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Influence of Silicon on the Development of Anthracnose of Grain Sorghum

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INFLUENCE OF SILICON ON THE DEVELOPMENT OF ANTHRACNOSE OF GRAIN SORGHUM

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To my sister Sharmila for her immense love and care

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Table of Contents

Abstract

Anthracnose caused by *Colletotrichum sublineolum* has been an economically important disease of sorghum (*Sorghum bicolor*) globally. Silicon (Si), a beneficial element found to alleviate heavy metal toxicity, enhance growth under stress conditions, and reduce diseases in several cereal crops, was used to determine its impact on anthracnose development. To study the effect of Si with and without a fungicide, several experiments were conducted in the greenhouse and field. In the initial study, different rates of Si [0 (control), 0 (lime control), 200, 400, 600, 800 kg Si ha^{-1}] were used in a Typic Albaqualfs soil (Alfisol, low-Si) to determine if inoculum densities were affected by Si levels. No differences were observed between inoculum densities of $1*10^5$ and $1*10^6$ conidia ml^{-1} in affecting anthracnose development in the greenhouse. Anthracnose severity was found to be lowest in plants treated with 800 kg Si ha^{-1}, regardless of inoculum density. In the second study, the effect of Si on moderately susceptible (Pioneer 84G62) and moderately resistant (Pioneer 84P80) hybrids was also examined with a fixed inoculum concentration of $1*10^5$ conidia ml^{-1} under greenhouse conditions. Fungicide (Pyraclostrobin) was also included to suppress the anthracnose development. Silicon had a significant effect on plant Si concentration and anthracnose development. Anthracnose severity was reduced as plant and soil Si levels increased. The highest Si application rate (800 kg Si ha^{-1}) reduced Final Disease Severity (FDS) and Area Under Anthracnose Progress Curve (AUAPC) by 18 and 36% as compared to the control for the first greenhouse experiment ($p<0.05$). Likewise, 800 kg Si ha^{-1} reduced FDS and AUAPC of the 2nd greenhouse experiment by 76 and 67%, respectively ($p<0.001$). Pyraclostrobin effectively reduced AUAPC by 50 and 36%, respectively, for the two greenhouse experiments. Similar Si + pyraclostrobin experiments were conducted under field conditions at two locations in Louisiana (Dean Lee (Inceptisols) and

Winnsboro (Alfisols)) with higher soil Si levels (120 µg g^{-1} and 40 µg g^{-1} respectively). Even though soil Si concentration increased with higher rates of Si for both fields, no significant increase in Si accumulation in sorghum leaves or grains was observed. At Dean Lee, pyraclostrobin reduced AUAPC by 44 and 39%; respectively, for Pioneer 84G62 and Pioneer 84P80 (p<0.001). Pyraclostrobin also reduced FDS by 50 and 48%; respectively, for the two hybrids (p<0.001). However, pyraclostrobin had no effect in reducing anthracnose at Winnsboro. Yield was higher for Pioneer 84G62 than Pioneer 84P80 at Dean Lee.

Silicon had a greater impact in suppressing anthracnose development on low-Si soils under greenhouse conditions in comparison to field experiments conducted on high-Si soils. Silicon application plays an important role directly or indirectly in enhancing anthracnose resistance in sorghum, especially in soils deemed to be low or limiting in plant-available Si. Thus, further research needs to be conducted in various soil types to determine the need for fertilizing with Si for managing anthracnose development.

Chapter 1: Introduction

1.1 Sorghum production in USA

Sorghum (*Sorghum bicolor* (L.) Moench) is an efficient, high-energy, drought-tolerant crop, which is generally used for either grain or forage (www.Sorghumgrowers.com, 2015). Sorghum grain is used primarily as a feed for livestock and for ethanol production in the United States and internationally. The gluten-free characteristic of sorghum makes it a good substitute for wheat (*Triticum aestivum*) for people with celiac disease. Additionally, it is used to produce cake, cookies, malted beverages, porridge and syrups, and unleavened bread. Sorghum is native to northeast Africa; however, it is also cultivated in Australia, China, India and United States, being produced in 21 states. Sorghum can tolerate growing on marginal lands under adverse environmental conditions and its high yielding capacity has increased its scope and importance worldwide. It is the fifth most important cereal crop in the world economy (Sasaki and Antonio, 2009) and third most important crop grown in United States (USDA, 2011). Grain sorghum production in 2015 was estimated at 15.2 million metric tons, approximately a 38% increase over the previous year. Likewise, the area planted to sorghum in 2015 is estimated at 3.4 million hectares, which is approximately a 19% increase over the previous year. Its productivity for 2015 was five metric tons per hectare, which is a 12% increase from the previous year (USDA, 2016). These data show that the production of sorghum is increasing every year, because of an increasing demand for sorghum products.

1.2 Severity of anthracnose in sorghum and other crop species

Many diseases limit the production of sorghum cultivation, which include anthracnose (*Colletotrichum sublineolum*), charcoal rot (*Macrophomina phaseolina*), downy mildew

(*Peronosclerospora sorghi*), gray leaf spot (*Cercospora sorghi*), head blight (many fungi) and zonate leaf spot (*Gloeocercospora sorghi*). Of all the diseases, anthracnose caused by *C. sublineolum* P. Henn. (Ngugi et al., 2000; Sherriff et al., 1995; Sutton, 1980) affects almost all sorghum growing areas worldwide (Wharton et al., 2001). Anthracnose is especially prevalent in America, eastern Africa, China, India, Pakistan, and South America (Crouch and Beirn, 2009) and has been found to reduce yield in excess of 50% on susceptible cultivars (Thomas et al., 1996) particularly under warm and humid conditions (Ali and Warren, 1987).

The genus *Colletotrichum* is found to infect at least 42 genera in the grass family (Crouch and Beirn, 2009). Anthracnose has been an economically important disease, especially in cereal corps. Corn (*Zea mays* L.) anthracnose caused by *C. graminicola*, was epidemic during the early 1970s (Wheeler et al., 1974). Likewise, red rot disease of sugarcane (interspecific hybrids of *Saccharum officinarum* L.) caused by *C. falcatum* is one of the most destructive diseases of sugarcane in Bangladesh, India, Pakistan and Taiwan (Crouch and Beirn, 2009). Similarly, common bean (*Phaseolus vulgaris* L.) anthracnose (*C. lindemuthianum* (Sacc. and Magnus) which is prevalent under warm, humid climates is an important disease in the tropical and sub-tropical regions. The pathogen can infect at any growth stage (Kumar et al., 1999; Tu, 1988) and can cause yield and quality loss in susceptible varieties up to 95% (Guzman et al., 1979). Another economically important disease is chili (*Capsicum annum* L.) anthracnose, which can cause yield loss up to 50% (Pakdeevaraporn et al., 2005). Likewise, other economically important diseases caused by *Colletotrichum* are cucumber (*Cucumis sativus* L.) anthracnose (*C. lagenarium* (Eii. and Halst) with yield loss up to 60% (Averre, 1980), cashew nut (*Anacardium occidentale* L.) anthracnose with more than 50% yield loss (Cardoso et al., 1994), pepper (*Capsicum annum* L.) anthracnose with losses up to 80% (Poonpolgul and Kumphai, 2007),

lentil (*Lens culinaris* Medik.) anthracnose (*C. truncatum* (Schwein.) causing 60-70% losses (Morrall and Pedersen, 1991; Morrall et al., 1990) and watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) anthracnose (*C. lagenarium*) with losses up to 63% (Amin and Ullasa, 1981).

1.3 Sorghum anthracnose (Symptoms and Fungal Morphology)

Sorghum anthracnose was reported in United States for the first time in 1911 in Texas (Ali and Warren, 1987). This fungus occurs in its mitosporic form and is found in crop debris and infected seeds as mycelium, conidia or sclerotia (Casela and Ferreira, 1998; Zanette et al., 2009). The pathogen may overwinter in soil and decaying plant residues as acervuli, melanized hyphopodia, sclerotia, micro-sclerotia and mycelia. The fungus, *C. sublineolum,* is capable of surviving in crop residues for 1.5 years, and in sorghum seeds at room temperature for around 2.5 years (Crouch and Beirn, 2009). The pathogen shows conidial dimorphism and hence produces two kinds of conidia, falcate and oval (Souza-Paccola et al., 2003). Transmission of the pathogen occurs through the transfer of falcate conidia, especially through water or rain drops (Crouch et al., 2009). *Colletotrichum sublineolum* also produces oval conidia that are smaller than falcate conidia (Panaccione et al., 1989). Even though oval conidia are present in the lesions and infected tissue, their role is unknown (Crouch and Beirn, 2009; Sukno et al., 2008).

The pathogen infects leaves, leaf sheaths, stalks, peduncles, panicles and grains (Ali and Warren, 1987; Resende et al., 2009). The infection initially appears particularly in leaves as small tan to reddish purple circular or elliptical spots (Warren, 1986). Gradually, the spots enlarge and coalesce where the center of the spots turns ashy-grey as the tissue dies. The leaf lesions are often small but numerous. The midrib also becomes discolored. Eventually, after heading, the stalks and peduncle also become infected. Lesions on the stem surface generally are

circular with a red to black border with a greyish center, which later penetrate to the center of the stem. Red to white marble symptoms are observed when the stems are cut longitudinally. Acervuli are produced in the center of leaf and stem surface lesions (Marley et al., 2001). Generally, setae are scattered within the acervuli, which can be seen easily with a 10x hand lens. High rainfall and relative humidity, moderate temperatures and high pathogen densities are the conditions that favor disease epidemics (Ngugi et al., 2000).

1.4 Differences in *Colletotrichum* species

Correct identification of the pathogen is the basic step in developing management strategies against anthracnose. The confusion about the causal agent of sorghum anthracnose hinders basic management strategies in disease reduction and eradication (Figueiredo et al., 2006**)**. Sorghum anthracnose was known to be caused formerly by *Colletotrichum graminicola* (Ces.), the pathogen responsible for causing anthracnose disease in cereals and maize. But the conidial morphology and rDNA sequences have concluded that the maize pathogen does not resemble the one causing anthracnose in sorghum; therefore, *Colletotrichum sublineolum* became the accepted name (Souza-Paccola et al., 2003). Isolates of *Colletotrichum* from maize and sorghum have been described as two separate species (Sutton, 1980). Maize isolates were described as *C. graminicola* whereas the sorghum isolates were referred to as *C. sublineolum* because the two species were morphologically different (Hsiang and Khan, 2003; Vaillancourt and Hanau, 1992). Likewise, the results of rDNA sequences, DNA fingerprints, appressorial morphology and mating tests showed differences between *C. sublineolum* and *C. graminicola* (Souza-Paccola et al., 2003; Sutton, 1968). The sclerotia of the two species also were different in shape and size. The species were not interfertile and could be distinguished by molecular markers (Vaillancourt

and Hanau, 1992). These species of *Colletotrichum* are found to be host specific and the isolates infecting maize were not pathogenic to sorghum and the sorghum isolates were not pathogenic to maize (Ali and Warren, 1987). Even if *C. sublineolum* is listed as the causal agent of anthracnose of several hosts, the results from molecular phylogenetic analysis show that the host range for this species is limited to sorghum species, both wild and cultivated (Crouch and Beirn, 2009).

1.5 Silicon, its uptake, transport and deposition in plants

Silicon (Si) is the second most abundant element in the earth's crust after oxygen (Epstein, 1999). Monosilicic acid/orthosilicic acid is the form of Si available for plant uptake. Silicon is deposited in cell walls, intercellular spaces of root and leaf cells and bracts. In sorghum, it is also deposited in the endodermal cells of roots and also on the outer shoot cell wall (Hattori et al., 2003) in forms of silica gel $SiO_2.nH_2O$. Silicon is immobile and not redistributed to actively growing tissues; hence, older leaves accumulate more Si. Silicon is deposited below the cuticle as a cuticle-Si double layer and will polymerize once the concentration of monosilicic acid exceeds 2 mM (Gao et al., 2004).

There are three proposed modes of Si uptake in plants: energy dependent process or the active mode, energy independent process or the passive mode and rejective mode which is slower than the water uptake (Takahashi et al., 1990). Active mode which includes the involvement of several transporter genes in Si uptake, accounts for the major differences in Si accumulators from Si excluders. Several transporter genes which are mainly expressed in roots are involved in uptake and translocation of Si in Si-accumulating plants, such as barley (*Hordeum vulgare* L.), maize (*Zea mays* L.) and rice (*Oryza sativa* L.) (Ma, 2007, 2009). Ma and his colleagues (2006 and 2007) found two transporters (influx and efflux) in Si uptake by the rice

roots. An influx transporter or Low Si-1 (*Lsi1*) mediates passive transport of Si across the plasma membrane between external source and plant cells and into the xylem. Efflux transporter or Low Si-2 (*Lsi2*) facilitates active transport of Si out of the plant cells. Therefore, once absorbed by plant roots, subsequent Si transport from root cortex to the stele is carried out. Silicon is then transported as silicic acid to the shoots through transporters (*Lsi6* in rice) and the transpiration stream in xylem. The transporter responsible for xylem loading of Si has not yet been identified. However, a transporter named *Lsi6* (Low silicon-6) was localized at the adaxial side of xylem parenchyma cells of leaf blades and leaf sheaths and found responsible for xylem unloading. Even though the function of these genes are the same in all crops, the different expression pattern and cell-type specificity of localization are the key factors that determine differences in Si uptake capacity of these species. Apart from the active mode, passive mode of Si uptake includes diffusion which includes movement of ion from higher concentration to lower concentration and mass flow which includes transportation of nutrients along with water to the root surface (Elawad and Green, 1979; Yoshida, 1975).

1.6 Silicon as a beneficial nutrient

Silicon is a beneficial plant nutrient and not only suppresses diseases and pests but also increases the resistance to lodging, and drought as well as dry matter accumulation in rice and cucumber (Datnoff and Rodrigues, 2005; Vasanthi et al., 2014). Silicon application helps in alleviation of heavy metal stress and improves salt tolerance of a number of grain crops (Yin et al., 2013).

1.6.1 Silicon's role in minimizing abiotic stresses

Plants of the Poaceae family, especially grain crops, are Si accumulators (Vasanthi et al., 2014). There are various physiological and metabolical benefits of Si application in plants. These include photo assimilation of carbon thus promoting the assimilation of carbon to rice panicles, improvement in salt tolerance and water balance of crops and reduction in the sodium ion accumulation and osmotic stress which are the effect of increase in salt concentration in the plants (Yin et al., 2013). Likewise, endodermal silicification in roots helped to prevent water movement from the stele and thus, ultimately increasing the drought tolerant capacity of sorghum (Lux et al., 2002) as well as inducing root elongation (Hattori et al., 2003). Furthermore, use of Si reduced carcinogen arsenic uptake in rice along with yield enhancements (Fleck et al., 2013).

Apart from the physiological benefits, Si accumulation in plants also increases rigidity and strength of the stem, prevents lodging of the grain crops, maintains water balance and reduces transpiration loss (Vasanthi et al., 2014). In sorghum, Si increases grain yield together with an increment in total biomass (Resende et al., 2013; Yin et al., 2013). In rice, according to Datnoff and his colleagues (1992), yield was increased by 53% for plants growing in silicon-amended soils compared to those non-amended.

1.6.2 Role of silicon in minimizing biotic stress

Application of Si to plants will delay pathogen infection and allow more time for the plant to develop a mediated defense response (Resende et al., 2013). This mediated response can result in the production of phenolics and phytoalexins, and Pathogenesis Related (PR) proteins, which are associated with increase in host-resistance (Fortunato et al., 2012; Rodrigues et al., 2004;2005)

and can efficiently reduce disease severity of various crops (Datnoff et al., 2007). Silicon promotes biosynthesis of defense compounds such as terpenoids and alkaloids (Epstein, 2009). Accumulation of peroxidase, glucanase and PR-1 transcripts resulted in limited colonization by *Magnaporthe grisea* in epidermal cells of rice supplied with Si (Rodrigues et al., 2005). The deposition of Si below the cuticle forms a cuticle-Si double layer which acts as a mechanical barrier against pathogen penetration (Yoshida, 1975). Severity of several economically important rice diseases such as brown spot, leaf scald, rice blast, sheath blight and stem rot were reduced with Si fertilization (Datnoff et al., 1997, 1992, 1991; Nanayakkara et al., 2008a). Likewise, the intensity of soil-borne diseases in cucumber, bell pepper and tomato (*Solanum lycopersicum* L.) plants were also reduced by Si fertilization (Belanger et al., 1995; Cherif et al., 1994, 1992; Dannon and Wydra, 2004; French-Monar et al., 2010). Silicon amendments increased resistance against leaf spot in bermudagrass (Datnoff and Rutherford, 2004). Silicon had an active role in enhancing wheat resistance against powdery mildew (Belanger et al., 2003). Furthermore, antifungal activity of Si by damaging the plasma membrane of some fungi such as *Penicillium digitatum,* resulting in leakage of protein and sugar, was effective in controlling green mold in citrus fruit (Liu et al., 2010), suggesting the beneficial aspects of Si in controlling postharvest diseases.

In the banana-Fusarium pathosystem, Si increased host resistance by reducing the relative lesion length (RLL) and asymptomatic fungal colonized tissue (AFCT). Increased resistance was also associated with enzymatic activities, pigments, and hydrogen peroxide concentration.

Silicon amendment in the soil where perennial ryegrass (*Lolium perenne* L.) was grown resulted in decreasing disease incidence and disease severity of gray leaf spot, regardless of Si sources used (Nanayakkara et al., 2008b).

In a study of gray leaf spot (*Magnaporthe grisea* (Hebert)) of St. Augustinegrass (*Stenotaphrum secundatum* (Walter) Kuntze), components of host resistance such as incubation period, latent period, lesion number, lesion area, daily rate of lesion expansion and number of conidia per lesion were studied to better understand the role of Si in disease management (Brecht et al., 2004). The percent leaf area diseased was also studied as a result of the components of disease resistance. They discovered that only the lesion number was the component of resistance affected by Si application, which was reduced by 61% when compared to a Si non-treated susceptible cultivar. Various rates (0, 0.5, 1, 2, 5 and 10 t ha^{-1}) of calcium silicate were used in this experiment, which were negatively correlated with the number of lesions. Number of lesions decreased significantly with an increase in rate of Si from 43 to 57%. Likewise, the percent leaf area diseased in the susceptible cultivar was reduced significantly (18 to 57%) with increasing Si rates (Brecht et al., 2007). The authors thus concluded that Si fertilization especially in soils with low Si content is a good management strategy for reducing gray leaf spot development in St. Augustinegrass.

For rice sheath blight development, the total number of sheath blight lesions, total area under the relative lesion extension progress curve, lesion height on main tiller and the overall disease severity were found to be drastically reduced with increasing Si rates. This effect was not due to calcium since the calcium content in the leaf tissue did not change (Rodrigues et al., 2003).

Increase in Si rates significantly increased the incubation period, reduced the number and size of sporulating lesions, rate of lesion expansion, diseased leaf area and number of spores per lesion, ultimately reducing the epidemic rate of rice blast (Seebold et al., 2001).

