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EFFECTS OF SILICON AND NITROGEN FERTILIZATION ON GROWTH,
YIELD, AND LEAF RUST DISEASE DEVELOPMENT IN WHEAT

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TABLE OF CONTENTS

ABSTRACT

Silicon (Si) is the second most abundant element present in the lithosphere and it is considered a nonessential element for plants. Field studies were conducted at multiple sites in the state of Louisiana in 2015 and 2016 to examine the impact of several sources of Si fertilizers and two rates of nitrogen (N) fertilizer on wheat (*Triticum aestivum* L.) growth, yield and the development of leaf rust, which is one of the most prevalent wheat diseases in Louisiana. The specific objectives were to : 1) evaluate the effect of soil Si amendments and foliar application of Si fertilizers, along with two rates of N fertilizer on agronomical parameters such as Si uptake, yield and scanning electron microscopic studies to determine %Si in epidermis, and 2) investigate the efficacy of different Si sources on wheat rust disease severity. The experimental design was randomized complete block design with four replications. The N source used in this study was urea (46% N) at 101 and 145 kg N ha^{-1}. The Si treatments consisted of two solid Si fertilizers (wollastonite- 23% Si, and silicate slag as Plant Tuff ®-12% Si) applied at 280 kg Si ha^{-1} and a foliar solution Si applied at 1000, 2000, and 4000 ml ha^{-1}. Wollastonite and slag applications significantly increased the soil Si content and soil pH in comparison with controls and plots treated with foliar Si. Analysis of variance (ANOVA) at *P*<0.05 showed that soil Si amendments significantly increased the plant Si uptake compared to non- treated plants at midseason. There was no significant effect of Si treatments either alone or in combination with N on grain yield in 2015 and 2016. The scanning electron microscopic (SEM) images indicated that Si fertilization significantly increased %Si deposited in epidermal regions of leaves compared to non-treated plants. Disease severity was significantly reduced in plots receiving Si treatments. These results may support the positive role of Si in cell wall reinforcement against fungal

penetration and the induction of plant defense responses which suggests that Si fertilization may

be considered a sustainable approach to improving plant disease resistance.

CHAPTER 1. INTRODUCTION

The global population has been expanding rapidly, and in order to meet food supply demands, agricultural approaches to increase food qualities and quantities are going to hold more importance than ever before. To match food supply with demand, production must double by 2050 (Bhalla, 2006). Plant diseases are considered major limiting factors in agriculture, and management practices utilizing chemicals raise environmental and food safety concerns (Aktar et al., 2009). Sustainable agricultural approaches focus on agronomic practices to improve production with minimal adverse effects on the environment. It suggests that supplemental application of some nutrients alone or in combination with other cultural practices can successfully manage some of the plant diseases Dordas (2008).

Maize and wheat constitute approximately two-thirds of human food consumption in the world (FAO, 2002 a). Wheat is one of the oldest domesticated food crops (Eckardt, 2010), making up a considerable portion of human food consumption in the world (Shewry and Hey, 2015). Global population growth and food demand require an increase in wheat production from 600 million tons to approximately 760 million tons by 2020 (CIMMYT, 2004). The United States is one of the world's largest wheat producers behind China, the European Union, India, and Russia, and wheat production ranked third among U.S. field crops following corn (*Zea mays* L.) and soybean (*Glycine max* L. Merr) (USDA- ERS, 2016). In 2015, over 19 million hectares of wheat was harvested where 13 million hectares were associated with winter wheat production (USDA-NASS, 2015).

Wheat is classified as a winter or a spring- type based on two different growing cycles. The vast majority of wheat production in the United States is winter wheat, which constitutes 70-80 percent of total wheat production (USDA- ERS, 2016). To achieve the flowering stage and

maximum yield potential, winter wheat plants require photoperiodic stimuli and exposure to 4°C for 6 weeks (Dubcovsky et al., 2006; FAO, 2002 b). Wheat has a high adaptation level under diverse environmental conditions due to the complexity of the genome (FAO, 2002 c; Feldman and Levy, 2012)

