a major problem of Alabama wheat in the past; however, in recent years, it has been commonly reported from the northern but not southern parts of the state. To date, no information is available concerning the distribution of wheat scab across Alabama as well as identity of the causal agent. Therefore, the objective of this study was to determine the incidence of wheat scab across Alabama as well as characterization of the causal pathogen. Wheat fields at different locations of the state were inspected for the presence of scab in 2011 and 2012. In 2011, fields were inspected in 7 locations (counties), whereas a total of 25 fields in 15 counties were surveyed in 2012. In 2012, scab incidence in North Alabama ranged from 5 to 30%. Fields in central and southwestern Alabama had few plants with signs of the disease. No signs of scab were found in wheat in southeastern Alabama. Wheat heads with sign and symptoms of the disease were collected during field inspections. Isolate characterization was conducted in the laboratory based on the morphological features of fungi grown on potato dextrose agar (PDA) medium. Morphological features of all isolates showed that the causal organism associated with wheat scab in Alabama is Fusarium graminearum. In conclusion, the findings of this study suggest that what scab appears as a potential threat for wheat production in north Alabama. Therefore, wheat growers in north Alabama are recommended to apply precautions against scab by regular following up of disease forecast and applying fungicide treatments as recommended.

Introduction

Wheat scab, also known as Fusarium head blight, is among the more destructive diseases of wheat worldwide. Wheat scab causes reduction in yield and grain quality because of *Fusarium*-incited damage to kernels and production of the mycotoxin, deoxynivalenol (DON) (McMullen *et al.*, 1997). During the 1990s, scab caused an estimated loss of \$3 billion in wheat and barley in the United States (Windels, 2000). Since then, scab has continued to cause economic losses in the northern Great Plains and central United States (Nganje *et al.*, 2002). Wheat scab has also appeared as a serious threat in the Mid-Atlantic States and the southeastern US (Cowger and Sutton, 2005).

Scab epidemics are greatly affected by local and regional environments, host factors such as the physiological stage at infection and genetic make-up, along with pathogen adaptation and virulence (Osborne and Stein, 2007). The combination of favorable weather conditions and abundant inoculum in the field during flowering of wheat enhances the development of scab (Windels, 2000). Warm and humid environments with temperatures from 15 to 30°C and RH levels around 90% are the ideal weather conditions for infection of wheat by *Fusarium graminearum* (De Wolf *et al.*, 2003). Cropping practices involving intensive cultivation of cereal crops in the same piece of land result in fortification and abundance of *F. graminearum* inoculum in the fields (Champeil *et al.*, 2004; Windels, 2000).

Wheat scab can be caused by several species of *Fusarium* of which *F. graminearum*, *F. culmorum*, *F. avenaceum*, and *F. poae* are the most common (Parry *et al.*, 1995). In addition to *Fusarium*, *Microdochium* species that incite scab include *M. nivale* and *M. majus* (Glynn *et al.*, 2005). *Fusarium* species produce mycotoxins, while *Microdochium* species are non-toxigenic (Simpson *et al.*, 2001). *F. graminearum* is the major pathogen causing wheat scab in the US (Gale *et al.*, 2007; Wilcoxson *et al.*, 1988; Zeller *et al.*, 2004) while *F. poae*, *F. culmorum*, *F. avenaceum* (Markell and Francl, 2003; Stack and McMullen, 1985; Wilcoxson *et al.*, 1988) and the newly discovered *F. gerlachii* and *F. asiaticum* (Gale *et al.*, 2011) are found at lower frequencies at a few locations. In the US, *F. graminearum* is distributed as a diverse population involving different chemotypes (Gale, 2003; Gale *et al.*, 2007).

Distribution of wheat scab pathogens differs among different geographic locations according to their specific biological and environmental requirements (Osborne and Stein, 2007). *F. graminearum* causes scab in warmer regions of the world such as the US, Australia and central Europe; whereas *F. culmorum* predominates in the cooler parts of northwestern Europe (Parry *et al.*, 1995). *F. graminearum* can grow well in temperatures up to 30°C while *F. poae*, a cool temperature fungus, can be found at temperatures around 20°C. Compared to other species, *F. graminearum* can be found over a wider range of temperatures and moisture regimes, preferring warmer and wetter conditions relative to other species (Osborne and Stein, 2007).