1.7 Relation of sorghum anthracnose and silicon

Various management practices to minimize the effect of anthracnose disease in grain sorghum are available, including Si application, which plays a role in minimizing biotic and abiotic stresses (Datnoff et al., 2007).

Sorghum accumulates Si in its plant tissue ranging from 0.8 to 2 dag kg^{-1} (Resende et al., 2013). Silicon positively affects sorghum plants by maintaining carbon fixation, enhancing the antioxidant system, and increasing the defense response of the plant against *C. sublineolum.* Higher Si deposition at infection sites of *C. sublineolum*, accumulation in extracellular spaces, epidermal walls, as well as the basal cells of the trichomes, has resulted in reducing fungal penetration. The incubation period and the latent period of sorghum anthracnose increased in plants amended with Si. Likewise, the relative infection efficiency and area under anthracnose index progress curve (AUAPC) were reduced (Resende et al., 2009).

Resende and colleagues (2009) studied the effects of different Si levels on various components of host resistance in two sorghum lines that were resistant and susceptible to *C. sublineolum*. They used an inoculum density of $1*10^6$ conidia ml^{-1} and the Si application rates were 0, 0.06, 0.12, 0.24, and 0.30 g Si kg^{-1} of soil. Although Si had no effect on the components of host resistance for resistant lines, all components of host resistance were affected by Si application for the susceptible line. A hypersensitive type resistant reaction, which limits the lesion growth only on the leaf blades without sporulation (Ali and Warren, 1987), was shown to occur in the resistant lines when inoculated with the pathogen (Resende et al., 2009). Similarly, Si was observed to increase the incubation period and latent period in the susceptible lines, which had negative correlation with the area under anthracnose progress curve. Likewise, the study showed that the Si content in plant tissue increased by 55 to 58% for susceptible and

resistant lines, respectively. The incubation period and latent period were found to be delayed longer at the highest Si rate. Consequently, a higher rate of Si resulted in a greater reduction in anthracnose development.

The production of a complex of phenols, whose components are the 3-deoxyanthocyanidin flavonoids: apigeninidin, luteolinidin, arabinosyl-5-O-apigeninidin, 7-methylapigeninidin and 5-methoxyluteolinidin phytoalexins, are the main defense response of sorghum plants reported against anthracnose. These complex phenols have been demonstrated to be fungitoxic to *C. sublineolum* (Nicholson et al., 1988) and phenolic compounds in general are believed to be a major form of resistance (Cherif et al., 1994; Fawe et al., 1998; Rodrigues et al., 2004).

As observed by Resende and her colleagues (2009 and 2013), Si was significant in reducing infection efficiency and AUAPC and delaying fungal colonization in sorghum. Santos and colleagues (2014) observed similar findings where Si reduced anthracnose severity in sorghum. Likewise, Si has been useful with and without fungicides in minimizing several diseases (Brecht et al., 2004, Resende et al., 2013; Seebold et al., 2004).

From all of the above mentioned benefits of Si, the objective of the current study was to evaluate its role in minimizing sorghum anthracnose under greenhouse and field conditions in Louisiana. One of the major concerns with anthracnose development under greenhouse conditions was inoculum density. There have been several pertinent studies with various inoculum densities. Resende and her colleagues (2009) used $1 * 10^6$ conidia ml^{-1} whereas Santos and his colleagues (2014) used $1* 10^5$ conidia ml^{-1}. Therefore, this study was conducted to: 1) evaluate anthracnose development at various inoculum densities in the greenhouse 2) evaluate the relation between soil Si concentration and Si accumulation in sorghum leaf tissues and sorghum grain and their impact on anthracnose development in the field, and 3) evaluate the role

of Si with and without a fungicide on anthracnose development in moderately susceptible and moderately resistant hybrids in the greenhouse and at various field locations in Louisiana.

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Chapter 2: Effect of silicon and inoculum densities of *Colletotrichum sublineolum* on anthracnose development of grain sorghum

2.1 Introduction

Sorghum (*Sorghum bicolor* (L.) Moench), the fifth most important cereal crop of world (Sasaki and Antonio, 2009), is grown in 21 states in USA (www.sorghumgrowers.com, 2015). Louisiana is one of the major sorghum growing states and a number of plant diseases such as anthracnose, charcoal rot and grain mold affect plant development and yield. Anthracnose caused by *Colletotrichum sublineolum* P. Henn, is the most important disease accounting for economic yield loss up to 28% over the past ten years, (Hollier, personal communication). This pathogen infects leaf, leaf sheath, peduncle, panicle as well as the grain (Gwary et al., 2003). *Colletotrichum sublineolum* survives on plant debris as conidia, mycelia or sclerotia, and is the source of primary infection disseminated by splashing rain on host leaves and wind (De Milliano et al., 1992). The disease is widespread in warm and humid climates. Foliar and stalk damage results in poor growth and once the panicle and grains are infected, yields per head are significantly reduced. As the plant approaches maturity, the disease destroys grain sorghum rapidly reducing seed weight and grain quality.

Various management practices to minimize the effect of anthracnose development in grain sorghum are available, including silicon (Si) nutrition, which plays a role in minimizing biotic and abiotic stresses (Datnoff et al., 2007). Silicon, a 'quasi-essential' element (Epstein and Bloom, 2005) has been found to reduce several soil-borne diseases such as root rot of banana (*Musa acuminata*) (Vermeire et. al., 2011), Phytopthora root rot of soybean (*Glycine max*) (Guerin et. al., 2014); foliar diseases such as rice (*Oryza sativa* L.) blast (Seebold et al., 2000),

powdery mildew of wheat (*Triticum aestivum*) (Guevel et al., 2007); bacterial blight of rice (Feng et al., 2010), and leaf streak of wheat (Silva et al., 2010).

Anthracnose development in a controlled environment, has been induced by using a foliar application of leaf powder or a conidial suspension of the pathogen (Dube et al., 2010). However, the conidial suspension was found to be more effective than leaf powder in promoting anthracnose development. Dube and his colleagues also found that the optimal temperature for fungal development was 27^{o}C within a controlled environment. Likewise, they also found that even if infection occurred in all plant growth stages, optimum disease development was found when the plants were inoculated at boot stage [when the head is extended into flag leaf sheath (50 days after emergence)]. However, plants inoculated with conidial suspension and maintained at 27^{o}C at 30 days after emergence had more pronounced disease development than plants inoculated 10 days after emergence (Dube et al., 2010).

In studies conducted by Singh et al. (2006) and Perumal et al. (2008), an inoculum concentration of $1 * 10^{6}$ conidia mL^{-1} was used in order to evaluate anthracnose severity, under a controlled environment. Likewise, Resende et al. (2009, 2013) and Li et al. (2013) used the same inoculum concentration to induce anthracnose on sorghum plants. However, to induce anthracnose in pepper (*Capsicum annum* L.), Hong and Hwang (1998) used $1*10^{4}$ spores mL^{-1}. Also, Santos and colleagues (2014) used $1*10^{5}$ conidia mL^{-1} to induce anthracnose development in sorghum. Therefore, a portion of this study was evaluated to determine if $1*10^{6}$ conidia mL^{-1} had a significant advantage over $1*10^{5}$ conidia mL^{-1} in enhancing anthracnose development. Therefore, the objectives of this study were to evaluate the effect of Si and inoculum densities of *C. sublineolum* on anthracnose development in grain sorghum

2.2 Materials and Methods

2.2.1 Greenhouse setup

For the greenhouse study, a low-Si soil was collected from a site in Eunice, Evangeline Parish, LA (30°32'50"N 92°30'33"W). The soil was Crowley-Vidrine (CV) complex and is classified as fine, thermic, smectitic, Typic Albaqualfs (SSURGO-USDA, 2015). The soil texture was silt loam to silt clay with a pH of 5.5, which is considered to be low for growing sorghum. Composite soil samples were analyzed for plant-essential nutrient composition using Mehlich-3 procedure, soil pH, and texture. Table 2.1 provides the soil concentration of selected plant-essential nutrients, sodium (Na), and Si, before planting sorghum.

Table 2.1. Initial nutrient content and pH of soil used for the greenhouse study.

Soil pH and Nutrient Content	Value	Soil Test Interpretation
pH (1:1 soil:water ratio)	5.5	Low
Phosphorus, mg kg^{-1}	17.4	Low
Potassium, mg kg^{-1}	101	Medium
Calcium, mg kg^{-1}	759	V. High
Magnesium, mg kg^{-1}	104	V. High
Sodium, mg kg^{-1}	28.3	Optimum
Sulfur, mg kg^{-1}	9.93	Low
Copper, mg kg^{-1}	1.36	High
Zinc, mg kg^{-1}	2.73	High
†Silicon mg kg^{-1}	8.19	Low

Plant essential nutrient content was determined based on Mehlich-3 extraction procedure and inductively-coupled plasma analysis (Mehlich, 1984).
† Determined based on 0.5 M acetic acid, 1-hr extraction procedure followed by Molybdenum Blue Colorimetry (Korndorfer et al., 2001).

Based on the initial soil test results in Table 2.1 and LSU AgCenter fertilizer recommendation for sorghum, nitrogen (N), phosphorus (P), and potassium (K) were applied at 110, 90, and 70 kg ha^{-1} as urea blended with ammonium sulphate (33-0-0 N), triple superphosphate (TSP, 0-46-0) and muriate of potash (MOP, 45-0-0), respectively.

For the greenhouse experiment, plastic pots (Hummert International, Earth City, MO) with an inside diameter of 15 cm and volume of 4 L were used. The bulk soil sample was processed with all debris removed. Three kg of ground, air-dried, soil was placed in each pot. Paper coffee filters (Brew Rite®) were used at the bottom of the pots to prevent significant soil loss through drainage holes. Pots were filled with 3 kg soil reaching up to 2.5 cm from the top rim of each pot. Lime was not recommended because the calcium (Ca) content of the soil was high. The following were applied per kg of soil: 0.188 g of ammonium sulfate blended with urea, 0.108 g of TSP, 0.0622 g of MOP to meet the requirement for N, S, P, and K. In the study, soil was treated with different Si levels at 200, 400, 600, and 800 kg Si ha^{-1}, including a check (0 Si) with and without lime. Wollastonite (Vansil® W10, Vanderbilt Minerals, Norwalk, CT) was used as source of Si and applied at 0.461, 0.922, 1.383, and 1.844 g kg^{-1} of soil, respectively for the above target rates. Wollastonite ($CaSiO_3$) is a powder containing 24% Si and 31% Ca. In order to differentiate Si effects from Ca effects, lime was incorporated into the treatment structure. In addition, depending on wollastonite application, lime was applied as Aglime at 0, 1.66, 1.38, 0.92, 0.46, 0 g kg^{-1} of soil to pots treated with 0, 0, 200, 400, 600, and 800 kg Si ha^{-1}, respectively to attain the same level of Ca equivalent across Si treatments. Pre-weighed soil contained in each pot was placed in a clean plastic 4 liter-zipper bag (Wal-Mart). Nutrients were then added and thoroughly mixed with the soil. The treated soil was then placed back to the corresponding pot and allowed to lay idle for a week to allow proper nutrient-soil establishment.

Sorghum seeds of Pioneer hybrid 84G62 were sown at four seeds per pot at a depth of 2.5 cm. Upon establishment, plants were thinned to two per pot. Six weeks after sowing, deficiency symptoms of NPK and boron (B) were observed. To correct the deficiencies, a basal application of Hoagland nutrient solution (Tubana, personal communication) was applied to all plants. Tap water (pH 8.0) was used to irrigate the plants maintaining adequate soil moisture. The plants were inoculated with a conidial suspension of *C. sublineolum* four weeks after sowing.

2.2.2 Isolation of the Pathogen

Leaves showing symptoms of anthracnose were collected from the field, and placed in a moist chamber for 24 hours. Two to four cm long pieces of leaf including acervuli were cut and washed with 10% bleach (Wal-Mart) for two minutes for surface sterilization. The surface sterilized leaf pieces were then washed in sterilized double distilled water (ddH_2O) for five minutes to remove the remaining bleach. The leaf pieces were placed on unsealed plates with PDA media and incubated at room temperature for 72 hours. A single acervulus was isolated and placed in sterilized ddH_2O and stirred using a sterilized needle. Few drops of water containing conidia were spread using a sterilized glass rod over the surface of the PDA media. Single spore culture was then prepared and the pathogen was grown on PDA as a pure culture which was used for genomic DNA extraction. Later, the fungus was cultured on PDA for 14 days to generate an adequate number of conidia for the inoculation test. A haemocytometer was used to standardize inoculum densities.

2.2.3 Preparation of media for culture growth

In vitro culture of *C. sublineolum* was accomplished by growing the isolates on PDA at 28^{o}C for 18-20 days. Full strength PDA was used for conidial production and was prepared by adding 19.5 g PDA to 500 ml of distilled water. Quarter strength PDA was used for culture maintenance and was prepared by adding 5.625 g agar to 4.875 g PDA and 500 ml of ddH_2O. The medium was stirred with a magnet for approximately 10 minutes and was then autoclaved at 121^{o} C for 20 minutes at 15 psi and then poured in petri dishes at 20-25 ml plate^{-1}.

2.2.4 Molecular identification of the pathogen using quantitative Polymerase Chain Reaction (qPCR) and DNA sequencing

Colletotrichum sublineolum was grown on PDA for a week at 28^{o}C. Mycelia was collected and used to extract genomic DNA using Promega Wizard Purification Kit (Promega Coorporation, Madison, WI), from which, the Internal Transcribes Spacer (ITS) region was amplified by PCR using forward primer ITS1F (CTTGGTCATTTAGAGGAAGTAA) (Gardes and Bruns, 1993) and reverse primer ITS4 (TCCTCCGCTTATTGATATGC) (White et al., 1990). Each PCR reaction contained one µl of genomic DNA (250 ng µl^{-1}), 12.5 µl of 2* PCR mix (GoTaq: Green Master (Ref: M782A), Promega), 1.25 µl of 10 µM forward and 1.25 µl of 10 µM reverse primers, and nine µl of sterilized ddH_2O in a total volume of 25 µl. The PCR program consisted of initial denaturation at 95°C for five minutes; 35 cycles of 95°C for 45 seconds, 55°C for 45 seconds and 72°C for one minute; and the final extension at 72°C for five minutes. Following, the PCR product was sent Louisiana State University School of Veterinary Medicine's Genelab, Baton Rouge, LA for sequencing. Upon receipt of the ITS sequence it was analyzed using Basic Local Alignment Search Tool (BLAST) of National Center for Biotechnology Information (NCBI) database to confirm that the pathogen was *C. sublineolum*.

2.2.5 Inoculation and incubation of greenhouse-grown grain sorghum

Two inoculum densities, $1*10^5$ and $1*10^6$ conidia ml^{-1}, were used for the experiments. The conidia were counted using a haemocytometer and the suspensions were collected in two 1L-spray bottle (The Bottle Crew, West Broomfield, MI) with an addition of Tween 20 at 0.05% to reduce surface tension. A whole plant inoculation was performed at the fourth leaf stage by atomizing the conidial suspension onto all treatment plants. Following inoculation, the plants were bagged using a 125 L-capacity white polythene bag (Berry Plastics, Evansville, IN) for 24 hours to maintain the relative humidity and establish fungal infection.

2.2.6. Anthracnose Final Disease Severity (FDS) and Area Under Anthracnose Progress Curve (AUAPC)

Final Disease Severity (FDS) which is the final proportion of area of infected plant tissue and Area Under Anthracnose Progress Curve (AUAPC) which is a quantitative measure of disease progress over time (Madden et al., 2007) were used to assess anthracnose development. Beginning five days after inoculation, four anthracnose severity measurements were made at five-day intervals. Anthracnose severity was determined as the percentage of total leaf area covered by symptoms of disease for all plants in each pot using the modified diagrammatic scale proposed by Sharma (1978). Sharma used standard visual ratings to score approximate percentage of leaf area affected by anthracnose. He used a scale of 0 to 9 where leaf with no symptoms was rated as ‘V’ (0%) and totally affected leaf as ‘9’ (100%). Intermediate ratings were ‘2’ (2.5%), ‘3’ (5%), ‘4’ (10%), ‘5’ (20%), ‘6’ (35%), ‘7’ (50%), and ‘8’ (75%). However, in this study, only the percentage measurement was used for calculation. Percentage of anthracnose infection was measured for each leaf and averaged for the two plants in a pot and used as a single value for each sample. After the final measurement (8 weeks after sowing), the

plants were cut at the base and left in the greenhouse for air drying. Area Under Disease Progress Curve was computed using the formula (Madden et al., 2007)

$$\text{AUAPC} = \sum_{i=1}^{n}\left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} - t_i)$$

Where,

n= total number of observations, y_i = disease severity at the i^{th} observation, and t= time at the i^{th} observation. Since the unit for y in these studies is %, and the unit for t is days, the unit of AUAPC here, is %-days unit.

2.2.7. Post-experiment soil silicon extraction

The main purpose of incorporating Si sources in the soil is to increase monosilicic acid in soil solution. Different rates of Si fertilization affect formation of different silicic acid species. The concentration of monosilicic acid increases with the increase in Si application up to a limit and then polymerizes thus being unavailable for plants (Tubana and Heckman, 2015). Therefore, to understand the availability of monosilicic acid in soil solution for sorghum growth, plant-available soil Si content was measured. To extract soil Si, soil samples were collected from each pot, dried, and two grams were weighed and placed in a 50-mL centrifuge tubes. A modified Molybdenum Blue Colorimetric (MBC) procedure as outlined by Korndorfer et al. (2001) was used to determine plant-available soil Si from the samples after extraction.

a) Soil silicon extraction procedure:

Twenty mL of 0.5 M acetic acid were added to the soil samples and placed on a reciprocal shaker (Eberbach; model number E6010.00) for one hour set at high speed. The samples were filtered through Whatman No. 1 filter paper into 50 mL centrifuge tubes, immediately after

shaking. A 0.5 mL aliquot from the filtered solution was pipetted into 50 mL centrifuge tubes for colorimetric procedure (Korndorfer et al., 1999).

b) Colorimetric procedure:

Two sets of Si standard series (0, 0.2, 0.4, 0.8, 1.2, 1.6, and 2.0 mg L^{-1}) were prepared using 0, 0.5, 1, 2, 3, 4, and 5 mL of 10 mg L^{-1} Si, respectively. For both sets, extracting solution was used as background matrix. These sets of standard series were treated similarly as the samples for the following procedures. Ten mL of deionized (DI) water was added into the aliquot followed by 0.5 mL of 1:1 HCl: DI water solution. One mL of 10% ammonium molybdate ($\{NH_4\}_6Mo_7O_{24}\cdot 4H_2O$) was then added to the tubes. After five minutes, one mL of 20% tartaric acid was added to the samples which were then hand-shaken for 10 seconds. After two minutes, one mL of the reducing agent, amino napthol n-sulfonic acid (ANSA) was added. Deionized water was then added to the samples to make the final volume of 25 mL. The tubes were capped and the solution was hand shaken for 10 seconds. After five minutes, the absorbance reading was measured using a Hach DR 5000 spectrophotometer at 630 nm.