Plant diseases are considered a major constraint to yield potential and future food security. Three wheat rusts, leaf, stem, and stripe, with the agents of *Puccinia triticina* (Eriksson), *Puccinia graminis* (Pers) and *Puccinia stiiformis* (Westend) respectively, have been the most damaging diseases impacting wheat production at the global level (Singh et al., 2016). More than one- hundred biotic and abiotic diseases affect wheat yields in the U.S.; among them the three wheat rusts leaf, stem, and stripe along with Fusarium head blight (*Fusarium graminearum* Schwabe) (Savary et al., 2012). According to Carefoot and Sprott (1967), historical records show that severe wheat rust epidemics occurred in Egypt causing significant yield losses centuries before the birth of Christ. The causal agent of wheat leaf rust, *Puccinia triticina* (Eriksson), is a parasitic heteroecious fungus which is characterized by infectious uredinospores scattering on both the upper and the lower leaf surfaces (Bolton et al., 2008). Yield losses caused by leaf rust are mostly due to reductions in net photosynthesis rates that lead to decreased numbers of kernels per head, lower kernel weights and lower grain quality compared to other diseases such as stem rust or Fusarium head blight (Bolton et al., 2008; Carretero et al., 2011). Leaf rust can adversely affect wheat photosynthesis and eventually yield production through damaging chlorophyll and decreasing light interception in the leaves (Carretero et al., 2011). A film of moisture on leaves for six to eight hours at temperature of 15.5 - 26.67°C are considered favorable environmental conditions for leaf rust (Groth et al., 2013). Susceptible varieties, favorable temperature and humidity and the presence of spores are the

most important factors affecting leaf rust disease development (Cook and Veseth, 1991). Leaf rust occurs most often in the northern United States and is also prevalent in the southeast, southern Great Plains and Pacific Northwest where wheat is grown between October and May (Cook and Veseth, 1991). Spore dispersal through wind and arising new races of pathogens due to constant breakdown of plant vertical resistance are critical attributes that make leaf rust a global wheat production concern (Chaves et al., 2013). Leaf rust management is achieved through the use of resistant varieties, fungicide applications and cultural practices, such as controlling volunteer wheat plants (USDA- ARS, 2016).

Silicon (Si) is the second most abundant element present in the earth's crust constituting one of the major inorganic nutrient elements of many plants (Epstein, 1999; Ma et al., 2004). Following uptake of monosilicic acid (H_4SiO_4) by the roots, Si is transported from the roots to the shoots where it is deposited into plant cell walls, intercellular spaces of cells and bracts in the form of amorphous silica gel ($SiO_2 \cdot nH_2O$) (Jones and Handreck, 1967; Epstein, 1999; Kim et al., 2002; Rodriguez and Datnoff, 2005). Plant molecular analysis has shown that in some plant species such as rice (*Oryza sativa* L.) and wheat, Si can also be taken up in the cells against a concentration gradient (Ma et al., 2004; Montpetit et al., 2012). Deposited Si in leaves cannot be remobilized or become available to other parts of the plant (Raven, 1983). Scanning electron microscopy and energy-dispersive X-ray spectroscopy analyses of dry ash samples of blueberry (*Vaccinum corymbosus* cv. Bluecrop) plants showed that Si accumulated in the form of phytoliths in the upper and lower epidermis (Morikawa and Saigusa, 2004). Silicon is considered a non- essential plant nutrient that may play a beneficial role in promoting plant growth by alleviating biotic and abiotic stresses in many plant species (Pilon-Smits et al., 2009). Plants deficient in Si have shown growth abnormalities and stress susceptibility (Epstein, 1999). Plants

only accumulate Si in 0.1 to 10% of the plant tissue, depending on the plant species (Ma et al., 2011). In plant Si accumulators the concentration of Si in plant exceeds 1.0%, and most of the species in *Gramineae and Cyperaceae* families are considered Si-accumulating plants (Yamaji and Ma, 2007). Guntzer et al. (2012) reported that Si concertation in rice, wheat, and maize shoot dry weights are 4.1%, 2.4% and 0.83% respectively. Silicon may increase plant tolerance to lodging, drought, salinity, metal toxicity stresses (Epstein, 1999; Kim et. al., 2014; Coskun et al., 2016) and micronutrient deficiencies (Hernandez-Apaolaza, 2014). It has been shown that Si supplementation facilitated leaf remobilization of iron in iron deficient cucumber plants (Pavlovic et al., 2016). Silicon application has been shown to improve soil fertility (Ning et. al., 2016), promote plant health and growth (Ma and Takahashi, 1989; Arthanari et al., 2002), alleviate plant abiotic stress (Liang et al., 2007) and enhance crop resistance to fungal and bacterial pathogens by inducing plant defense mechanisms (Datnoff and Rodrigues 2005; French-Monar et al., 2010). For example, Si as a soil amendment can reduce the incidence of Phytophthora root rot in bell peppers (*Capsicum annuum*) and enhance plant dry matter (French Monar et al., 2010).