Wheat scab pathogens can be distinguished from each other morphologically on the basis of colony color, size, shape, and septation of macroconidia, presence or absence of microconidia, chlamydospores and perithecia, as well as with the help of molecular tools (Kammoun *et al.*, 2009; Leslie and Summerell, 2006). Macroconidial structures are usually used to differentiate *Fusarium* species as they are easy to distinguish on the basis of size, shape and segmentation (Summerell *et al.*, 2003).

Wheat is an important grain crop in Alabama and ranks 5th in terms of acreage (USDA-NASS, 2011). In the past, scab had not been considered as a major disease in Alabama wheat (Gazaway, 1997). However, during recent years, destructive scab occurrences have been reported from northern parts of Alabama, especially in the Tennessee Valley region (Hagan, 2011). Scab has been found at lower levels at a few locations in south Alabama (Glass *et al.*, 2012). In addition, *F. graminearum*, the major causal agent of wheat scab, was detected in fields in Autauga (central) and Baldwin (southwestern) counties of Alabama (Gale *et al.*, 2011).

Although there have been reports of scab outbreaks in a few locations of Alabama, no comprehensive study has yet been conducted regarding the distribution of scab in the wheat growing areas of the state. Further, no information is available on the characteristics of *Fusarium* species associated with wheat scab in Alabama. Characterization of causal organisms helps in understanding the mechanisms of pathogenesis and types of mycotoxin that could be produced by a particular isolate (Nicholson *et al.*, 2004). Further, isolate characterization helps when choosing an appropriate fungicide and application procedure to control the disease and mycotoxin contamination (Jennings *et al.*, 2000). Therefore, the overall goal of this study was to determine the status of wheat scab distribution in Alabama as well as characterization of the causal fungal isolates. The findings of this study would bring better understanding of

wheat scab distribution in Alabama, which could be helpful to the growers for adopting proper control procedures.

Materials and Methods

Field inspection. Wheat fields were inspected for scab in 2011 and 2012 at the soft dough stage. In 2011, inspected fields included seven wheat fields located at Auburn University Research Centers in Baldwin, Henry, Macon, Autauga, DeKalb, and Limestone counties; one additional location was a grower's field in Colbert county. Unlike in 2011, several growers' fields were included in the 2012 survey. A random route was prepared in accordance with wheat cultivation acreage in Alabama (Fig. 1). Wheat fields found around roadsides were examined for the presence of scab. In 2012, 16 fields from 8 counties of north Alabama (Talladega, DeKalb, Madison, Morgan, Limestone, Lauderdale, Colbert, and Franklin) and 9 fields from 7 counties of central and south Alabama (Baldwin, Coffee, Dale, Henry, Macon, Autauga, and Dallas) were inspected (Table 1). From each inspected field, wheat heads containing signs and/or symptoms of scab were collected and taken to the laboratory for isolation and further characterization of the pathogen isolates.

Characterization of isolates. Individual wheat spikes were cut into two halves and surface-disinfested by soaking in 0.8% sodium hypochlorite for 1 minute. Heads were then rinsed with sterile water and air dried. Each wheat head was put onto a petri dish containing *Fusarium* selective medium (FSM) (Schmale *et al.*, 2006) and incubated for 3 to 5 days at room temperature. Fungal colonies that developed on FSM were transferred to plates containing quarter strength potato dextrose agar (½PDA). After 3

days, a small plug of mycelium was removed from the PDA and put onto another plate containing water agar. After 12 hours, a single growing hyphal tip was cut with a sterile needle under the dissecting microscope and transferred to quarter strength PDA. Pure cultures were incubated for 10 to 15 days and used for the morphological identification. Morphological characterization was conducted based on the color of colony, shape, size and septation of macroconidia, and presence or absence of sporodochia, microconidia, or chlamydospore.

Results

Scab occurrence in 2011. Scab incidence was minimal in fields inspected in 2011. Of the seven locations, few scabby heads were found in wheat at Sand Mountain Research and Extension Center, Crossville (DeKalb county), Tennessee Valley Research and Extension Center, Belle Mina (Limestone county), Gulf Coast Research and Extension Center, Fairhope (Baldwin county), as well as a production field in Colbert county (Table 1). With the exception of the Baldwin county site, however, no signs of disease were found in wheat in central and south Alabama, which included Prattville Agricultural Research Unit, Prattville (Autauga county), E. V. Smith Research Center, Shorter (Macon county), and Wiregrass Research and Extension Center, Headland (Henry county).