2.2.8. Plant silicon extraction

Plants were excised at soil level and left in the greenhouse for air drying. Plant samples were further dried in an oven at 65^{o}C for 96 hours to remove residual moisture and then ground into fine powder using a Thomas-Wroy Laboratory Mill, Model-4 grinder.

a) Plant Digestion Procedure:

A modified version of Kraska and Breitenbecks' Oven-Induced Digestion (OID) procedure (2010) was followed to digest the plant samples. A 100 mg sample was weighed into 50-mL

centrifuge tubes. Weighed samples were dried at 60^{o}C for 15 minutes in a mechanical convection oven (Yamato; model number DKN600). Five drops of octyl-alchohol were added to the samples to reduce foaming. Two mL of 30% hydrogen peroxide (H_2O_2) was added. The tubes were tightly capped and placed in oven at 95^{o}C for 30 minutes. Four mL of 50% sodium hydroxide (NaOH) was added into the samples. The samples were loosely capped and placed in the oven at 95^{o}C for four hours. The samples were mixed every 15 minutes using a vortex mixer during the 4-hour digestion. The samples were removed from the oven after four hours. One mL of 5 mM ammonium fluoride was added and the samples were vortexed. Finally, the samples were diluted with DI water to make up the volume to 50 mL.

b) Plant Si colorimetric procedure:

The Si content in the plant digest samples was determined by the modified MBC procedure (Hallmark et al., 1982). Two mL aliquot of the digested solution was taken into a 50 mL polyethylene screw-cap centrifuge tube. Ten mL of 20% acetic acid was added to the aliquot. The solution was then mixed by swirling the tubes for 10 seconds. Four mL of 0.26 M ammonium molybdate was then added to the solution. After five minutes, two mL of 20% tartaric acid was added. The solution was again mixed by hand swirling for 10 seconds. After two minutes, two mL of the reducing agent ANSA was added. Twenty percent acetic acid was added to the solution to bring the final volume to 30 mL. The tubes were then capped and shaken by hand for 10 seconds. After 30 minutes, the absorbance reading of the samples was made using the UV visible spectrophotometer (Hach DR 5000) at 630 nm wavelength. Likewise, Si standard series consisting of (0, 0.4, 0.8, 1.6, 3.2, 4.8 and 6.4 µg Si mL^{-1} were prepared by pipetting 0, 0.5, 1.0, 2.0, 4.0, 6.0, and 8.0 mL of 24 µg mL^{-1} Si, respectively into a 50-mL centrifuge tubes with a digested blank as background matrix.

2.2.9. Experimental design and data analysis

The experiment was conducted once as a factorial in a completely randomized design with five replications. Factors were Si rates (control, lime control, 200, 400, 600, 800 kg Si ha^{-1}) and inoculum densities ($1*10^5$ and $1*10^6$ conidia ml^{-1}). Statistical analysis was performed using SAS 9.4 (SAS Institute, Cary, NC, 2014). Analysis of variance was performed using PROC MIXED to determine the effects of Si, inoculum densities, and their interactions on measured parameters which are soil Si, soil pH, plant Si, AUAPC, and FDS. Orthogonal polynomial contrasts (linear, quadratic, cubic and quartic) analysis were performed between lime control and four rates of Si. Also, contrast between control and lime control was analyzed to determine if any differences existed. Simple linear regression analysis was performed with PROC REG procedure to determine the relationship between soil Si and plant Si.

2.3. Results

2.3.1 Confirmation of pathogen

A BLAST search of the sequence against NCBI database showed the highest sequence identity to *Colletotrichum sublineolum* (99 % homology). The sequence was deposited in the Genebank database with Genebank accession number AJ301978.1.

2.3.2 Effect of Si rates and inoculum densities on soil Si, pH, plant Si, AUAPC and FDS

Incorporation of Si into the soil as wollastonite had a significant effect ($p<0.05$) on soil Si concentration, plant Si concentration, soil pH, AUAPC, and FDS (Table 2.2). However, there was no effect of inoculum densities on any of these parameters. Treatment interaction was not significant for soil pH, plant Si, AUAPC and FDS; however, there was a significant treatment

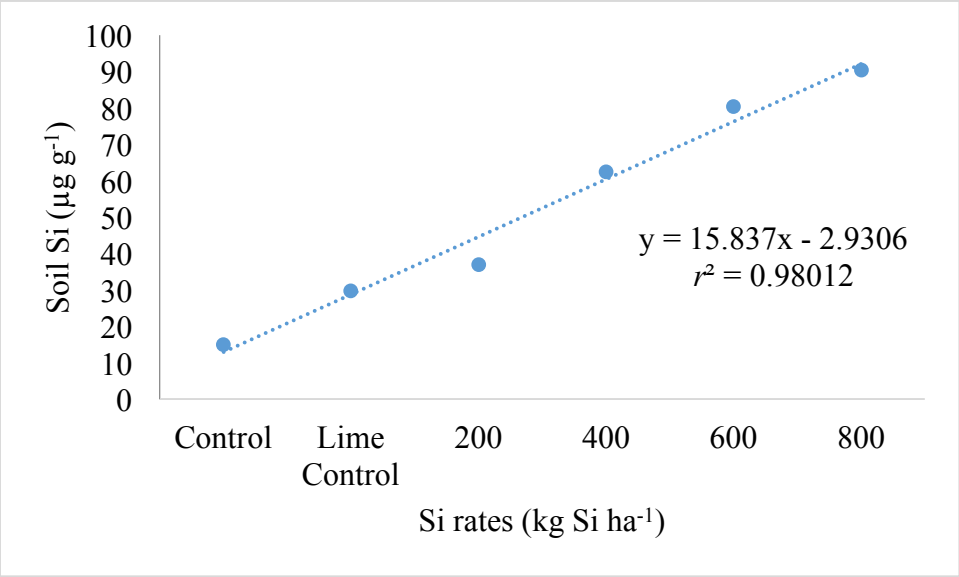
interaction effect for soil Si. The two controls were significantly different for soil Si and pH. However, there was no significant difference between control and lime control for other measured parameters. All the measured parameters showed distinct linear response to Si rates. Furthermore, soil pH, and plant Si showed quadratic response, and soil Si and AUAPC showed cubic response to Si rates.

Table 2.2. Summary of two-way factorial ANOVA analyzing the effects of Si rates and inoculum densities on measured parameters.

Effect	Pr>F				
	Soil Si	Soil pH	Plant Si	AUAPC	FDS
Si	<0.0001	<0.0001	<0.0001	0.0017	0.0009
Control vs lime control	0.0030	<0.0001	0.2186	0.8643	0.5927
Linear	<0.0001	<0.0001	<0.0001	0.0048	0.0040
Quadratic	0.8761	0.0100	<0.0001	0.6361	0.7769
Cubic	0.0156	0.9920	0.1086	0.0124	0.0517
Quartic	0.3654	0.8282	<0.0001	0.3388	0.2322
Inoculum densities	0.2181	0.9585	0.6642	0.7349	0.4780
Si*Inoculum densities	0.0206	0.9205	0.7571	0.7632	0.5151

2.3.3 Effect of Si rates on soil Si and pH

There was a significant increase in soil Si with increasing Si rates (Table 2.2, Figure 2.1). A positive linear relation described the effect of Si rates on soil Si. Soil Si was highest at the highest Si rate, whereas the control had the lowest concentration. The two controls were significantly different in terms of soil Si (Table 2.2). A positive quadratic relation described the effect of Si rates on soil pH and pH generally increased as the Si rate increased.



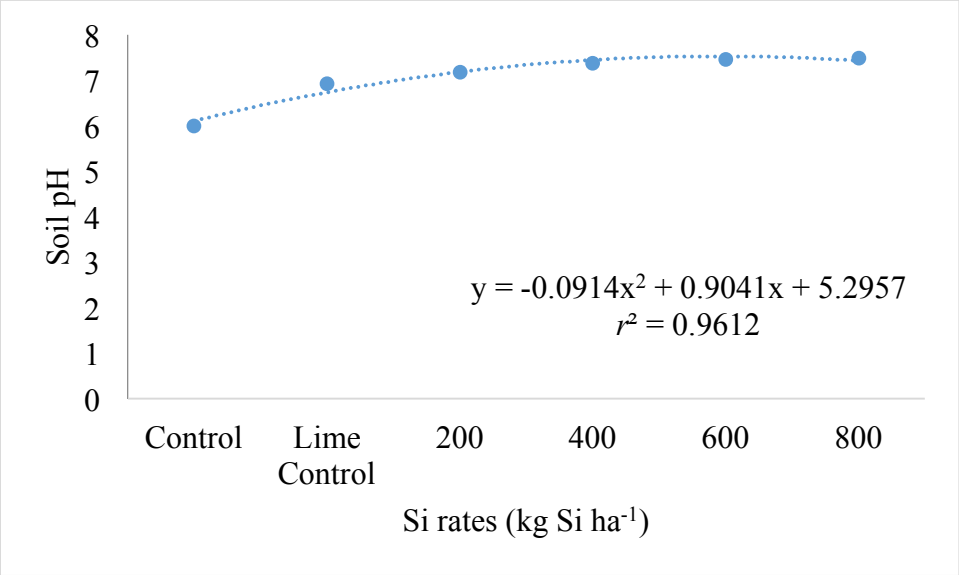


Figure 2.1. Relationship between Si rates on soil Si and soil pH.

2.3.4 Relationship between soil Si and pH

The relationship between soil Si and pH are shown in Figure 2.2. Soil with low pH (6.0) had the lowest Si concentration; whereas, soil Si increased with increasing Si rate when pH was

between 6.8-7.8. This result suggests that the effect of Si rate to plant available Si concentration in an acid Alfisol is more pronounced as the soil pH becomes more alkaline.

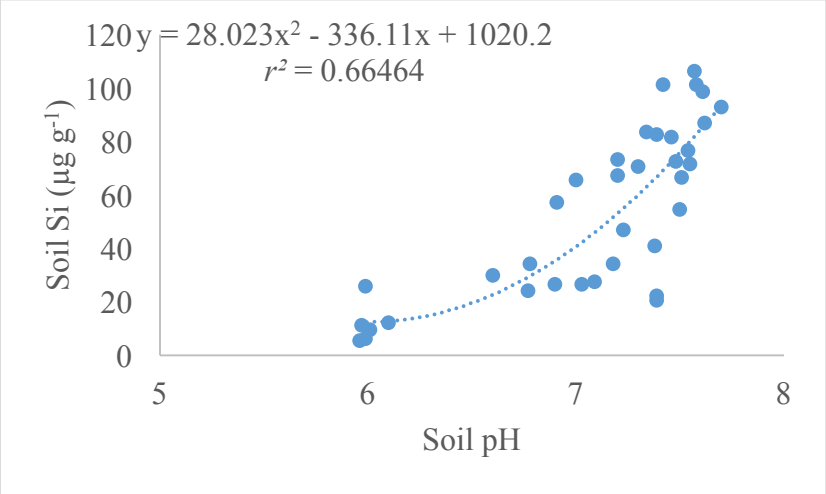


Figure 2.2. Relationship between soil Si and pH.

2.3.5 Relationship between soil Si and plant Si

A cubic relationship was observed between plant tissue and soil Si (Figure 2.3). Plant tissue Si increased slowly when soil Si was below 40 µg g^{-1}, and increased at a higher rate when soil Si was between 40-80 µg g^{-1}. However, plant tissue Si began to level off at about 80 µg g^{-1}soil Si level.

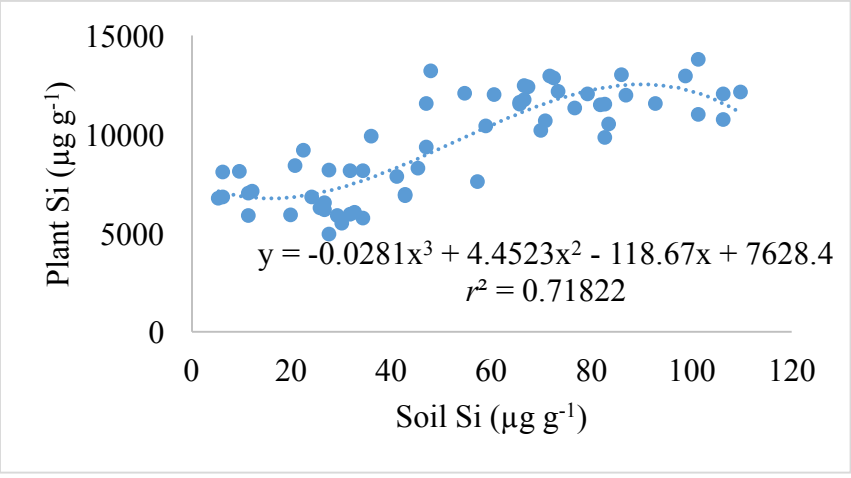


Figure 2.3. Relationship between plant and soil Si.

2.3.6 Effect of Si rates on FDS and AUAPC

A negative linear relation was observed between Si rates with FDS and AUAPC (Table 2.2, Figure 2.4). The two controls were not significantly different and had the highest FDS and AUAPC values. Likewise, the highest Si rate had lowest FDS and AUAPC values.

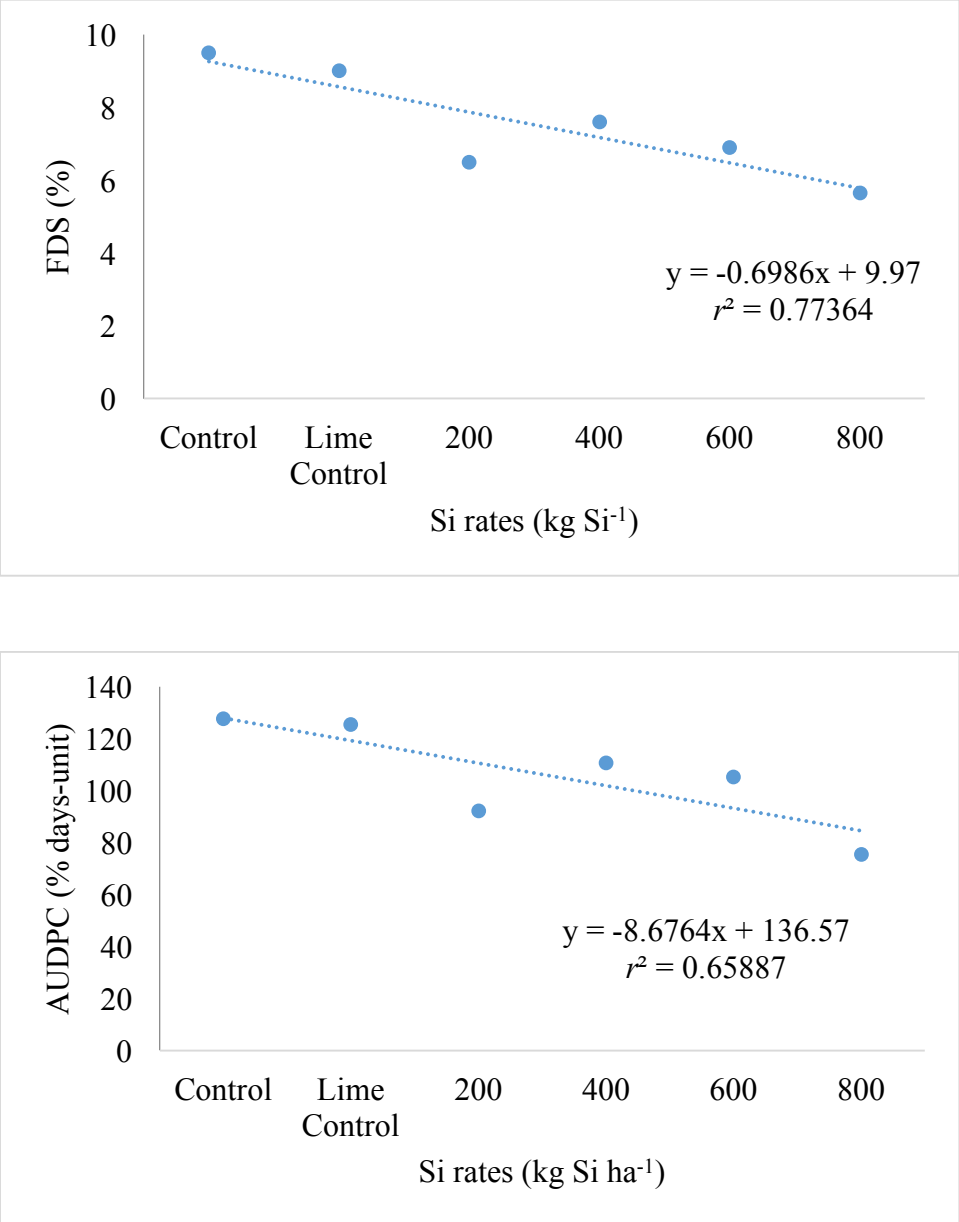


Figure 2.4. Relationship between Si rates and FDS and AUAPC.

2.4 Discussions

2.4.1. Effect of Si rates on plant-available soil silicon concentration and pH

There was a linear relationship between Si rates and soil Si concentration. Soil treated with the highest Si rate (800 kg Si ha^{-1}) obtained the highest plant available soil Si. Lime control and four Si rates had similar soil pH values because the calcium content of the soil with these treatments was adjusted to the same level. However, the two controls were significantly different from each other in terms of plant available soil Si and pH (Table 2.2). Even though Si was not applied to both of the controls, the higher pH in lime control might have contributed to an increase in plant available soil Si. Apart from this, a positive relationship was observed between soil pH and plant available soil Si (Figure 2.2). This result is in agreement with the findings of Miles et al. (2014) which showed a positive relationship between soil pH and Si solubility or extractability. Soil Si was lowest at the lowest pH and increased when pH was between 6.8-7.8. Monosilicic acid is mostly available in soil (pH 6.5-8.5) but low in soil with low pH because of leaching (Frings et al., 2014; Haynes, 2014). Korndorfer and colleagues (2005) also explained a negative relationship between plant available soil Si and soil acidity, due to the decrease in dissolution of Si in soil. This might explain the lower Si value for the control vs. the lime control. Even though there was no effect of inoculum densities on plant available soil Si, a significant treatment interaction was observed indicating that the effect of Si rate on soil Si was not consistent at the two inoculum densities (Table 2.2).