Silicon fertilization and straw incorporation increased disease resistance of pot-grown winter wheat plants to a number of diseases (Rodgers-Gray and Shaw, 2004). Resistance mechanisms were induced in tomatoes (*Solanum lycopersicum* L.) by Si application, leading to reductions of *Ralstonia solanacearum* (Smith) populations within xylem vessels and reduction in bacterial wilt disease severity (Diogo and Wydra, 2007). Transmission electron microscopy studies revealed that silicified rice epidermal cell walls inhibited fungal penetration and reduced rice blast (*Magnaporthe oryzae* B.C. Couch) severity (Kim et al., 2002). Foliar applications of Si led to reduced powdery mildew (*Podosphaera xanthii,* Castagne) disease development in melons

(*Cucumis melo* L.) through the cell wall fortification and promoting of the antioxidant defense system (Dallagnol et al., 2012). It was reported that foliar application of Si resulted in decreased bacterial speck symptoms by directly affecting on *Pseudomonas syringae* pv. *tomato* (Okabe) without any effects on host defense enzymes (Anderade et al., 2013).

Several studies have shown that Si fertilization may have beneficial effects on wheat plant growth. Mali and Aery (2008) in a potted study showed a significant increase in K and Ca absorption in plants treated with increasing doses of sodium metasilicate (50–800 mg Si kg^{-1}) in a potted study. In wheat plants treating with various Si compounds such as pyrolitic fine silica particles [aerosil®], sodium silicate or silica gel, Si mainly deposited in the leaves, and the intensity of silica bodies was higher on the abaxial side versus the adaxial side (Mecfel et al., 2007). Silicon increased antioxidant defense systems and alleviated oxidative damage in drought stressed wheat plants (Gong et al., 2005).

Foliar and soil Si fertilizers are commercially available, while the most common Si sources are slag materials. Calcium silicate slags are by-products of the metallurgical smelting process, contain varying percentages of Si (Ma and Takahashi, 2002), and have positive effects on correcting soil acidity (Nolla et al., 2013), plant growth and alleviation of stresses (Raid et al., 1992; Alvarez et al., 1988; Ning et al., 2014). Another commonly used Si fertilizer is wollastonite, which is a natural calcium silicate (Maxim et al., 2008). Wollastonite contains higher fractions of easily soluble Si compared to slags (Tubana et al., 2016). Torlon et al. (2016) observed that wollastonite was effective in reducing powdery mildew severity in pumpkin (*Cucurbita pepo* L.). Nanayakkara et al. (2008) reported that application of silicon fertilizers such as calcium silicate slag and wollastonite reduced gray leaf spot (*Magnaporthe oryzae* B.C.

Couch) incidences and severity in perennial ryegrass (*Lolium perenne* L.) suggesting that the material may be considered in integrated management programs.

Nitrogen is an essential element for plant growth that is assimilated into organic compounds following uptake (Rentsch et al., 2007). Two forms of inorganic N sources in the soil that are taken up by roots are ammonium (NH_4^+) and nitrate (NO_3^{-2}) (Li et al., 2015). Plants are capable of absorbing organic N through organic N transporters (Rentsch et al., 2007). It was observed that N fertilization increased leaf size, light interception, and grain yield in maize (Tajul et al., 2013). Nitrogen fertilization improved plant chlorophyll content and the activity of Phenyl-Alanine-Lyase enzyme in three varieties of Kacip Fatimah (*Labisia pumila* Blume), a traditional herbal medicine grown in Malaysia (Ibrahim et al., 2011). It was reported that N fertilization improves plant growth and grain yield in spring wheat (Pearman et al., 1977). Although N plays a key role in plant health and productivity (Long et al., 2000), a number of studies show contrasting effects of plant tissue N concentration on disease development (Dordas, 2008; Veresoglou et al., 2013). This inconsistency could be attributed to plant cultivars, pathogen types or timing of N application (Long et al., 2000; Dordas, 2008; Hoffland et al., 2000).