Scab occurrence in 2012. Scab was commonly found in 2012 at varying incidence levels in the growers' fields in north Alabama (Table 1). Similar low levels of disease were found in Auburn University research centers as with previous year, except for Autauga (Prattville Research Center) and Macon (E. V. Smith Research Center)

county, where disease was not found in 2011. In a few growers' fields in Madison, Morgan, Lauderdale, and Colbert counties, scab was found at higher incidences compared to other locations, where the disease incidence ranged from 5 to 30%. While few symptomatic heads were found in fields in Talladega, Autauga, Macon, Dallas, and Baldwin counties, none were found in fields inspected in Henry, Dale, and Coffee counties in southeast Alabama. The weather information collected from three locations: Belle Mina, E. V. Smith and Fairhope, representing north, central, and south Alabama, respectively were not noticeably different from one-another with respect to the disease occurrences (Table 2).

Isolate characterization: Characterization of isolates was carried out from pure cultures grown on quarter strength PDA. Colonies grew onto the PDA with white, orange, or yellow colored mycelium (Fig. 2). When in contact with agar, the mycelium produced a pinkish pigment. None of the isolates produced microconidia. Macroconidia, which were produced by all isolates, were 45 to 65 μm long, 3 to 5 μm diameter and were moderately curved to straight with the presence of 4 to 6 segments. The apical and basal cell of macroconidia were somewhat tapered and foot shaped, respectively. Sporodochia and chlamydospores were not commonly formed and rarely formed in the cultures older than 30 days. These morphological characteristics are characteristic of *F. graminearum*.

Discussion

Results from this study, which showed that wheat scab incidence in Alabama declined from northern to southern portion of Alabama, confirmed previous observations (Glass *et al.*, 2012; Hagan, 2011). Scab was commonly observed in fields inspected in

northern counties at varying level of incidences. Scab was found at lower indices in the central and southwestern parts of the state and was not observed in wheat in southeastern region of Alabama. Scab development in wheat is influenced by weather conditions and cultural practices. Frequent rainfall or high humidity (~90%) coupled with temperatures between 15 and 30°C during flowering are the ideal conditions for *F. graminearum* infection of wheat (De Wolf *et al.*, 2003). Therefore, scab outbreaks vary depending upon the local weather conditions during flowering period of wheat. In addition to the weather variables, agronomic factors such as cultivar genetics, crop rotation, and tillage practices significantly affect the occurrence of scab (Blandino *et al.*, 2012; Osborne and Stein, 2007; Pereyra and Dill-Macky, 2008).

The levels of scab in the fields, which were located in Auburn University research centers, were found similar in 2011 and 2012. The fields showing higher levels of disease were among the arbitrarily selected growers' field in north Alabama, which were inspected only in 2012. We were not able to explain the exact reasons behind the disease levels in these fields due to the unavailability of information on the cultural practices and weather conditions during flowering of the wheat. However, the record of weather variables (rainy days, average relative humidity, and rainfall amounts) during March-April (time of wheat flowering) in three locations (Belle Mina- north Alabama, E. V. Smith- central Alabama, Fairhope- south Alabama) failed to explain the trend of declining disease incidence from northern to southern parts of the state. In these locations, the average March-April temperature ranges were within the favorable range for scab development, however, other major factors, the number of rainy days and total rainfall amounts were not noticeably different from one another. The average RH ranges

in each of locations were not high enough for ideal scab development. Therefore, the possible factors causing variable disease levels could have been associated with the local source of inoculum and cultural practices such as crop rotation and tillage.

Both wheat and corn are more densely cultivated in northern than in southern Alabama (USDA-NASS, 2009), which could have resulted in a higher incidence of wheat scab in north than in south Alabama. Corn is an important part of this system since *F. graminearum*, the major causal agent of wheat scab, can also cause ear rot disease in corn. After harvesting corn, *F. graminearum* survives in corn debris as a saprophyte and later can infect wheat causing the head scab (Goswami and Kistler, 2004; Sutton, 1982). In addition to the local sources of inoculum in the field, wheat can also be infected through the inoculum carried through air from neighboring states (Schmale *et al.*, 2006; Schmale *et al.*, 2011a). Future studies are recommended to evaluate if any of such mechanisms are involved in the scab outbreaks in north Alabama.