2.4.2. Relationship between plant tissue Si concentration with Si rates and soil Si

Silicon rates had a significant effect on plant Si concentration. The two controls were not significantly different in terms of plant Si. However, a positive relationship was observed

between soil Si and plant Si content (Figure 2.3). Osuna-Canizalez and colleagues (1991) found a similar relationship between plant and soil Si where there was a significant increase in Si content of rice leaf blades with increasing soil Si. Similarly, Brecht and colleagues (2004) found 1.2 to 1.3% Si in leaves of St. Augustinegrass supplied with calcium silicate whereas, the levels of Si in non-amended plants were from 0.6 to 0.7%. Furthermore, Nanayakkara and colleagues (2008) also observed increases in Si concentration in shoot tissues of perennial grass when grown in soil, especially with low silicon content amended with increasing rates of either calcium silicate slag or wollastonite. Plant tissue Si appeared to be substantially increase when soil Si was between 40-60 $\mu g\ g^{-1}$ (Figure 2.3). Above 65 $\mu g\ g^{-1}$, plant Si began to decline. Depending on the soil type and crop grown, plant tissue Si may not necessarily increase with increasing Si application (Iller et al., 1979). With the increasing levels of applied Si, dissolution of the fertilizer materials in silt loam soils having low adsorption capacity may have lead to polymerization thus reducing plant available Si (monomeric form). Hence, even though total Si content of soil increases, Si might become polymerized or might remain in forms unavailable for plant uptake.

2.4.3. Effect of silicon application and inoculation on FDS and AUAPC

Silicon application significantly reduced sorghum anthracnose in this study. There was a negative relation between Si rates and FDS as well as AUAPC. Both FDS and AUAPC values were lowest for sorghum treated with the highest Si rate. Anthracnose was reduced by 41% when compared with the control (Figure 2.4). This result is in agreement with those found by Resende et al. (2009; 2012) and Santos et al. (2014) who found a significant reduction in AUAPC values of sorghum anthracnose when plants were grown in soil amended with different levels of Si.

Resende et al. (2009) also found that Si rates reduced FDS along with AUAPC indirectly, suggesting that fungal colonization was affected with some kind of resistance mechanism of the host. Furthermore, Seebold et al. (2001) reported that relative rice blast infection efficiency reduced in a linear manner with increasing Si rates. Grey leaf spot on St. Augustinegrass was also significantly linearly reduced by Si application (Brecht et al., 2007).

Even if the application of Si to sorghum resulted in reduction in anthracnose, the mechanism behind the suppression was not elucidated in this study. Silicon might have acted as a physical barrier to prevent pathogen penetration (Yoshida, 1975). Silicon might have enhanced the production of phenolics and phytoalexins which would have improved the defense mechanism of sorghum against *C. sublineolum* (Resende et al., 2013).

No significant difference in anthracnose development was observed between the two inoculum densities used in this study. Resende et al. (2009, 2013) and many other researchers had used $1 * 10^6$ conidia ml^{-1} to promote anthracnose development under controlled environments. However, Santos et al. (2014) used $1* 10^5$ conidia ml^{-1} for infecting sorghum plants in Brazil. This study demonstrated that either inoculum density was effective for promoting anthracnose development in sorghum.

2.5 Conclusions

This study supported other studies concerning Si's impact on managing diseases. Increasing Si rates significantly reduced anthracnose of sorghum. Furthermore, a negative linear relation between Si rates and anthracnose development was observed in this study supporting the findings from previous studies. Even though other studies have used different inoculum densities to induce disease development, this study found no differences in inoculum densities suggesting

that lower inoculum densities may be used. This study aimed to evaluate Si's role in disease management, however the mechanism behind the observable negative impact of Si on sorghum anthracnose was not elucidated. Thus, future research should focus in understanding the mechanism of resistance mediated by Si.

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Chapter 3: Influence of silicon and fungicides on anthracnose development in grain sorghum

3.1 Introduction

Silicon is a beneficial plant nutrient that helps in minimizing several abiotic and biotic stresses (Datnoff et al., 2007). Silicon application helps in alleviation of heavy metal stress and improves salt tolerance of a number of grain crops (Yin et al., 2013). It also increases resistance to lodging, and drought as well as dry matter accumulation in rice and cucumber (Datnoff and Rodrigues, 2005; Vasanthi et al., 2014). Likewise, Si has been found to reduce severity of several economically important diseases of rice such as brown spot, leaf scald, rice blast, sheath blight and stem rot (Datnoff et al., 1997; Datnoff et al., 1991; Datnoff et al., 1992). It also reduced incidence and severity of gray leaf spot in perennial ryegrass (*Lolium perenne* L.) (Nanayakkara et al., 2008). Furthermore, Si application increased host-resistance against Fusarium wilt of banana (*Musa* sp.) (Fortunato et al., 2012). Silicon amendments increased resistance against leaf spot in bermudagrass (Datnoff and Rutherford, 2004); powdery mildew in wheat (Belanger et al., 2003). Silicon has been found to reduce diseases either by delaying fungal infection to allow more time for the plants to develop defense mechanisms or by acting as a mechanical barrier against pathogen penetration. (Yoshida, 1975; Polanco et al., 2012).

Sorghum is a Si-accumulator with Si concentrations ranging from 0.8 to 2 dag kg^{-1} (Resende et al., 2013). In sorghum plants infected by anthracnose, Si application helps in maintaining carbon fixation, enhancing the antioxidant system and increasing the defense response of the plant against *C. sublineolum.* Higher Si deposition at infection sites of *C. sublineolum*, its accumulation in extracellular spaces, epidermal walls as well as the basal cells of the trichomes, ultimately helps in inhibiting fungal penetration. The incubation period and the latent period of

sorghum anthracnose increased in plants amended with silicon. Likewise, the relative infection efficiency and area under anthracnose index progress curve were reduced (Resende et al., 2013).

Silicon, when applied to fungicide-treated and untreated plants, has shown significant effect alone or in combination in reducing diseases of beans (*Phaseolus vulgaris* L.), rice, and sorghum (Rodrigues et al., 2015; Seebold et al., 2004; Resende et al., 2012). Datnoff et al. (1997) showed reduction in rice blast incidence from 73% to 36% in presence of Si without fungicide (benomyl) and from 27% to 13% with fungicide. Likewise, Seebold et al. (2004) found that Si alone and in combination with edifenphos reduced severity of leaf blast of rice by 22% and 75% when compared with the untreated controls. They also found that Si alone was equally or more effective than full rate of edifenphos in suppressing leaf blast. Furthermore, Resende et al. (2013) also found that Si alone and in combination with fungicide Opera effectively reduced area under anthracnose progress curve in sorghum by 37 and 44%, respectively.

There has been an increasing trend of using foliar fungicides on grain sorghum over the past few years in most parts of Louisiana (Fromme et al., 2014). From all of the above mentioned benefits of Si, the objective of the current study was to evaluate the role of Si alone and in combination with fungicide for suppressing anthracnose development under greenhouse and field conditions in Louisiana.

3.2 Materials and Methods

3.2.1. Greenhouse studies

For the greenhouse studies, a low-Si soil was collected from a site in Evangeline Parish, LA (30^{o}32’50”N 92^{o}30’33”W). The soil was Crowley-Vidrine (CV) complex and is classified as fine, thermic, smectitic, Typic Albaqualfs (SSURGO-USDA, 2015). The soil texture was silt

loam to silt clay with a pH of 5.5, which is low for growing sorghum. Soil samples collected were analyzed for pH, plant-essential nutrients, sodium (Na) composition based on Mehlich-3 procedure, and Si content based on 0.5 M acetic acid extraction procedure (Table 3.1), before planting sorghum.

Table 3.1. Initial nutrient content and pH of soil used for the greenhouse study

Soil pH and Nutrient Content	Value	Soil Test Interpretation
pH (1:1 soil: water ratio)	5.5	Low
Phosphorus, mg kg^{-1}	20	Low
Potassium, mg kg^{-1}	165	Very High
Calcium, mg kg^{-1}	546	Very High
Magnesium, mg kg^{-1}	96	Very High
Sodium, mg kg^{-1}	41	Optimum
Sulfur, mg kg^{-1}	22	Medium
Copper, mg kg^{-1}	2.4	High
Zinc, mg kg^{-1}	2.1	Medium
†Silicon (mg kg^{-1})	25.2	Low

Plant essential nutrient content was determined based on Mehlich-3 extraction procedure and inductively-coupled plasma analysis (Mehlich, 1984).
† Determined based on 0.5 M acetic acid, 1-hr extraction procedure followed by Molybdenum Blue Colorimetry (Korndorfer et al., 2001).

Based on the initial soil test results in Table 3.1 and LSU AgCenter fertilizer recommendation for sorghum, nitrogen (N) and phosphorus (P) were applied at 110 and 90 kg ha^{-1} as urea blended with ammonium sulphate (33-0-0 N) and triple superphosphate (TSP, 0-46-0), respectively. Lime and potassium were not recommended because calcium (Ca) and potassium (K) content of the soil were high.

For the greenhouse experiment, plastic pots (Hummert International, Earth City, MO) with an inside diameter of 15 cm and volume of 4 L were used. The low-Si soil was air-dried and processed with all debris removed. Paper coffee filters (Brew Rite®) were placed at the bottom of each pot to prevent significant soil loss through the drainage holes. Pots were filled with 3 kg soil

reaching up to 2.5 cm from the top rim of each pot. The following were applied per kg of soil: 0.188 g of ammonium sulfate blended with urea and 0.108 g of TSP to meet the requirement for N, S, and P. In the study, soil was treated with different Si rates at 200, 400, 600, 800 kg Si ha^{-1} applied as wollastonite (Vansil® W10, Vanderbilt Minerals, Norwalk, CT) as source of Si at 0.46, 0.92, 1.38, and 1.84 g kg^{-1} of soil, respectively. A check (0 Si) with and without lime was also included. Wollastonite ($CaSiO_3$) is a powder containing 24% Si and 31% Ca. In order to differentiate Si effects from Ca effects, lime was incorporated into the treatment structure. In addition, depending on wollastonite application, lime was applied as Aglime at 0, 1.66, 1.38, 0.92, 0.46, 0 g kg^{-1} of soil to pots treated with 0, 0, 200, 400, 600, and 800 kg Si ha^{-1}, respectively to attain the same Ca equivalents and lime effect across Si treatments. Pre-weighed soil contained in each pot was placed in a clean plastic 4 L-zipper bag (Wal-Mart). Nutrients were then added and thoroughly mixed with the soil. The treated soil was then placed back into the corresponding pot and allowed to lay idle for a week to allow proper nutrient-soil establishment. Sorghum seeds of Pioneer hybrid 84G62 and 84P80 were sown at four seeds per pot at a depth of 2.5 cm. Upon establishment, plants were thinned to two per pot. Six weeks after sowing, deficiency symptoms of NPK, and boron (B) and zinc (Zn) were observed. To correct the deficiencies, a basal application of Hoagland nutrient solution (Tubana, personal communication) was applied to all plants. Zinc sulfate at 0.0186 g kg^{-1} of soil as a Zn source and 0.618 g L^{-1} of boric acid was as a stock solution for B. Tap water (pH 8.0) was used to irrigate the plants maintaining adequate soil moisture. The plants were inoculated with a conidial suspension of *C. sublineolum* at $1*10^5$ conidia mL^{-1} four weeks after sowing.

3.2.2. Field Design

Field experiments were conducted at two locations during June-September, 2015 in Louisiana. Dean Lee Research Station, Alexandria (field coordinate: 31°10’21’’ N 92°24’16’’ W) and Macon Ridge Research Station, Winnsboro (field coordinate: 32°08'33.0"N 91°42'23.8"W). The soil at Dean Lee was Coushatta silty clay loam: fine-silty, mixed, superactive, thermic Fluventic Eutrudept (Inceptisol), whereas Winnsboro was a Gigger-Gilbert silt loam: fine-silty, mixed, active, thermic, Typic Glossaqualfs (Alfisol) (Weindorf, 2008). Two sorghum hybrids were used at each location. Each field was divided into two parts for each hybrid. There were five replicates for each Si rates and the fungicide (Pyraclostrobin) (12 treatment combinations) for each hybrid, yielding 120 plots at each location. Each plot was 9.15*3.05 m^2 at Dean Lee and 10.67*3.05 m^2 at Winnsboro. Experimental design was a randomized complete block. There were four rows in each experimental unit. The outer rows acted as a border to prevent interplot interference. Silicon was broadcasted by hand as wollastonite for each plot. However, only the two middle rows were used to measure disease, sample collection and fungicide application. Standard sorghum cultivation practices (www.lsuagcenter.com) were followed at each field (Table 3.2).

Table 3.2. Sorghum cultivation practices at Dean Lee and Winnsboro, 2015.

Cultivation Practices	Dean Lee	Winnsboro
Fertilized (10/22/14)	0:18:36 NPK @ 170 kg ha^{-1}	
Planted (6/5/15)	170,000 seeds ha^{-1}	105,000 seeds ha^{-1}
Pre-emergence herbicide (6/5/15)	Atrazine @ 2.93 L ha^{-1} Dual II Mac @ 1.12 L ha^{-1}	Atrazine @ 2.34 L ha^{-1} Roundup @ 2.34 L ha^{-1} Charger Basic @ 1.17 L ha^{-1}
Fertilized (6/18/15)	30:0:0:2 NPKS @ 561 kg ha^{-1}	120:50:50:8 NPKS before planting
Insecticide (7/16/15)	Transform @ 0.073 L ha^{-1}	Baythroid XL@ 0.095 L ha^{-1}
Insecticide (7/30/15)	Sivanto @ 0.37 L ha^{-1}	Sivanto @ 0.23 L ha^{-1}
Insecticide (8/5/15)	Grizzly Z @ 0.22 L ha^{-1}	Belt @ 0.22 L ha^{-1}
Insecticide (8/28/15)	Transform @ 0.073 L ha^{-1}	Baythroid XL @ 0.095 L ha^{-1}

3.2.3. Selection of hybrids

The hybrids used in both greenhouse and field studies were Pioneer 84G62 and Pioneer 84P80. Pioneer 84G62 is high yielding hybrid, and is moderately susceptible to sorghum anthracnose (Mascagni et al., 2012, Kruse et al., 2012). This hybrid is short-statured with plant height up to 133 cm, and is resistant to lodging and drought. It was declared as the best performing sorghum hybrid of the Pioneer team according to National Grain Sorghum Producer (www.myplainview.com, 2003). Pioneer 84P80 is slightly taller and moderately resistant to anthracnose. The yield capacity for both hybrids is almost similar (www.lsuagcenter.com, 2013). Due to similarities in growth and yield, but with differences in anthracnose susceptibility, these hybrids were chosen for the current study.

3.2.4. Inoculation, incubation and fungicide application for greenhouse study

Based on previous research reported in Chapter 2, inoculum density had no significant effect on anthracnose development. Hence, in this greenhouse study, plants were inoculated with $1*10^5$ conidia ml^{-1}. The conidia were counted using a haemocytometer and the suspensions were collected in two 1L-spray bottle (The Bottle Crew, West Broomfield, MI) with an addition of Tween 20 at 0.05% to reduce surface tension. A whole plant inoculation was performed at fourth leaf stage by atomizing the conidial suspension onto all treatment plants. Following inoculation, the plants were covered with a white polythene bag of 125 L (Berry Plastics, Evansville, IN) for 24 hours to maintain relative humidity and establish fungal infection. Headline, a suspension concentrate (SC) fungicide (ai: pyraclostrobin) (BASF EPA Reg. No. 7969-289) was applied in 3 mL L^{-1} water using a 1L-spray bottle (The Bottle Crew, West Broomfield, MI), two days after

inoculation in the greenhouse. Half of the plants in the greenhouse were left untreated. However, in the field study, pyraclostrobin was applied once at 0.44 L ha^{-1} at grain sorghum heading stage (25% flowering), to plants in middle two rows in each experimental unit using a CO_2 backpack sprayer-40 psi@ 3.22 km hr^{-1} (R and D Sprayers) with a TeeJet 8002 nozzle.

3.2.5. Anthracnose severity measurement, final disease severity (FDS) and area under anthracnose progress curve (AUAPC) calculation

Final Disease Severity (FDS) which is the final proportion of area of infected plant tissue and Area Under Anthracnose Progress Curve (AUAPC) which is a quantitative measure of disease progress over time (Madden et al., 2007) were evaluated. Symptoms were observed five days after inoculation. Hence, starting five days after inoculation, four anthracnose severity measurements were made at five-day intervals in the greenhouse whereas in the field, severity was measured twice before and twice after fungicide applications. The first assessment in the field was done at 53 days after planting and then at 2 week intervals. Anthracnose severity was determined as the percentage of total leaf area covered by anthracnose symptoms for all plants in each pot in the greenhouse using a modified diagrammatic scale proposed by Sharma (1978). Sharma used standard visual ratings to score approximate percentage of leaf area affected by anthracnose. He used a scale of 0 to 9 where leaf with no symptoms was rated as ‘V’ (0%) and totally affected leaf as ‘9’ (100%). Intermediate ratings were ‘2’ (2.5%), ‘3’ (5%), ‘4’ (10%), ‘5’ (20%), ‘6’ (35%), ‘7’ (50%), and ‘8’ (75%). However, in this study, only the percentage measurement was used for calculating anthracnose severity. Percentage of anthracnose infection was measured for each leaf and averaged for the two plants in a pot and used as a single value for each sample. After the final anthracnose assessment (8 weeks after sowing), the plants were excised at the soil line and left in the greenhouse for air drying.

For the field study, only the top 3 leaves of the plant canopy were used for anthracnose assessment because of their better exposure to fungicide application. The plants were harvested after final anthracnose assessment. Area Under Disease Progress Curve was determined using the formula (Madden et al., 2007)

$$\text{AUAPC} = \sum_{i=1}^{n} \left(\frac{y_i + y_{i+1}}{2}\right)(t_{i+1} - t_i)$$

Where

n= total number of observations, y_i = disease severity at the i^{th} observation, and t= time at the i^{th} observation. Since the unit for y in these studies is %, and the unit for t is days, the unit of AUAPC is %-days unit.

3.2.6. Yield measurement and plant, grain, and soil sample collection

To measure soil Si, one sample of soil from the furrow between the middle two rows of each experimental unit was taken at a depth of 15-20 cm using a soil probe (AMS, Forestry suppliers, Inc., MS) at both field locations. The soil was dried before extraction. The third leaf from the top of the plant was collected from 5 randomly selected plants from the middle two rows to determine leaf Si content. All heads of the respective 5 plants were excised and used to determine grain Si content. Yield was measured as weight of sorghum grains harvested from all plants of the middle two rows of each plot.

For the greenhouse studies, a 15 cm- soil depth sample was collected using a soil probe from each pot. Whole plants were used to determine plant Si content.