Plant nutrition management has considerable contributions to sustainable agriculture, which encompasses many aspects such as increasing food production and economic benefits while reducing adverse environmental impacts (Goulding et al., 2008; White and Brown, 2010). Goals of sustainable agriculture include promoting environmental stewardship by protecting and improving soil quality, reducing dependence on non-renewable resources, such as synthetic fertilizers and pesticides, and minimizing adverse impacts on environmental resources (Dordas, 2008). This study addresses many of these goals and approaches the challenges of managing leaf

rust at the systems level. Silicon has the potential to improve soil fertility, reduce disease severity

by enhancing wheat plant defense mechanisms and improve yield stability. Alvarez and Datnoff

(2001) provided convincing evidence that the benefits of using Si as part of an integrated disease

management (IDM) program can outweigh the costs of Si. Since then, several reports revealed

the positive effects of N and Si fertilizations on plant growth, yield and alleviation of biotic

stress, yet we know very little of the combined effects of these two nutrient elements on leaf rust.

This study aimed to: 1) evaluate the efficacy of individual and combined Si and N applications

on agronomic parameters such as Si uptake, yield and silica body distribution in the epidermal

cells, and 2) evaluate the effects of individual and combined Si and N applications on disease

incidence and severity.

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CHAPTER 2. EFFECTS OF DIFFERENT SILICON SOURCES ON AGRONOMIC PARAMETERS AND SILICON UPTAKE OF WHEAT GROWN UNDER DIFFERENT NITROGEN RATES

2.1 Introduction

Wheat (*Triticum aestivum* L.), covering 217 M ha, was the third main crop cultivated in the world in 2012, ranked after maize (*Zea mays* L.) and rice (*Oryza sativa* L.) (Jones, 2015). One of the largest global agricultural challenges will be increasing food production to support a growing population that will reach 9 billion by the year 2050 (Fischer et al., 2014). In this context, the global population growth and the food demand require an increase in wheat production from 600 million to approximately 760 million tons by 2020 (CIMMYT, 2004). According to Cakmak (2000), at least 60% of soils under farming systems are challenged with nutrient deficiencies or toxicities. Therefore, balanced nutrient management can be a sustainable approach in the context of secure food production (Goulding et al. 2008). Plant pathogens individually, or combined with other biotic and abiotic factors, can cause severe yield losses worldwide (Teng and Shane, 1984). In addition to being a major constraint to food security (Strange and Scott, 2005), plant diseases are also major threats to global food safety (Wild and Gong, 2010). Several soil environmental factors such as nutrients, moisture, and pH can affect plant disease development (Dordas, 2008).

Pathogen infection leads to a decline in photosynthesis and accelerates assimilatory metabolism which then increases respiration and provides the required secondary metabolites for defense (Berger et al., 2007). Plant pathogens obtain soluble assimilates from their hosts and can cause plants to become nutrient deficient and susceptible to disease (Hancock and Huisman, 1981). Wheat rust diseases are considered among the global constraints of wheat production and are a matter of concern in the realm of food security (Chaves et al., 2013). Oerke et al. (1994)

suggested that annual wheat yield losses caused by diseases worldwide were about 12.4%. The three wheat rusts, leaf, stem, and stripe, with the agents of *Puccinia triticina* (Eriksson). *Puccinia graminis* (Pers) and *Puccinia stiiformis* (Westend) respectively are the most devastating diseases threatening wheat yield at the global level (Singh et al., 2016). More than one hundred biotic and abiotic diseases affect wheat yields in the United States with the three wheat rusts (leaf, stem, and stripe) and Fusarium head blight (causing the largest losses (Savary et al., 2012). Unlike stem rust or Fusarium head blight, the pathogenic mechanisms of the leaf rust pathogen is associated with reductions in a net photosynthesis rate, which leads to decreased numbers of kernels per head, lower kernel weights and lower grain quality (Bolton et al., 2008; Carretero et al., 2011).

Silicon (Si) makes up 28% of the weight of the Earth's crust, and after oxygen it is the second most abundant element found in the Earth's hard outer layer (Epstein et al., 1994). Silicon content in the soil ranges from 1 to 45 % dry weight (Sommer et al., 2006). Silicon is released into soil through the weathering of silicate minerals (Cornelis et al., 2001), the biogeochemical cycle of Si and through recycling by vegetation (Sommer et al., 2006). In warm sub-humid and humid tropical areas, weathering may lead to low Si concentration in the soil (Datnoff and Rodrigues, 2005).