Variable occurrence of wheat scab between the northern and southern regions of Alabama could also be associated with the growth and survival potential of *Fusarium* inoculum. Decomposition of crop residue is responsible for reducing survival and recovery of *G zeae* inoculum (Pereyra *et al.*, 2004). Furthermore, higher rainfall means wetter soil, and warmer temperatures accelerate crop residue decomposition (Adiku *et al.*, 2010; Stott *et al.*, 1990; Stroo *et al.*, 1989). In general, Alabama has higher rainfall and temperatures in the south compared to the north (Chaney, 2013; USDA-NASS, 2011). This can be an important factor minimizing disease incidence in south Alabama due to accelerated residue decomposition which results in low inoculum levels. Therefore, one

possible reason for differences in scab occurrence could be the variability of in-field inoculum sources resulting from the differential rate of crop residue decomposition.

Morphological characterization of all isolates confirmed that the causal agent associated with wheat scab in Alabama is *F. graminearum*. Previous studies have also confirmed *F. graminearum* as the primary *Fusarium* sp. associated with wheat scab in the US (Schmale *et al.*, 2011; Stack and McMullen, 1985; Zeller *et al.*, 2004). Gale *et al.* (2011) also reported finding isolates of *F. graminearum* from two locations of Alabama: Autauga and Baldwin counties. Recent studies have shown that *F. graminearum* in the US is present not only as a single population but also is divided into three subpopulations producing three different trichothecenes (15ADON, 3ADON, and nivalenol), i.e., 'chemotypes' (Gale *et al.*, 2007; Gale *et al.*, 2011; Puri and Zhong, 2010). Therefore, further studies on wheat scab in Alabama are might be directed towards characterizing *F. graminearum* isolates for the presence of particular chemotypes.

In conclusion, apart from north Alabama, wheat scab levels across the state, in the years of this study, were insufficient to reduce wheat yield or increase the risk of mycotoxin contamination. However, this finding is limited and epidemics could differ from year to year depending upon favorable weather conditions. It is reported that scab occurrences in north Alabama during 2010 were more destructive than those observed in 2011 or 2012 (Hagan, personal communication). In addition, in this study, higher scab incidences were found in growers' fields as compared to fields at Auburn University Experimental Stations. Such differences could have resulted due to lack of appropriate cultivar selection and proper decisions on fungicide application. Therefore, wheat growers in Alabama, particularly in the northern region, are reminded to plant cultivars

that are found to have some levels of scab resistance. Information on wheat cultivar performance in Alabama can be found online at www.ag.auburn.edu/agrn/alabamavarietytesting. Further, growers can make proper decisions on fungicide application by following the information from national Fusarium head blight prediction center available at www.wheatscab.psu.edu.

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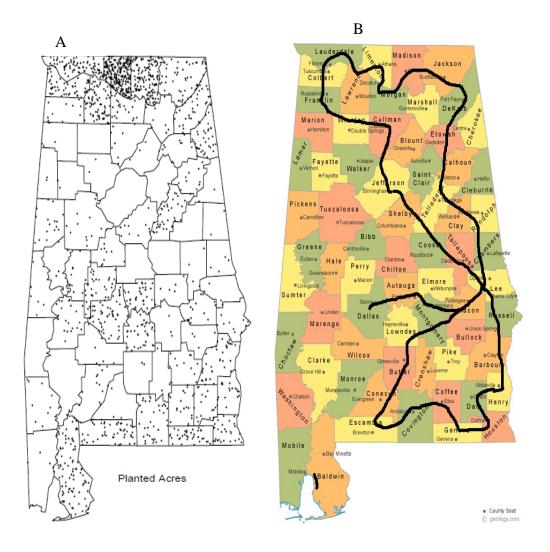


Figure 1. A, black dots indicate the distribution of wheat acreage in Alabama in 2007 (USDA-NASS); B, black line represents road map used to inspect wheat fields in order to check for scab occurrence in 2012.