3.2.7. Post-experiment soil Si extraction

Plant-available soil Si content was measured based on 0.5 m acetic acid procedure (Korndorfer et al., 2001). Two grams of air-dried soil were weighed in 50-ml centrifuge tubes. A modified Molybdenum Blue Colorimetric (MBC) procedure was used to determine plant-available soil Si.

a) Soil Si extraction procedure:

Twenty mL of 0.5 M acetic acid were added to the 2 g air dried soil samples and placed on a reciprocal shaker (Eberbach; model number E6010.00) for one hour set at high speed. The samples were filtered through Whatman No. 1 filter paper into 50 mL centrifuge tubes immediately after shaking. A 0.5 mL aliquot from the filtered solution was pipetted into 50 mL centrifuge tubes for colorimetric procedure (Korndorfer et al., 1999).

b) Colorimetric procedure:

Two sets of Si standard series (0, 0.2, 0.4, 0.8, 1.2, 1.6, and 2.0 mg L^{-1}) were prepared using 0, 0.5, 1, 2, 3, 4, and 5 mL of 10 mg L^{-1} of reagent grade Si (Fisher Scientific), respectively. For both sets, extracting solution was used as background matrix. These sets of standard series were treated similarly as the samples for the following procedures: Ten mL of deionized (DI) water was added into the aliquot followed by 0.5 mL of 1:1 HCl: DI water solution. One mL of 10% ammonium molybdate ($\{NH_4\}_6Mo_7O_{24}\cdot 4H_2O$) was then added to the tubes. After five minutes, one mL of 20% tartaric acid was added to the samples which were then hand-shaken for 10 seconds. After two minutes, one mL of the reducing agent, amino napthol n-sulfonic acid (ANSA) was added. Deionized water was then added to the samples to make the final volume of 25 mL. The tubes were capped and the solution was hand-shaken very well. After five minutes, the absorbance reading was measured using a Hach DR 5000 spectrophotometer at 630 nm.

3.2.8. Plant and grain Si extraction

To extract plant Si, plants were excised at soil level and left in the greenhouse for air drying. The plant samples were further dried in an oven (Yamato; model number DKN600) at 65^{o}C for 96 hours to remove residual moisture. The plants were then ground into fine powder using a Thomas-Wroy Laboratory Mill, Model-4 grinder. A one hundred milligram sample was used for the plant digestion procedure (Kraska and Breitenbeck, 2010). Likewise, the grains harvest from the field study were ground using a coffee grinder (Sunbeam Products©).

a) Plant Digestion Procedure:

A modified version of Kraska and Breitenbecks' Oven-Induced Digestion (OID) procedure (2010) was followed to digest the weighed plant samples. Weighed samples were dried at 60^{o}C for 15 minutes in a mechanical convection oven (Yamato; model number DKN600). Five drops of octyl-alcohol were added to the samples to reduce foaming. Two mL of 30% hydrogen peroxide (H_2O_2) was added. The tubes were tightly capped and placed in oven at 95^{o}C for 30 minutes. Four mL of 50% sodium hydroxide (NaOH) was added into the samples. The samples were loosely capped and placed in oven at 95^{o}C for 4 hours. The samples were mixed every 15 minutes using a vortex mixer during the 4-hour digestion. The samples were removed from the oven after four hours and one mL of 5 mM ammonium fluoride was added and the samples were vortexed (Fisher Vortex Mixer, Fisher Scientific) for 5 seconds. Finally, the samples were diluted with DI water to make up the volume to 50 mL.

b) Plant Si colorimetric procedure:

The Si content in the plant digest samples was determined by the modified Molybdenum Blue Colorimetric (MBC) procedure (Hallmark et al., 1982). Two mL aliquot of the digested solution was placed into a 50 mL polyethylene screw-cap centrifuge tube. Ten mL of 20% acetic

acid was added to the aliquot. The solution was then mixed by swirling the tubes by hand for 10 seconds. Four mL of 0.26 M ammonium molybate was then added to the solution. After five minutes, two mL of 20% tartaric acid was added. The solution was again mixed by hand swirling for 10 seconds. After two minutes, two mL of the reducing agent ANSA was added. Twenty percent acetic acid was added to the solution to bring the final volume to 30 mL. The tubes were then capped and shaken by hand for 10 seconds. After 30 minutes, the absorbance reading of the samples was made using the UV visible spectrophotometer (Hach DR 5000) at 630 nm wavelength. Likewise, Si standard series consisting of 0, 0.4, 0.8, 1.6, 3.2, 4.8 and 6.4 μg Si mL^{-1} were prepared by pipetting 0, 0.5, 1.0, 2.0, 4.0, 6.0 and 8.0 mL of 24 μg mL^{-1} Si, respectively, into a 50-mL centrifuge tubes with a digested blank as background matrix. These sets of Si standard series were treated similarly as the plant digested samples.

3.2.9. Experimental design and data analysis

For greenhouse studies, the experiment was repeated once and each experiment had 2 x 2 x 6 factorial treatment structure arranged in a completely randomized design with five replications. Factors were Si rates (control, lime control, 200, 400, 600, 800 kg Si ha^{-1}), hybrid (Pioneer 84G62 and Pioneer 84P80) and pyraclostrobin (with and without). Therefore, there were 24 treatment combinations and five replications yielding 120 pots. Statistical analysis was performed using SAS 9.4 (SAS Institute, Cary, NC, 2014). Analysis of variance was performed using PROC MIXED to determine the effects of Si, hybrids, pyraclostrobin and their interactions on the following measured parameters: soil Si, soil pH, plant Si, AUAPC, and FDS. Since lime control and four Si rates had same Ca equivalents, but had differences in Si, orthogonal polynomial contrasts (linear, quadratic, cubic and quartic) analysis were performed between lime

control and four rates of Si. Control was not included in orthogonal polynomial contrasts. Also, contrast between control and lime control was analyzed to determine if differences existed between these two treatments. Simple linear regression analysis was performed with PROC REG procedure to determine the relationship between soil Si and plant Si. Coefficient of determination denoted by r^2 was used as a measure of goodness of fit of the trend line to the data. Value of r^2 as 1, determined a perfect fit of the trend line.

For the field studies, treatments consisting of six Si rates, two hybrids, and two pyraclostrobin rates (with and without) replicated five times and laid out in randomized block design. Fixed effects were hybrids, pyraclostrobin, and Si rates and their interactions. Statistical analysis was performed in a similar manner as the greenhouse experiments. Furthermore, yield and grain Si were also measured as parameters apart from those listed above. Student's t-test was used to identify if differences existed for fungicide and hybrids. Tukey's test was used for multiple comparision of means. Pdmix800 was used to show the significant level between the treatments.

3.3 Results

a. Greenhouse studies

3.3.1 Effect of Si rates, hybrids, and fungicide on soil Si and pH

Increasing Si rates had a significant effect ($p<0.05$) on both soil Si and pH. No effects of hybrids or a fungicide was observed for soil Si and pH. Control and lime control were significantly different from each other for soil pH for both experiments, however, significant differences were observed between the two controls only for soil Si for the first experiment.

Lime control and four rates of Si showed significant linear (p<0.001), and quadratic (p<0.05) response for soil Si and pH except for pH in experiment 2 (Table 3.3).

Table 3.3. Summary of two-way factorial ANOVA analyzing the effects of Si rates, hybrids and a fungicide on soil Si and pH.

Effect	Pr>F			
	Experiment 1		Experiment 2	
	Soil Si	Soil pH	Soil Si	Soil pH
Hybrid	0.0575	0.8385	0.6345	0.1840
Fungicide	0.1105	0.0940	0.5412	0.7718
Fungicide*hybrid	0.9814	0.8112	0.0123	0.0744
Si levels	<.0001	<0.0001	<0.0001	<0.0001
Control vs lime control	0.1034	<0.0001	0.0002	<0.0001
Linear	<0.0001	<0.0001	<0.0001	<0.0001
Quadratic	0.0123	<0.0001	0.0025	0.7903
Cubic	0.0034	<0.0001	0.7700	0.0453
Quartic	0.1903	0.0992	0.4610	0.7214
Si levels*hybrid	0.5944	0.4188	0.7987	0.0002
Si*fungicide	0.7688	0.9747	0.4899	0.0006

The highest Si rate had the highest soil Si concentration for both experiments (Figure 3.1). Soil Si increased gradually with increasing Si rates. There was a highly positive quadratic relation between soil Si and pH for both experiments for all six Si rates. There was a very positive quadratic relation between soil Si and pH for the greenhouse experiments (Figure 3.2). Soil Si was lowest for soil pH<6.0. However, soil Si appeared to drastically increase when the pH was between 6.5-7.5.

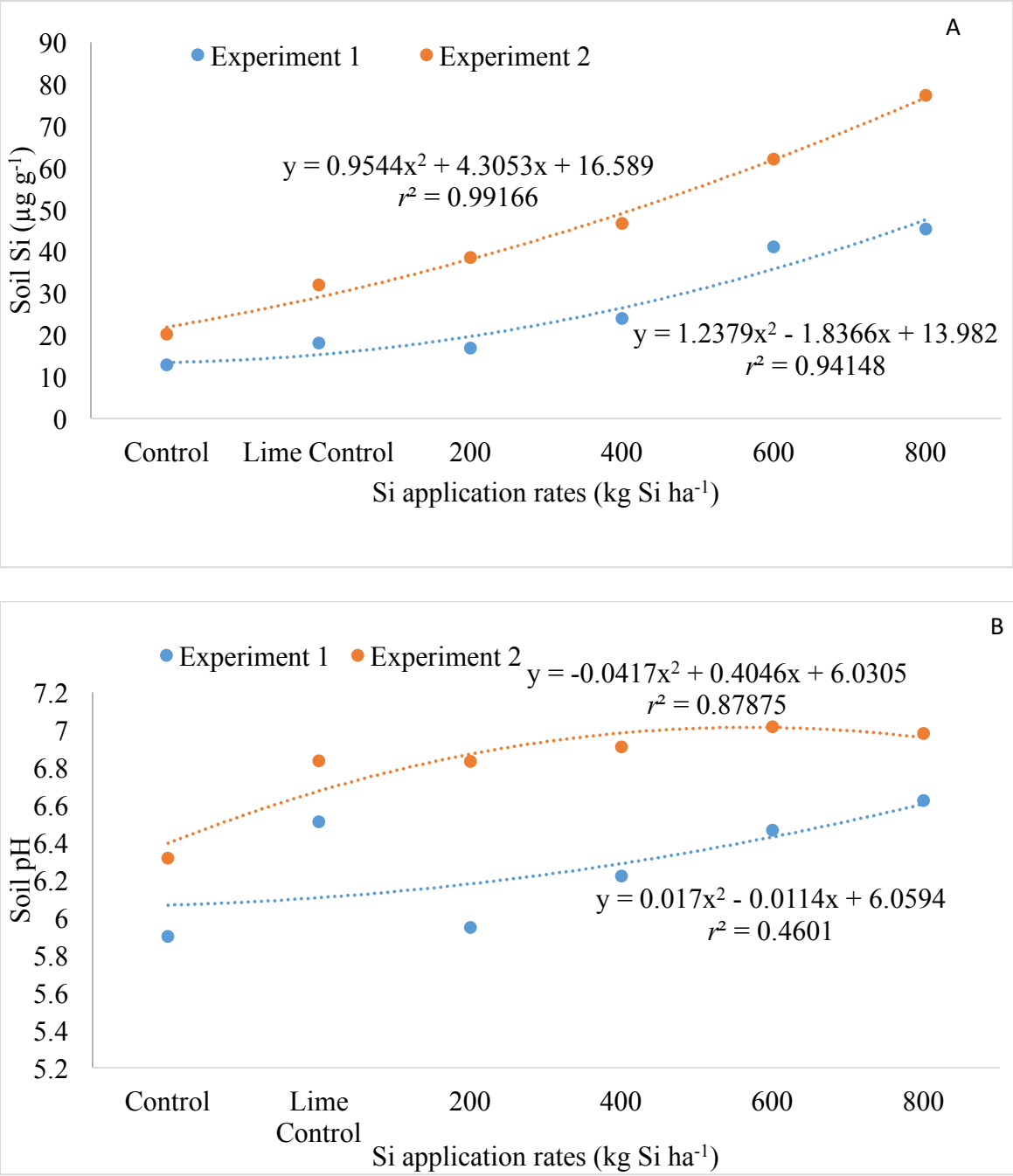


Figure 3.1. Effect of Si rates on (A) soil Si (B) soil pH for two experiments in the greenhouse.

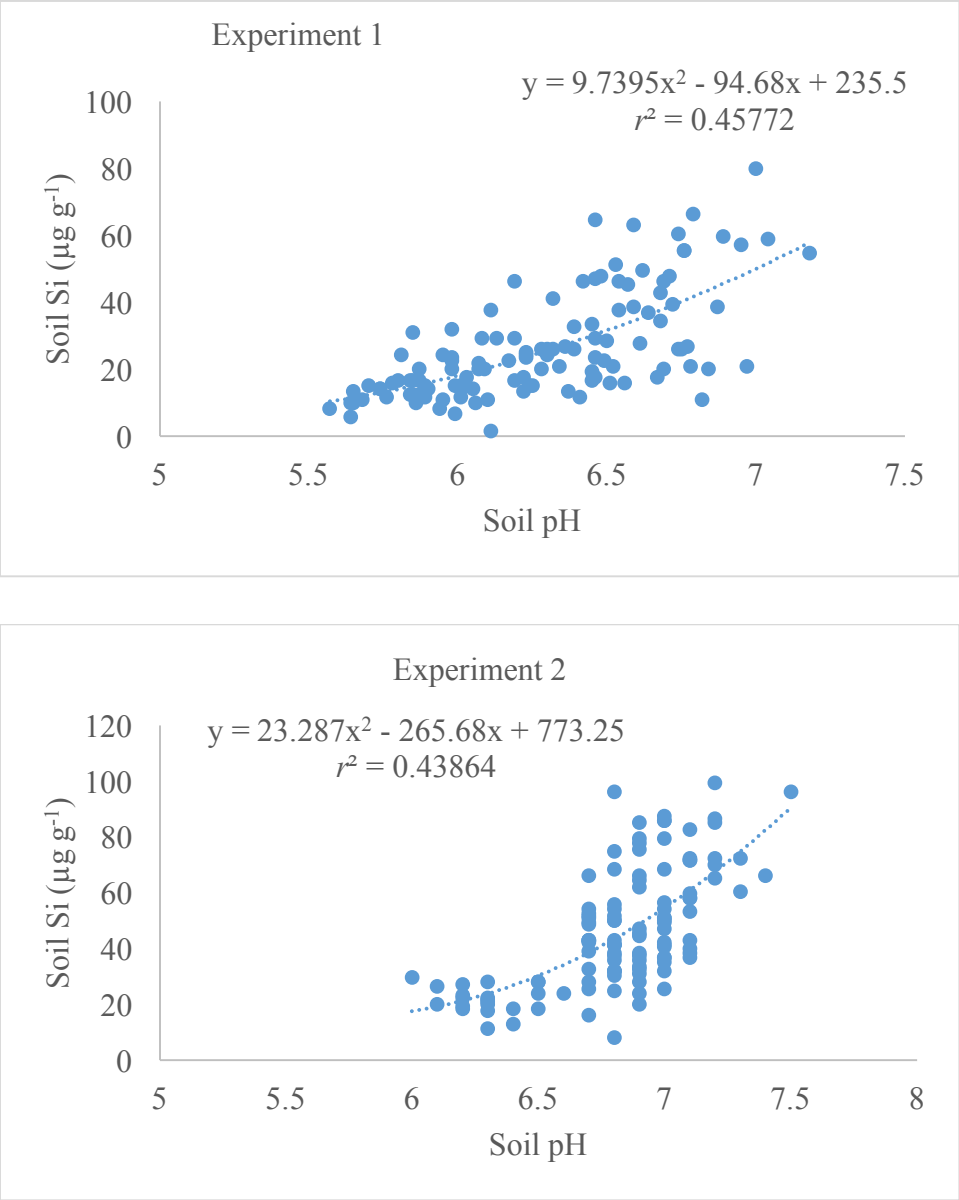


Figure 3.2. Relationship between soil Si and pH.

3.3.2 Effect of Si rates, hybrids, and a fungicide on plant tissue Si content

Silicon rates had a significant effect on plant tissue Si content for both experiments (Table 3.4). The orthogonal contrast analysis showed that the control and lime control did not differ significantly for plant tissue Si content. Significant differences in plant Si among the two hybrids was observed in second experiment (p<0.001) but not in the first one. Lime control and four rates

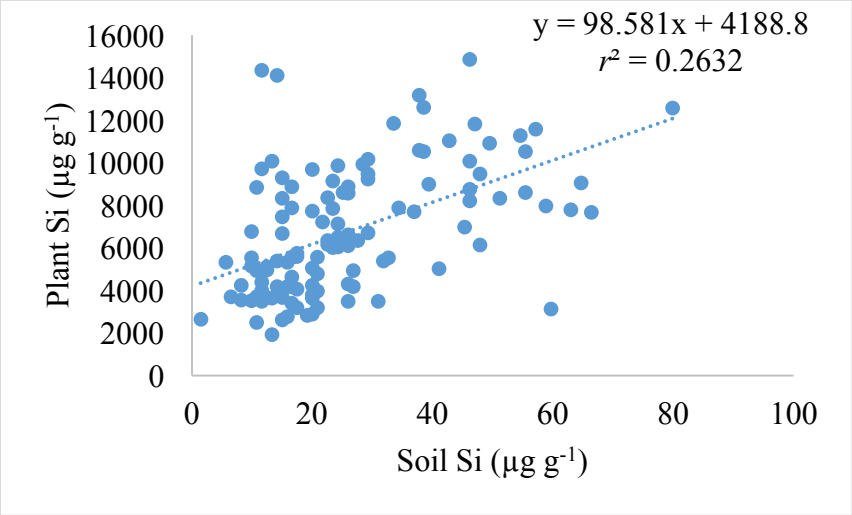
of Si had significant linear and cubic response for plant Si content for the first experiment (p<0.05); whereas there were significant linear and quadratic responses for the second experiment (p<0.05). Treatment interaction had no effect on plant Si content.

Table 3.4. Summary of two-way factorial ANOVA analyzing the effects of Si rates, hybrids and a fungicide on plant tissue Si content

Effect	Pr>F	
	Experiment 1	Experiment 2
	Plant Si	Plant Si
Hybrid	0.4992	<0.0001
Fungicide	0.0984	0.9048
Fungicide*hybrid	0.9879	0.1195
Si levels	<.0001	<0.0001
Control vs lime control	0.2199	0.2903
Linear	<0.0001	<0.0001
Quadratic	0.9121	0.0046
Cubic	0.0128	0.3097
Quartic	0.1074	0.7541
Si levels*hybrid	0.0915	0.1903
Si*fungicide	0.5014	0.6164

A linear relation (p<0.0001) best described the relationship between soil Si concentration and plant tissue Si content for both experiments (Figure 3.3). The hybrids were not significantly different for plant Si in first experiment. A linear relationship best described the relationship between plant Si and Si rates for the first experiment (Figure 3.4). However, Pioneer 84G62, the moderately susceptible hybrid, had a higher plant Si content for all Si rates than Pioneer 84P80 for second greenhouse experiment only (Figure 3.4).