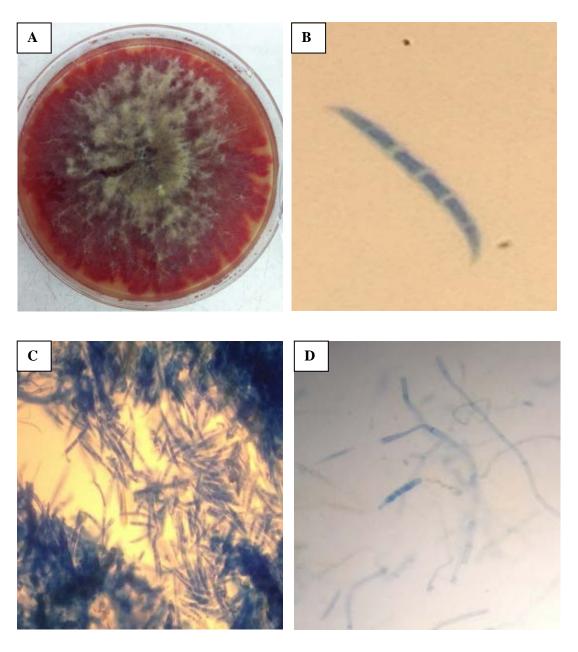


Figure 2. Morphological characterization of *Fusarium graminearum*. A, colony characters of isolates on quarter strength PDA, B, a single macroconidium with 6 segments, C, sporodochial mass formed on >30 days old culture, D, a single chlamydospore formed on >30 days old culture.

Table 1. Location of wheat fields inspected for scab and incidence levels of the disease.

		2011	2011 2012		
		No. of	Scab	No. of	Scab
Region	County	fields	incidence ^a	fields	incidence ^a
South	Baldwin	1	Low	1	Low
	Coffee	0	_	1	None
	Dale	0	_	1	None
	Henry	1	None	3	None
Central	Autauga	1	None	1	Low
	Dallas	0	_	1	Low
	Macon	1	None	1	Low
	Talladega	0	_	2	Low
North	Colbert	1	Low	2	Medium to severe
	DeKalb	1	Low	1	Low
	Franklin	0	_	1	Medium
	Lauderdale	0	_	3	Medium to severe
	Limestone	1	Low	2	Low to medium
	Madison	0	_	4	Medium to severe
	Morgan	0	_	1	Severe

^alow = 1 to 5 % disease incidence, medium= 5 to 20 % disease incidence, severe = 20 to 30% disease incidence.

Table 2. March-April weather data from three different locations representing north (Belle Mina), central (E. V. Smith) and south (Fairhope) Alabama (source: www.awiss.com).

Location	Month	Mean temperature (°C)	Total rainfall (mm)	Rainy days	Average RH (%)
Belle Mina	March	17.5	75.69	8	65.5
	April	18.22	37.85	6	66.4
E. V. Smith	March	17.56	81.03	11	70.2
	April	18.17	24.13	7	70.9
Fairhope	March	19.66	55.12	13	79.2
	April	19.28	35.81	4	74

Summary

This work described herein was initiated with the goal of addressing the epidemiology and management of wheat scab in Alabama. The specific objectives of the study were: 1) to evaluate the efficacy of fungicides in control of wheat scab and foliar diseases of wheat in Alabama; 2) to evaluate perithecial development by *Gibberella zeae* on wheat straw; and 3) to determine the distribution of wheat scab incidence across Alabama, and collect and characterize *Fusarium* species associated with the disease.

Fungicide trials were conducted for two years at each of three locations. The three locations were: E. V. Smith Research Center, Tallassee, in east-central Alabama; Sand Mountain Research and Extension Center, Crossville, in northeastern Alabama; and Tennessee Valley Research and Extension Center, Belle Mina, in north-central Alabama. Five fungicides were applied in a completely randomized design with five replicates. In addition to wheat scab, fungicides were tested for their ability to control common foliar diseases of wheat. Different diseases were found across locations and years, at varying intensities. Due to the minimal levels of disease at most locations, effects of fungicide application generally were non-significant for disease severity and the yield components, kg/ha and 1000 kernel weight (g). However, in the locations with relatively higher disease pressures (Tennessee Valley or E. V. Smith in 2011), fungicide treatments tended to reduce disease severities. Fungicides showed consistently positive effects on yield components irrespective of the disease levels. None of the fungicides were found

 $SC^{\mathbb{B}}$ consistently superior another. **Absolute** 500 over one however. StrategoYLD[®] (prothiconazole+trifloxystrobin) (tebuconazole+trifloxystrobin) and showed better response for reducing diseases and improving yield components. Wheat scab was found at trace levels at all locations in both years, but was not significantly affected by fungicides. Septoria blotch was the most commonly occurring disease at all locations followed by leaf rust. In 2012, a few of the wheat samples from three experimental fields plus an additional two fields (Prattville and Fairhope) from a different study were tested for the presence of the mycotoxin, DON. Only a few samples of wheat from Sand Mountain showed detectable levels of DON and these were within the safe limits for human consumption.