Experiment 1



Experiment 2

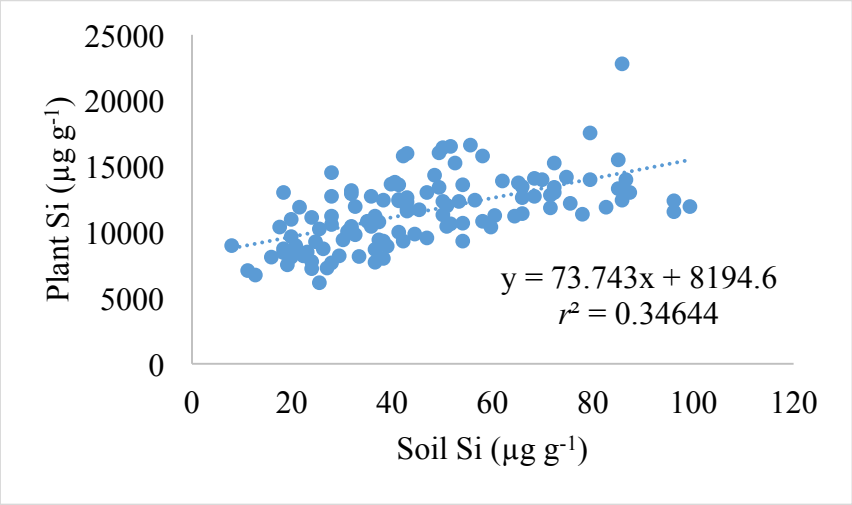
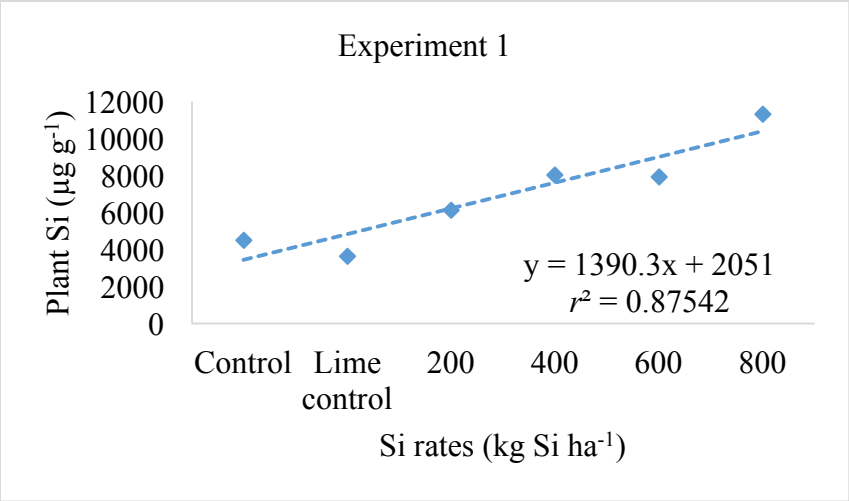


Figure 3.3. Relationship between soil Si concentration and plant Si tissue content.



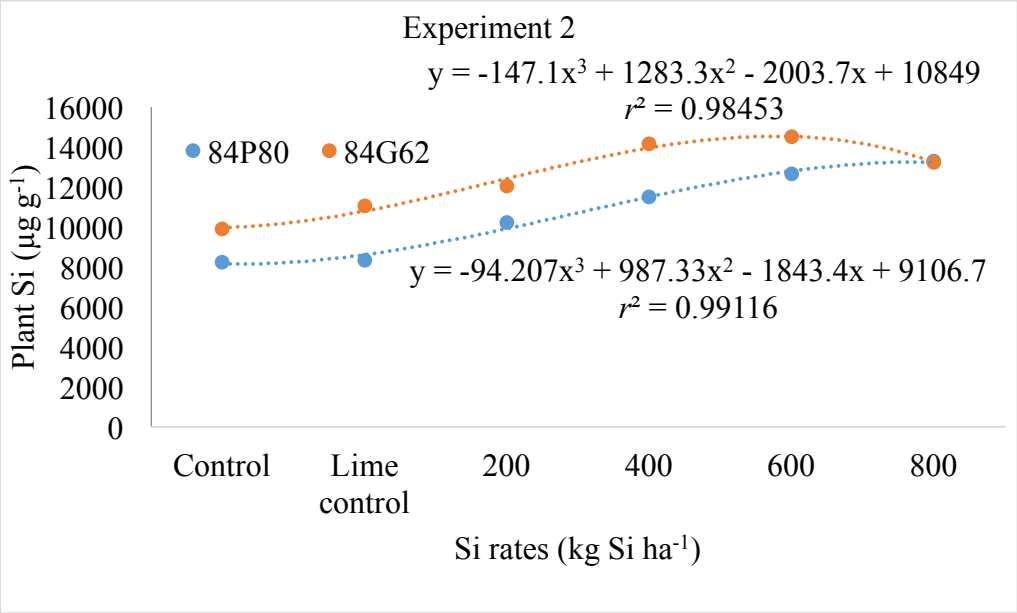


Figure 3.4. Effect of Si rates on plant Si accumulation for the two hybrids combined (1st experiment) and separated (2nd experiment) in the greenhouse.

3.3.3. Effect of Si rates, hybrids, and fungicide on AUAPC and FDS

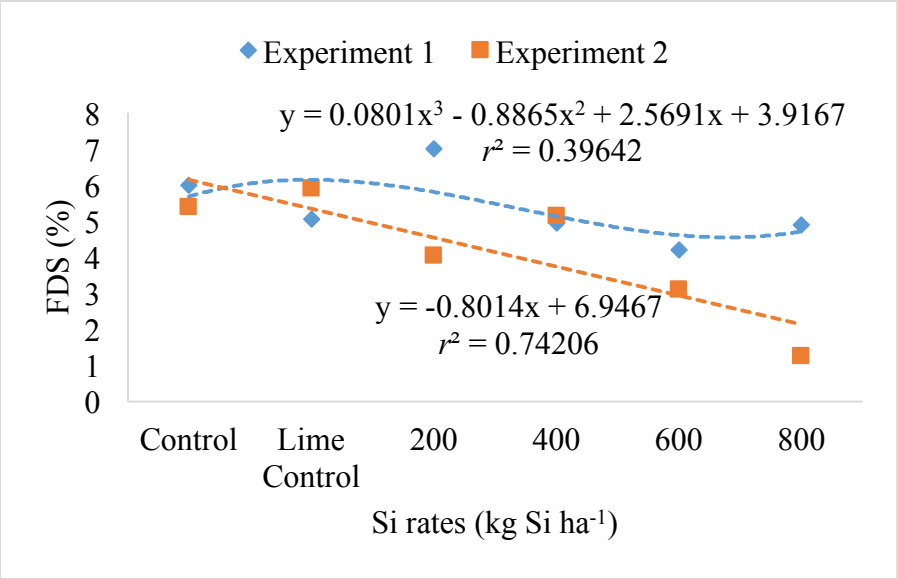
Fungicide and Si rates had significant effect on anthracnose development (Table 3.5). Even though, no differences were observed between the two hybrids for AUAPC and FDS, the interaction of fungicide and hybrid had significant differences in AUAPC and FDS for only the first experiment (p<0.05). The two controls had significant differences in AUAPC for the first

experiment (p<0.05) but were not significantly different for the second experiment. Although a significant linear response was observed to AUAPC in the first experiment, the cubic response was the best fit for AUAPC and FDS. A significant linear response was observed for AUAPC and FDS in second experiment.

Table 3.5. Summary of two-way factorial ANOVA analyzing the effects of Si rates, hybrids and fungicide on AUAPC and FDS. (* Student's t-test used for mean comparison)

Effect	Pr>F			
	Experiment 1		Experiment 2	
	AUAPC	FDS	AUAPC	FDS
Hybrid	0.1093	0.9463	0.8052	0.4167
*Fungicide	<0.0001	<0.0001	<0.0001	0.0002
Fungicide*Hybrid	0.0319	0.0391	0.1926	0.0869
Si levels	0.0018	0.0255	<0.0001	<0.0001
Control vs lime control	0.0440	0.2696	0.4090	0.5733
Linear	0.0437	0.1084	<0.0001	<0.0001
Quadratic	0.8102	0.5972	0.1052	0.1815
Cubic	0.0134	0.0054	0.4122	0.1677
Quartic	0.5624	0.3114	0.0813	0.0741
Si levels*hybrid	0.1437	0.4144	0.0285	0.6372
Si*fungicide	0.3784	0.6211	0.1177	0.2612

AUAPC and FDS values declined as Si rates increased for both experiments (Figure 3.5). The highest Si rate reduced FDS and AUAPC by 18 and 36% for first greenhouse experiment, whereas, FDS and AUAPC were reduced by 76 and 67% for second greenhouse experiment, respectively.



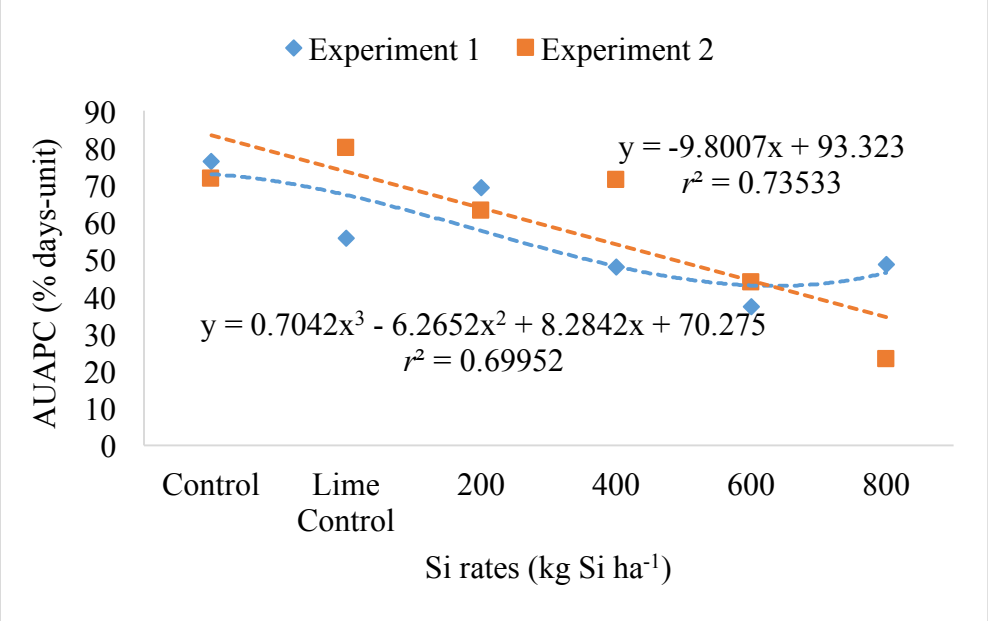
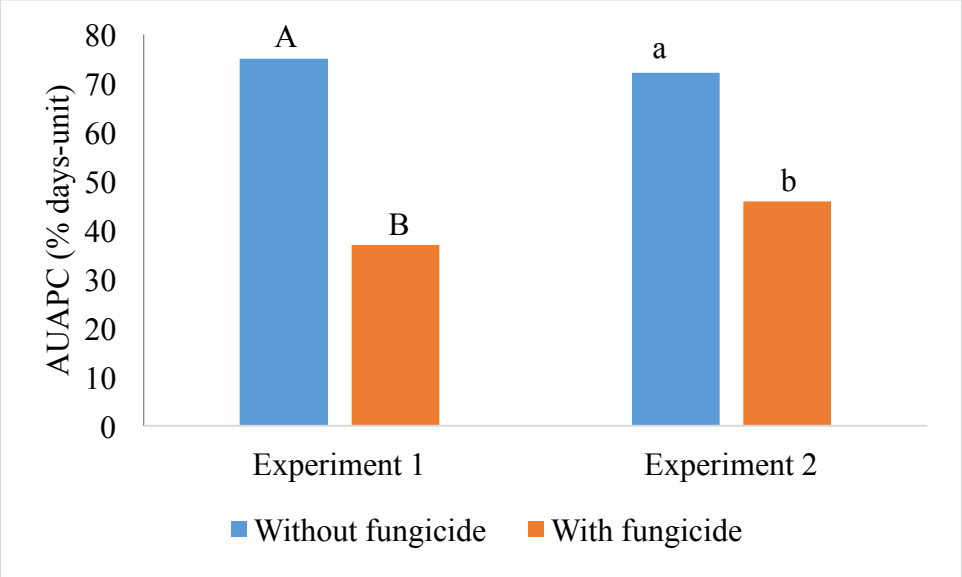


Figure 3.5. Effect of Si rates on FDS and AUAPC.

There was a significant effect of fungicide on anthracnose development on AUAPC and FDS (p<0.001). Fungicide reduced AUAPC by 50 and 36%, respectively, for the two experiments (Figure 3.6). Likewise, fungicide reduced FDS by 40 and 38%, respectively, for the two

experiments (p<0.001 and p<0.05). Even though Si and fungicide were both effective in reducing disease, no interaction existed between these two factors (Table 3.5).



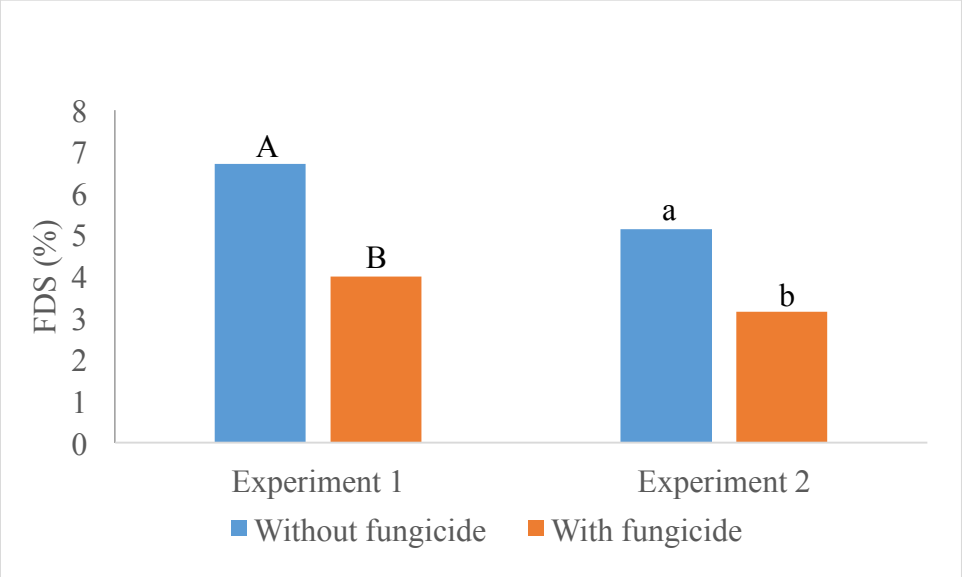


Figure 3.6. Effect of fungicide on AUAPC and FDS. Bars with different letters under same case are significantly different based on Student's t-test (p≤0.05).

b. Field studies

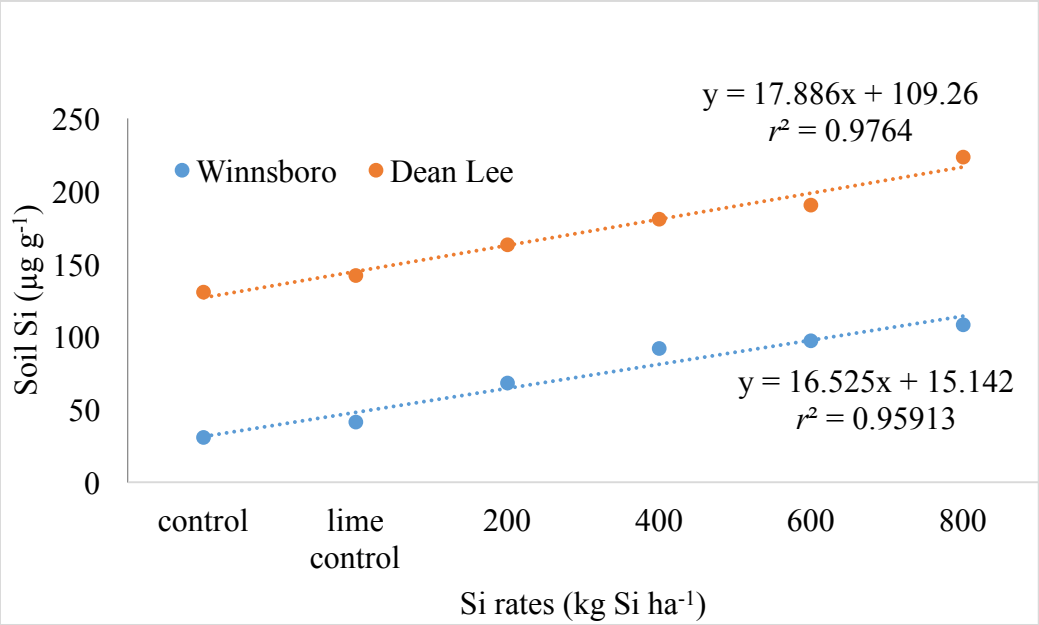
3.3.4 Effect of Si rates, hybrids, and fungicide on soil Si and pH

There was a significant effect of Si rates on soil Si and pH (Table 3.6). Fungicide had a significant effect on both soil Si and pH at Winnsboro but not at Dean Lee. Likewise, soil pH was significant for hybrids at Dean Lee. Control and lime control had no significant difference in soil Si concentration. However, they had pH differences at Winnsboro. Lime control and the four Si rates had linear response to Si application rates for soil Si and pH (p<0.05).

Table 3.6. Summary of two-way factorial ANOVA analyzing the effects of Si rates, hybrids and fungicide on soil Si and pH.

Effect	Pr>F			
	Winnsboro		Dean Lee	
	Soil Si	Soil pH	Soil Si	Soil pH
Hybrid	0.4067	0.1952	0.9962	0.0002
Fungicide	0.0030	0.0222	0.7114	0.3197
Fungicide*hybrid	0.9289	0.8630	1.0000	0.6362
Si levels	<.0001	<0.0001	<0.0001	0.0128
Control vs lime control	0.2514	0.0027	0.2505	0.3648
Linear	<0.0001	0.0107	<0.0001	0.0171
Quadratic	0.0962	0.1935	0.5524	0.8302
Cubic	0.5411	0.5691	0.2331	0.4078
Quartic	0.1849	0.4228	0.5516	0.3614
Si levels*hybrid	0.9304	0.7196	0.9666	0.7144
Si*fungicide	0.5590	0.7134	0.5495	0.6913

Soil Si increased in a linear manner with increasing Si rates for both locations. It was highest at the highest Si rate (800 kg Si ha^{-1}) and lowest for the controls at both locations (Figure 3.7). Similarly, there was a linear trend for soil pH as Si rates increased. Soil pH was highest at the highest Si rate. Controls had the lowest pH at both locations. The relationship between soil pH and soil Si was more pronounced in Winnsboro and it was observed that soil Si increases dramatically between pH 7.5-8.2 (Dean Lee) and 6.5-7.5 (Winnsboro) (Figure 3.8). The soil Si-soil pH relation in Winnsboro was similar to that of greenhouse experiments (Figure 3.2).



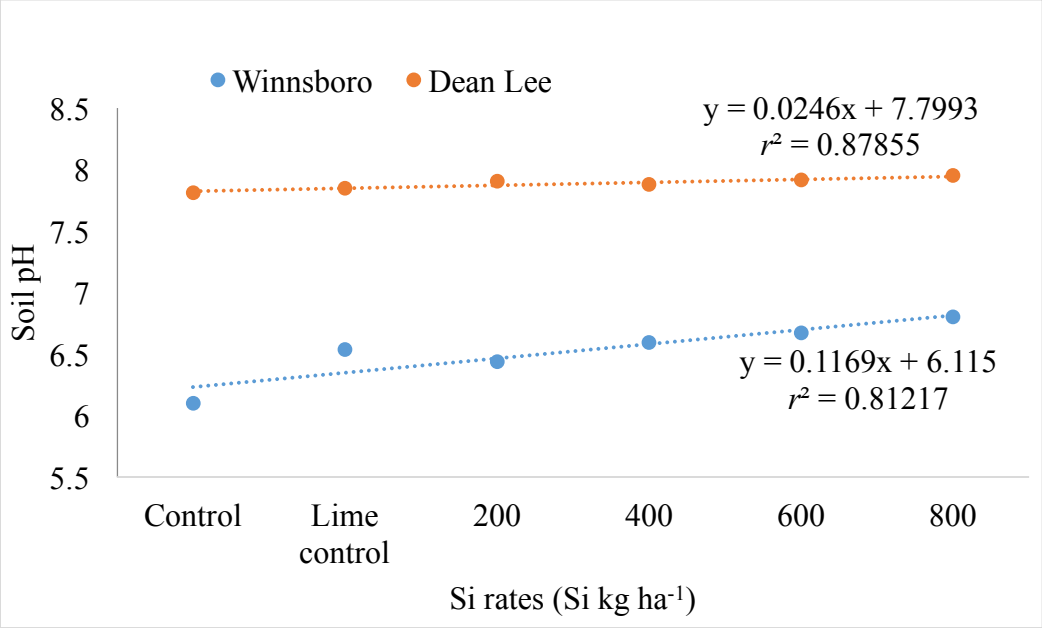
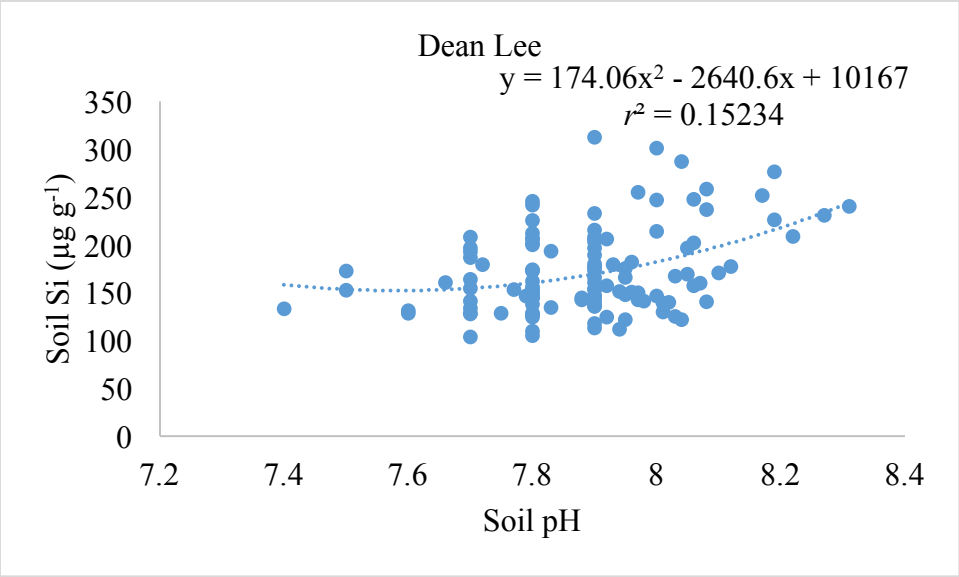


Figure 3.7. Effect of Si rates on soil Si and pH at two field locations.



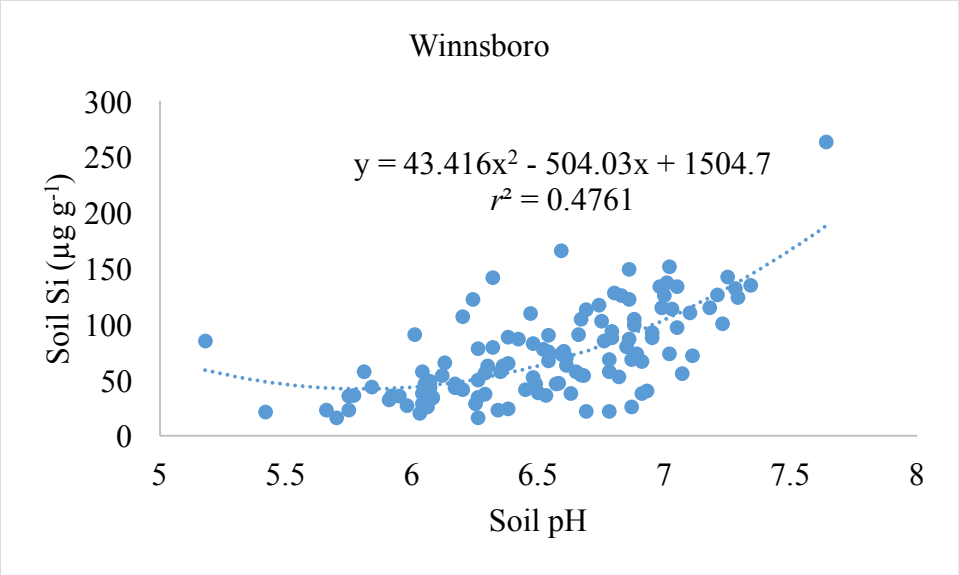


Figure 3.8. Relationship between soil Si and pH for the two field locations.

3.3.5 Effect of Si rates, hybrids, and fungicide on leaf and grain Si content

Increasing Si soil rates had no effect on increasing leaf or grain Si content at both field locations (Table 3.7). However, hybrids were significantly different in terms of both leaf and grain Si content at Winnsboro only. Pioneer 84G62 had the highest accumulation of Si in leaf

tissue and grain in comparison to Pioneer 84P80 (Figure 3.9). Since there was no Si effect, orthogonal polynomial contrast was not measured for leaf and grain Si content. No relationship was observed between leaf Si and soil Si at both locations (Figure 3.10).

Table 3.7. Summary of two-way factorial ANOVA analyzing the effects of Si rates, hybrids and fungicide on leaf and grain Si content. (* Mean comparison conducted using Student's t-test).

Effect	Pr>F			
	Winnsboro		Dean Lee	
	Leaf Si	Grain Si	Leaf Si	Grain Si
*Hybrid	<0.0001	<0.0001	0.0607	0.8984
Fungicide	0.9856	0.9404	0.5869	0.9712
Fungicide*hybrid	0.1299	0.9660	0.1616	0.2988
Si levels	0.5626	0.5387	0.5429	0.9566
Control vs lime control	-	-	-	-
Linear	-	-	-	-
Quadratic	-	-	-	-
Cubic	-	-	-	-
Quartic	-	-	-	-
Si levels*hybrid	0.7844	0.6979	0.2764	0.1867
Si*fungicide	0.7070	0.6234	0.9542	0.3881

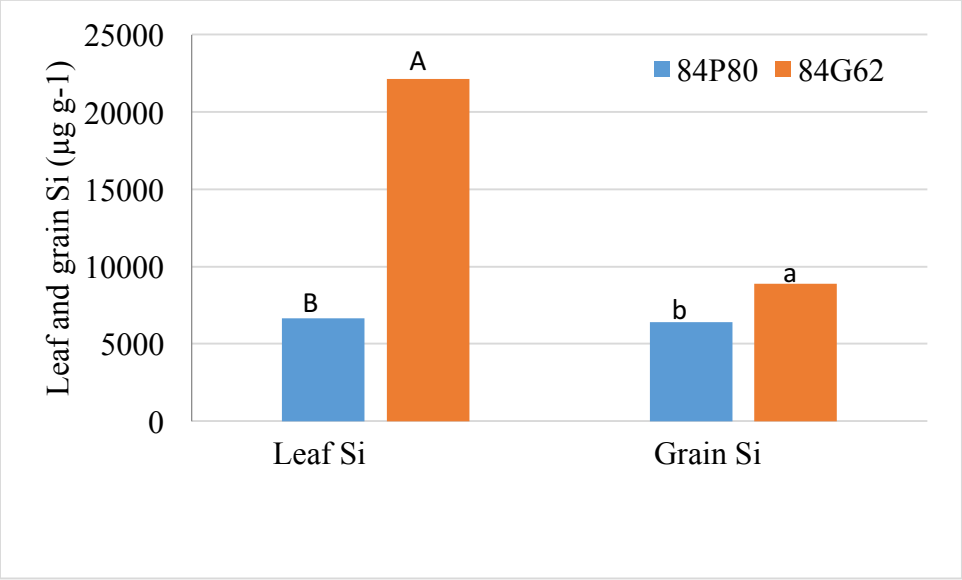


Figure 3.9. Differences in leaf and grain Si content among two hybrids at Winnsboro. Bars with different letters under same case are significantly different based on student's t- test ($p \leq 0.05$).

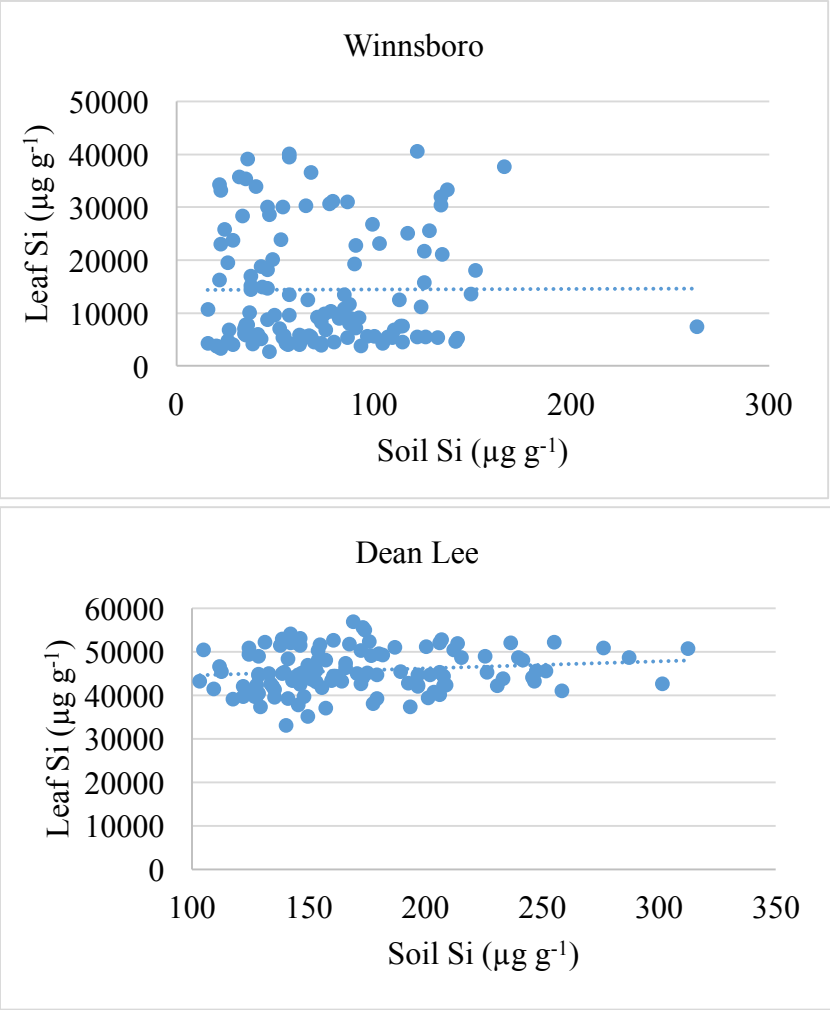


Figure 3.10. Relationship between soil Si and leaf tissue Si at both field locations.

3.3.6 Effect of Si rates, hybrids, and fungicide on Anthracnose development

The two hybrids were significantly different for AUAPC and FDS at both field locations (Table 3.8). However, fungicide was only effective for suppressing anthracnose development at Dean Lee. A significant interaction of fungicide and hybrid on AUAPC and FDS was observed at Dean Lee. A significant linear relationship was observed between Si rates with AUAPC and FDS at Dean Lee but not at Winnsboro. Significant interaction effect of Si and fungicide was observed for FDS at Dean Lee.

Table 3.8. Summary of two-way factorial ANOVA analyzing the effects of Si rates, hybrids and fungicide on AUAPC and FDS (* Student's t-test used for mean comparison).

Effect	Pr>F			
	Winnsboro		Dean Lee	
	AUAPC	FDS	AUAPC	FDS
*Hybrid	<0.0001	<0.0001	<0.0001	0.0394
Fungicide	0.6302	0.8358	<0.0001	<0.0001
Fungicide*hybrid	0.1360	0.9559	0.0002	0.0455
Si levels	0.7044	0.1317	0.0473	0.0048
Control vs lime control	-	-	0.7775	0.8993
Linear	-	-	0.0147	0.0036
Quadratic	-	-	0.3151	0.0504
Cubic	-	-	0.2670	0.0957
Quartic	-	-	0.5407	0.3162
Si levels*hybrid	0.8934	0.3609	0.5310	0.5175
Si*fungicide	0.6977	0.8802	0.1983	0.0024

The moderately susceptible hybrid (Pioneer 84G62) had a higher AUAPC compared to the moderately resistant hybrid (Pioneer 84P80) (Figure 3.11). Pioneer 84P80 had a lower AUAPC with and without a fungicide. Similarly, fungicide- treated plants had lower AUAPC in comparison to those non-treated. Pioneer 84P80 with fungicide had the lowest AUAPC value. Fungicide reduced AUAPC by 44 and 39%; respectively, for Pioneer 84G62 and Pioneer 84P80. Similar effect of fungicide and hybrid were observed for FDS on both hybrids too (Figure 3.11). Fungicide reduced FDS by 48 and 50% for Pioneer 84P80 and Pioneer 84G62, respectively.

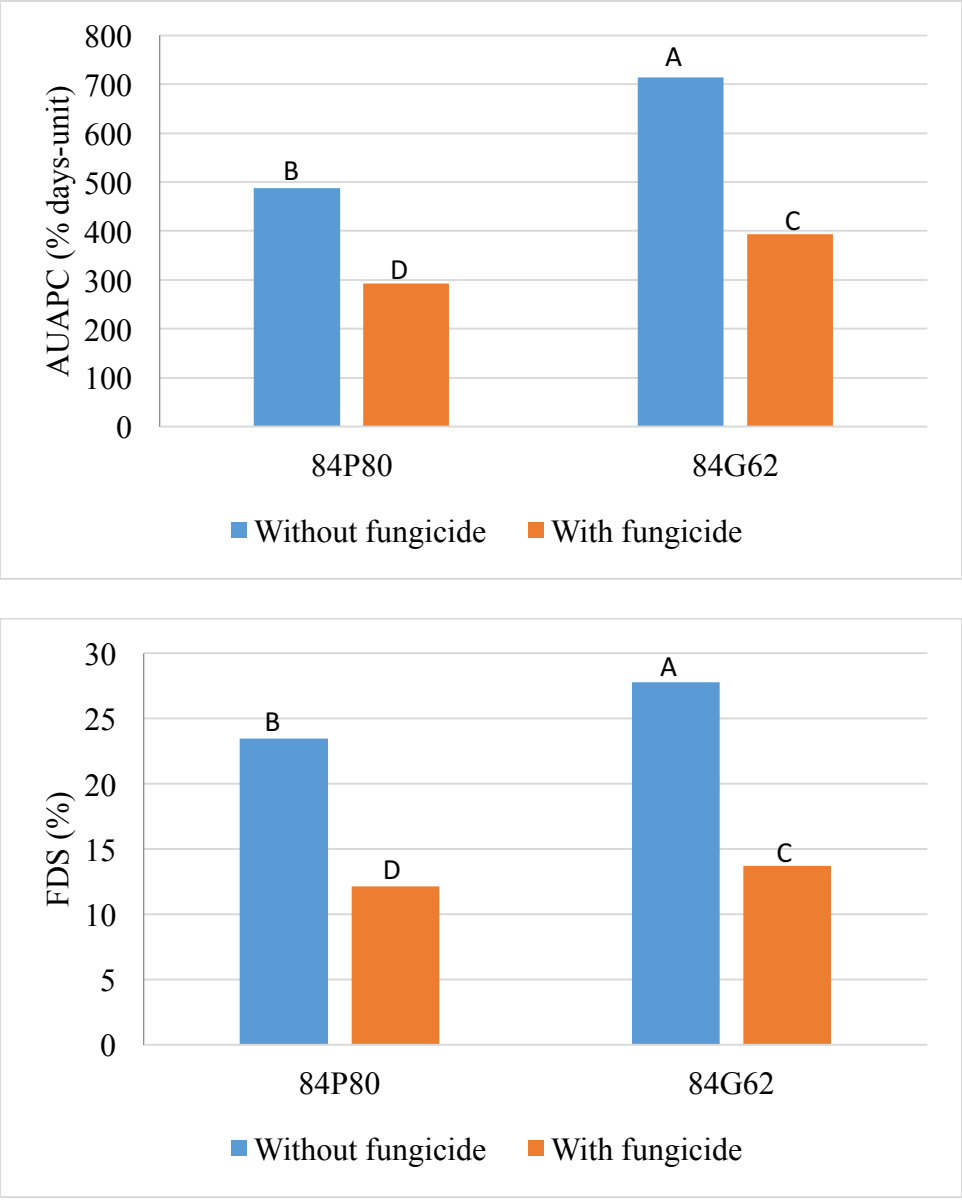
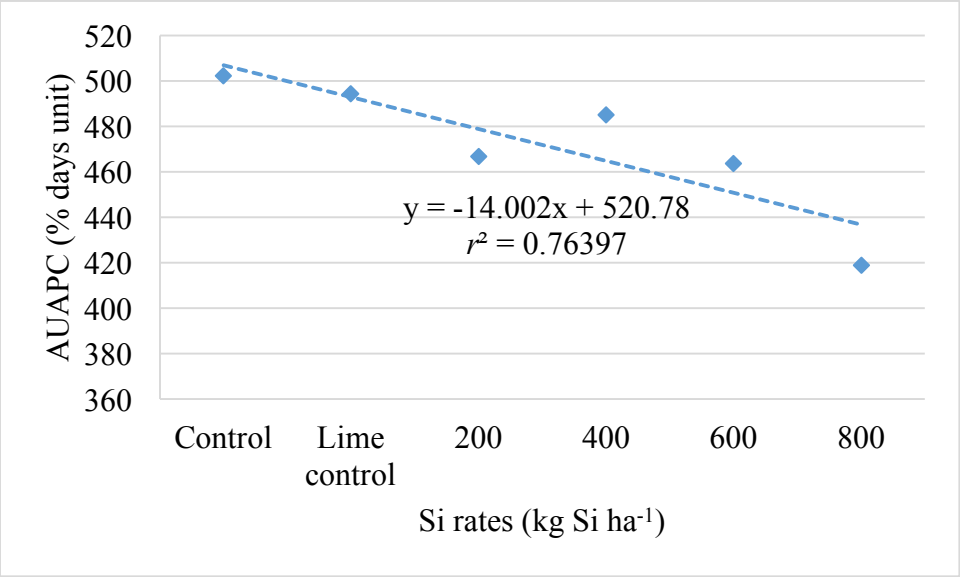


Figure 3.11. Effect of fungicide and hybrid on AUAPC and FDS at Dean Lee. Bars with different letters are significantly different based on Tukey's test ($p \leq 0.05$).