Crop residues in the field are considered the principle source of inoculum for G. zeae. Decomposition of these crop residues in the field negatively impacts the saprophytic growth and development of F. graminearum and reduces the chances of scab in wheat. Soil type and prevailing soil moisture and temperature can impact the differential rate of crop residue decomposition in the field, which could impact scab occurrence in different regions. In Alabama, wheat scab is commonly found in northern regions but is found to a lesser extent in southern regions. We hypothesized that due to higher annual rainfall and temperatures, residue degradation is greater and fungal development is reduced in southern fields than in northern fields. Two experiments were conducted in order to evaluate the ability of degraded wheat straw to support perithecial development by G. zeae. In experiment I, treatments comprised a $3\times2\times3$ factorial with three levels of temperature (20, 26, and 32°C), two soil types (Independent loamy fine sand and Dothan sandy loam), and three levels of soil moisture (5, 10, and 15%, wt./wt.).

For experiment II, treatments with non-sterile soil comprised a 3×2×4 factorial with three levels of temperature (20, 26, and 32°C), two levels of soil type (Decatur silt loam and Dothan sandy loam), and four levels of soil moisture (0, 4, 8, and 12%, wt./wt.). Uniform straw pieces were buried in different combinations of soil type, soil moisture, and temperature conditions. Incubated straw were taken out in one monthly interval for three months and inoculated with a macroconidial suspension of F. graminearum. Inoculated wheat straws were incubated in growth chambers at 26°C and a diurnal light cycle. Perithecia were counted for up to 90 days after inoculation. Perithecial numbers on straw were significantly impacted by soil type and moisture. Perithecial numbers were higher on straw incubated in finer textured than coarser textured soil. Perithecial numbers were reduced on straw incubated at higher soil moistures. Temperature had a significant effect on perithecia production only in experiment I, where lower temperature increased numbers of perithecia. These findings could be useful for understanding the occurrence of wheat scab based upon the status of prevailing crop residues in the fields with varying soils, temperatures and rainfall patterns.

In order to find the distribution of wheat scab incidence in Alabama, wheat fields were inspected for the presence of scab in two years: 2011 and 2012. In 2011, fields were inspected at 7 locations (7 counties); whereas, a total of 25 fields (in 15 counties) were examined in 2012. In both years, scab was commonly found with varying levels of incidences in north Alabama. Scab incidence in this region ranged from 5 to 30%. However, fields in central and southwestern Alabama had only a few plants with signs of the disease. No sign of scab was found in wheat fields in southeastern Alabama. Scabby heads were collected from inspected fields for isolation and characterization of the

pathogen. Isolates were characterized based on their morphological features when cultured on PDA medium. Morphological features of all of our isolates revealed that the causal organism associated with wheat scab in Alabama is *F. graminearum*. Based on the production of particular trichothecenes (15ADON, 3ADON, and nivalenol), future studies would be worthwhile characterizing these isolates into different chemotypes.

The findings of this two-year study reinforces that, apart from a few fields in north Alabama, wheat scab levels across the state did not seem problematic with regards to yield reduction or mycotoxin contamination. However, although noted at lower intensities in most locations, wheat scab poses a potential threat to Alabama wheat, if weather conditions are favorable. Our results suggest that fungicide applications could be beneficial in reducing multiple fungal diseases as well as increasing wheat yield. Therefore, wheat growers in Alabama, particularly in the northern region, are reminded to treat their fields with fungicides and plant available cultivars with known resistance against wheat scab. The results also suggest that crop residues in soils that are exposed to higher moisture and temperature levels, and for longer periods of time, will not support high *G. zeae* perithecial numbers. This could be one of the reasons for the more common occurrence of scab in north Alabama compared to southern parts of the state.