Reduced AUAPC and FDS levels were significantly associated with increasing rates of soil applied Si (Figure 3.12). A negative linear relationship was observed between Si levels and AUAPC and FDS. The AUAPC and FDS values were highest for the control and lowest at Si rate of 800 kg Si ha^{-1}. The highest Si rate (800 kg Si ha^{-1}) appeared to reduce AUAPC and FDS by 17 and 19%, respectively.



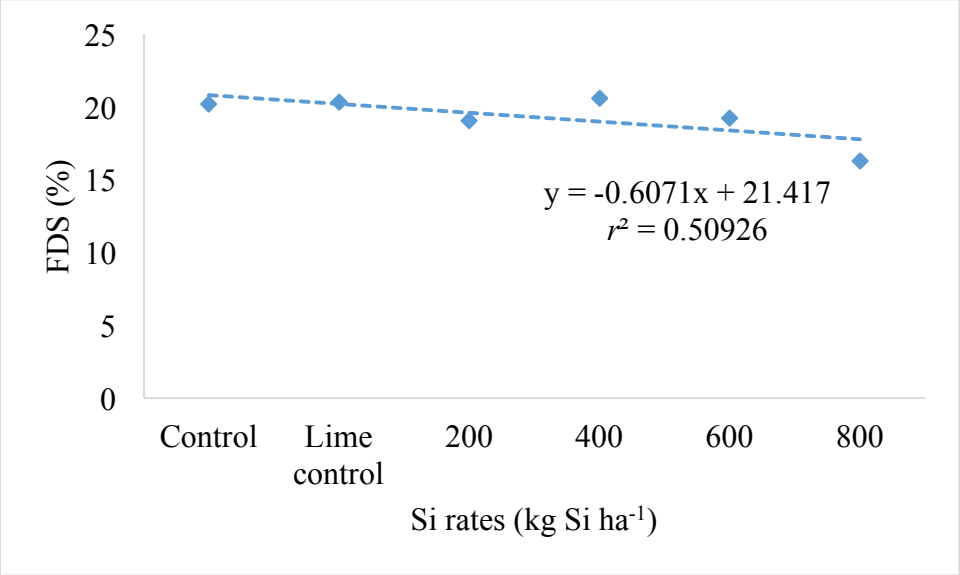


Figure 3.12. Relationship between Si rates and AUAPC and FDS at Dean Lee.

The two hybrids were significantly different for anthracnose development at Winnsboro (p<0.001). The moderately resistant hybrid Pioneer 84P80 had 13 and 33% lower FDS and AUAPC values; respectively, in comparison to Pioneer 84G62 (Figure 3.13).

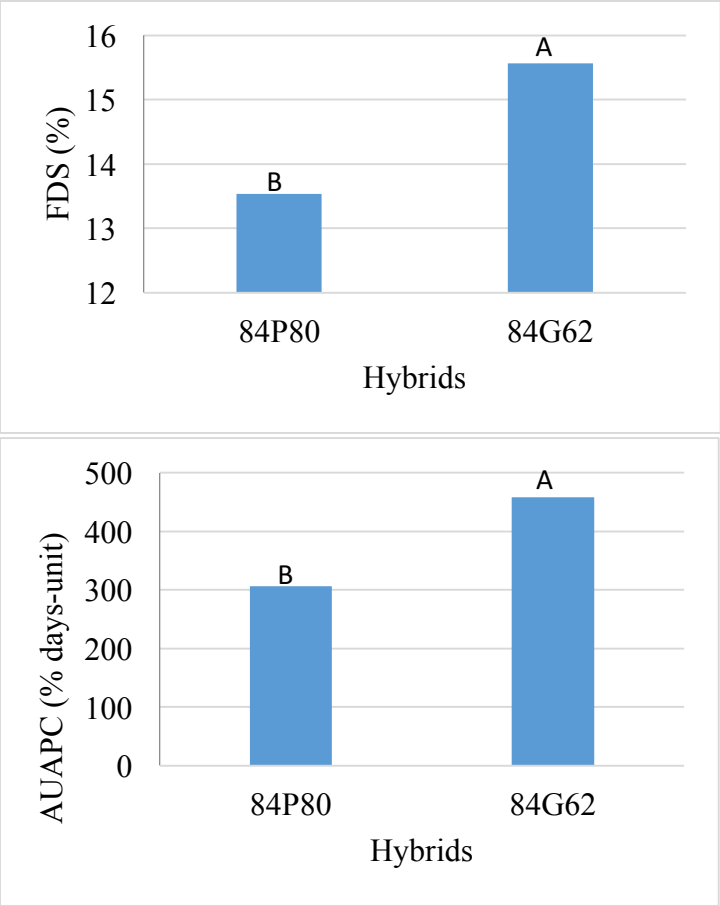


Figure 3.13. Anthracnose development (FDS and AUDPC) on the two hybrids at Winnsboro. Bars with different letters are significantly different based on student's t-test ($p \leq 0.05$).

3.3.7 Effect of Si rates, hybrids, and fungicide on yield

Silicon rates had a significant effect on grain yield at Dean Lee (Table 3.9). Likewise, the two hybrids had significantly different yield. No yield differences were detected between the two controls. Pioneer 84G62 had higher yield when compared to Pioneer 84P80 at Dean Lee (Figure 3.14). Yield was not measured at Winnsboro because there was a heavy aphid infestation and insecticides failed to control this insect. Fungicide also had no effect on increasing yields at Dean Lee.

Table 3.9. Summary of two-way factorial ANOVA analyzing the effects of Si rates, hybrids and fungicide on grain yield at Dean Lee.

Effect	Pr>F
	Dean Lee Yield
Hybrid	<0.0001
Fungicide	0.4575
Fungicide*hybrid	0.9281
Si levels	0.0244
Control vs lime control	0.0523
Linear	0.1866
Quadratic	0.1934
Cubic	0.0525
Quartic	0.0160
Si levels*hybrid	0.2950
Si*fungicide	0.0922

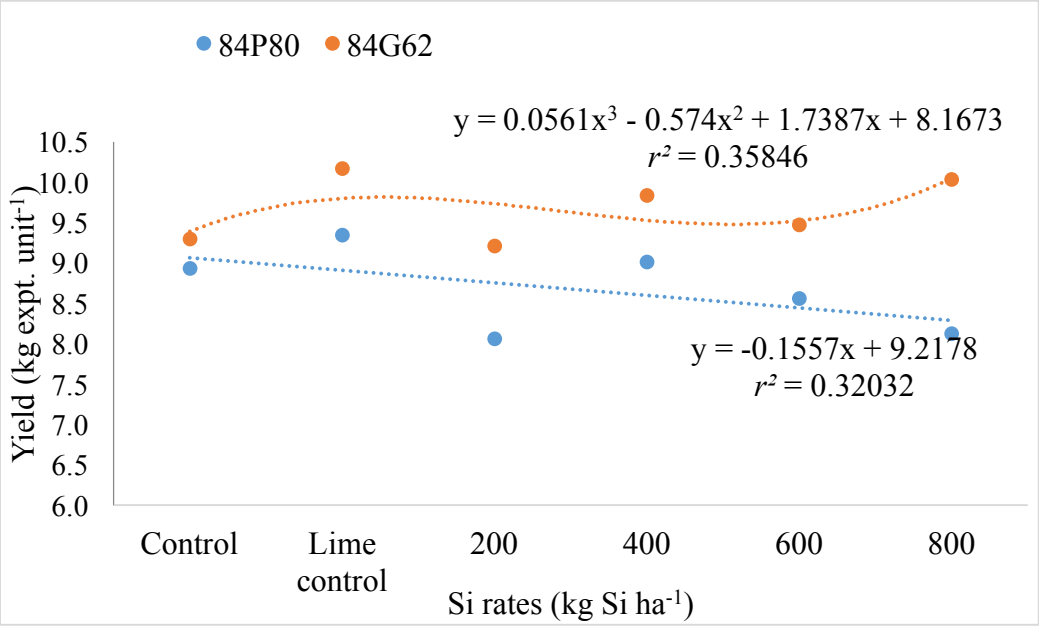


Figure 3.14. Relationship between Si rates and yield of the two hybrids at Dean Lee.

3.4 Discussions

3.4.1 Effect of Si rates on plant available soil Si and pH

Soil Si and pH increased gradually with increasing Si rates for both experiments in greenhouse and field. Even though the control and lime control had a significant difference in soil Si levels for the second greenhouse experiment (Table 3.3), no differences between these two treatments were observed in first greenhouse experiment as well as for the field experiments. A quadratic relationship was observed between soil pH and soil Si for both experiments in the greenhouse and field. Since initial soil pH at Dean Lee was higher (>7.5) than at Winnsboro (pH 6.0), the addition of lime (lime-control) at Dean Lee had no impact on soil pH. Hence, the control and lime control treatments had no pH differences at Dean Lee, but had a significant difference at Winnsboro. Likewise, these treatments were significantly different for soil pH in both greenhouse experiments where the initial soil pH was low (5.5). Initial soil Si levels were lower in the greenhouse (25.2 $\mu g\ g^{-1}$) and at Winnsboro (40 $\mu g\ g^{-1}$) and both had a low soil pH. However, at Dean Lee the soil Si level was greater (120 $\mu g\ g^{-1}$) and had higher soil pH. These results are in agreement with the findings of Haynes (2014) who reported that soils with a high pH will have a higher soil Si level in comparison to soils having pH of 6 or lower. Low pH soils are believed, in part, to be lower in plant-available Si due to leaching (Haynes, 2014).

Even though soil Si levels increased with increasing Si rates for both greenhouse experiments and field experiments, there was no relationship between soil Si levels and plant Si content for the field experiments. At Dean Lee, the soil type was an Inceptisol with high soil Si level (120 $\mu g\ g^{-1}$) while at Winnsboro, it was an Alfisol with higher soil Si level (40 $\mu g\ g^{-1}$) than the low-Si Alfisol (25.2 $\mu g\ g^{-1}$) used in the greenhouse studies. The low-Si Alfisol used in the greenhouse studies had a better response to Si fertilization in terms of increasing plant tissue Si content and

reducing anthracnose development in comparison to the Alfisol in Winnsboro. Similar results were observed for Histosol with low soil Si at South Florida, where sugarcane, rice and citrus production benefitted from Si fertilization (Elawad et al., 1982; Matichenkov et al., 1999). Furthermore, in India, Ultisols having low (~20.53 μg g^{-1}) to medium (~35.76 μg g^{-1}) soil Si responded better to Si fertilization than the soils having a higher (~60.0 μg g^{-1}) Si content (Narayanaswamy et al., 2009). Korndorfer and his colleagues (2001) also noticed similar results in organic soils (Histosol) of Florida. Likewise, Si fertilization of rice grown in low Si soils offered promising results in terms of reducing disease susceptibility and improving yields (Datnoff and Rodrigues, 2005).

Thus, different soil types may have a different response to Si application and even within the same soil order; soils with low Si levels will response better to Si fertilization than a soil with a high Si level. Apart from soil order, soil texture and cropping duration are other criteria that may provide information about plant-available Si status (Tubana et al., 2016). Furthermore, continuous farming for decades results in soils with low quantities of plant-available Si (Datnoff et al., 1997).

3.4.2 Effect of Si rates on plant and grain Si concentration

A significant effect of Si rates was observed on plant Si concentration in both experiments conducted in the greenhouse. Moreover, a positive linear trend best described the relation between soil Si and plant Si content (Figure 3.3). However, no such results were observed in the field; there was no relationship between soil Si and leaf tissue Si content. The application of Si had no effect on grain Si content. This could be due to high initial soil Si level in the field compared to the greenhouse soil. Initial soil Si level in the fields were 40 and 120 μg g^{-1} for

Winnsboro and Dean Lee, respectively. Consequently, because of higher initial soil Si level, application of increasing rates of Si had no effect on both leaf and grain Si content. With this result, we can assume that increasing Si application rates probably has a greater influence on plants when grown in low-Si soil. With the increasing levels of Si application, the dissolution of the fertilizer materials in silt loam soils having low adsorption capacity may lead to polymerization thus reducing plant available Si (monomeric form) (Iller et al., 1979). Hence, even though total Si content of soil increases, Si might get polymerized or may remain in forms unsuitable for plant uptake.

3.4.3 Effect of Si rates, fungicide and hybrids on anthracnose development (AUAPC, FDS)

The application of Si significantly reduced both AUAPC and FDS over the control in both greenhouse experiments (Figure 3.5). This result is in agreement with those found by Resende et al. (2012) and Santos et al. (2014) who found significant reduction in AUAPC values of sorghum anthracnose when plants were grown in low-Si soil incorporated with different Si levels. Resende et al. (2009) also found that Si rates reduced FDS along with AUAPC indirectly suggesting that, fungal colonization was affected with some kind of resistance mechanism of the host. Even though Si and fungicide were noted effective in reducing anthracnose in both greenhouse experiments, there was no significant interaction effect observed. This result contrasts the results of Datnoff et al. (1997) who showed reduction in rice blast incidence from 73% to 36% in presence of Si without fungicide (benomyl) and from 27% to 13% with fungicide. Likewise, Seebold et al. (2004) found significant interaction effect of Si and edifenphos in reducing rice leaf blast severity. They also found that Si alone was equally as effective as full rate of fungicide edifenphos. Furthermore, Resende et al. (2013) also found that

Si alone and in combination with fungicide Opera effectively reduced area under anthracnose progress curve in sorghum.

The two hybrids had no significant differences in anthracnose development in the greenhouse whereas, the moderately susceptible hybrid (Pioneer 84G62) had higher anthracnose development at both field locations (Figure 3.11, Figure 3.13). Plants were grown for a shorter period in greenhouse than in the field. This could be one of many possible reasons for no differences among hybrids in the greenhouse in terms of anthracnose development. Furthermore, there was a significant effect of fungicide and hybrid interaction at Dean Lee where, moderately susceptible hybrid without fungicide had higher anthracnose development than the susceptible hybrid (Figure 3.11). Similar results were observed for FDS. Final disease severity was greater for moderately susceptible hybrid at Dean Lee. However, fungicide was not effective in minimizing anthracnose at Winnsboro. The field at Winnsboro had various environmental challenges such as heavy aphid infestation.

For the field experiments, increasing Si rates were found to significantly reduced the disease only at Dean Lee (Figure 3.12). However, since no relationship was observed between leaf Si and plant available Si for both field locations, it is doubtful that Si had any influence on anthracnose development in the field. Application of Si in soil was found to increase availability of plant essential nutrients such as Calcium (Ca) and Magnesium (Mg) (Ferreira et al., 2015). Even though wollastonite has ideal composition of Ca and Si, some minor amounts of aluminum (Al), iron (Fe), manganese (Mn), magnesium (Mg), potassium (K), sodium (Na) are also found in wollastonite (Virta, 2004). So, Si application might have also increased availability of Ca and Mg. Silicate fertilizers improve soil fertility (Matichenkov and Calvert, 2002) and hence, improvement of soil fertility and increased availability of nutrients, might have improved

performance in host resistance to anthracnose. Furthermore, increase in pH with Si application (Figure 3.7) might have also aided the availability of Ca and Mg. Unfortunately, Ca and Mg were not measured in this study so it would be difficult to know whether Ca or Mg minimized anthracnose development.

3.4.4 Effect of silicon and fungicide on yield

There was no yield obtained for the greenhouse experiment because the plants were grown for only eight weeks. There was no yield for Pioneer 84G62 in Winnsboro because of heavy aphid infestation which could not be controlled even with insecticide application. Furthermore, as mentioned earlier, because of higher initial soil Si status, and no differences in plant Si concentration among various Si rates, Si had no such pronounced effect on grain yield (Figure 3.14). This result is in contrast with the research by Resende and colleagues (in 2012) on sorghum where yield increased with Si application. Furthermore, Pioneer 84G62 had higher yield than Pioneer 84P80 at Dean Lee. There was no effect of fungicide in grain yield at both locations.

3.5 Conclusions

Silicon application increased plant available Si in the greenhouse experiments. Silicon and fungicide were effective in reducing both AUAPC and FDS, however, no interaction effect was observed in reducing anthracnose development. Although soil Si had a linear relation with Si application for both field experiments, Si had no significant effect on leaf tissue Si, grain Si, and yield at both locations. There was a significant effect of fungicide and hybrid interaction on anthracnose development. However, fungicide had no effect on minimizing anthracnose at

Winnsboro. Even though statistically, anthracnose development was lower for higher rates of Si at Dean Lee, since there was no relation between plant available Si and leaf Si content, Si might have enhanced availability of beneficial nutrient such as Ca and Mg which might have ultimately improved performance of sorghum plants against anthracnose. Furthermore, Si showed a better impact on the low-Si soil used in the greenhouse along with controlled environmental conditions than under field conditions with soil testing higher in Si content(40-120 $\mu g\ g^{-1}$). Since Si has been found beneficial for minimizing diseases of various crops under different soil types, future studies should be conducted at various field locations with different soil Si levels to determine which would be best for receiving Si fertilizer for minimizing anthracnose development in sorghum.

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Chapter 4: Conclusions

Low-silicon (Si) soil, (low pH Alfisol) when amended with wollastonite, increased soil Si levels which increased plant tissue Si content and ultimately reduced sorghum anthracnose development in the greenhouse. A negative linear relationship between Si rates and anthracnose development also was observed in the greenhouse. Soil Si had a significant linear relationship with plant tissue Si only in the greenhouse. No relation was observed between soil and plant tissue Si in the field. This is probably due to the high-Si soils found in the fields at Dean Lee and Winnsboro. For the greenhouse study, no differences in inoculum densities were observed between 10^5 and 10^6 conidia ml^{-1} suggesting that a lower concentration may be used.

Silicon and fungicide were effective in reducing both AUAPC and FDS; however, no interaction effect was observed in reducing anthracnose development. A linear relationship was observed for soil Si and pH for both field experiments. Silicon application had no significant effect on leaf tissue Si, grain Si, and grain yield at both field locations. However, soil Si levels were observed to have a negative linear relationship with anthracnose development at Dean Lee. However, since no relationship between plant tissue and soil Si was observed at either field location, wollastonite might have enhanced soil fertility and improved availability of other important nutrients such as Ca and Mg. These elements might have improved the host's ability to resist anthracnose development. Furthermore, there was significant interaction effect of fungicide and hybrids on anthracnose development. However, fungicide had no effect on minimizing anthracnose at Winnsboro.

Based on the findings obtained from these studies, future research should be conducted at various field locations with different Si soil levels to determine when to use Si for managing anthracnose development in sorghum.

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