Plants can absorb Si in the form of silicic acid [$Si(OH)_4$], which is present in the soil as an uncharged monomeric molecule below pH 9 (Ma and Yamaji, 2015) with the concentration in soil varying between 0.1 to 0.6 mM (Epstein et al., 1994). Silicon deficiency can occur in strongly weathered or acidic soils (Nanayakkara and Uddin, 2008). Approximately 0.1 – 10 % of shoot dry weight in plants can consist of Si, and plant families: *Poaceae, Equisetaceae* and *Cyperaceae* show high Si accumulation (>4 % Si) (Currie and Perry, 2007). According to

Matichenkov and Calvert (2002), around 210 to 224 million tons of Si are adsorbed by plants and removed from cultivated areas annually. Among the Si accumulators in *Gramineae* family, sugarcane (*Saccharum officinarum* L.) showed the highest rate of Si absorption (300-700 kg of Si ha^{-1}), followed by rice (150-300 kg of Si ha^{-1}), and wheat (50-150 kg of Si ha^{-1}) (Barker and Pilbeam, 2007).

Silicon is taken up by the root system through a NIP group of the aquaporin family transporters called low Si 1 (*Lsi1*), which are involved in the Si influx, while the efflux transport of silicon is carried out by putative anion Si transporters called low Si 2 (*Lsi2*)(Ma et al.,2011). The *Lsi1* is involved in passive transport of Si, however *Lsi2* is considered as an active transporter of Si (Ma et al., 2007). Homologous proteins to the *Lsi1* channel proteins have been identified and characterized in other plants such as barley (*Hordeum vulgar* L.; HvLsi1) (Chiba et al., 2009), wheat (TaLsi1) (Montpetit et al., 2012), maize (ZmLsi1, ZmLsi6) (Mitani et al., 2009), and soybean (*Glycine max* L.; GmLsi1) (Deshmukh et al., 2013). The *Lsi2* channel proteins homologous in barley (HvLsi2), maize (ZmLsi2) (Mitani et al., 2009) and pumpkin (*Cucurbita pepo* L.; CmLsi2) (Mitani-Ueno et al., 2011) have been identified. The various capabilities of plant species in Si uptake can be associated with Si transporters that differ in their expression levels in the cells and localization in plant tissues (Chiba et al., 2009).

The presence of Si in cell walls and firm linkages with the cell wall matrix were revealed by an inductively coupled plasma mass spectrometry (ICP-MS) and X-ray photoelectron spectroscopy (XPS), suggesting the crucial role of this nutrient element in maintaining cell integrity (He et al., 2013). Scanning electron microscopy showed that Si was deposited beneath the cuticle of rice epidermal cells (Ma et al., 2011). If the concentration of silicic acid exceeds 2 mM, polymerization occurs, leading to precipitation of amorphous silica particles called

phytoliths (Ma and Yamaji, 2006). Scanning electron microscopy and energy-dispersive X-ray spectroscopy analyses of dry ash samples showed the presence of phytoliths in the upper and lower epidermis of blueberry leaves (*Vaccinum corymbosus* L. cv. Bluecrop) (Morikawa and Saigusa, 2003). Electron microscopy and *in situ* X-ray microanalysis showed that following treatment Si was deposited in epidermal cell walls, middle lamellae, and intercellular spaces within sub-epidermal tissues rice leaves (Kim et al., 2002).

Slag-based fertilizers are an inorganic source of Si (Haynes, 2014). Calcium silicate slag ($CaSiO_3$), a byproduct of steel production (Devi and Gnanavel, 2014), provides plants with a high level of bioavailable Si and may be applied as a liming material to correct soil pH (Ma and Takahashi, 2002; Torlon et al., 2016). Wollastonite, which is a naturally occurring $CaSiO_3$ mineral with lower trace elements and higher fractions of easily soluble Si compared to slags (Tubana et al., 2016), can also be used as another source of Si fertilizer (Pereira et al., 2004). Many studies have demonstrated the beneficial effects of Si application on yield, crop productivity and plant tolerance to adverse environmental conditions. The Si deficient plant showed growth abnormalities and susceptibility to stresses (Epstein, 1999). Application of silicate slag in a rice-sugarcane rotation led to higher rice yields and increased sugar content in cane (Alvarez et al., 1988). Detmann et al. (2012) demonstrated that Si fertilization led to increased sink strength, grain yield and nitrogen (N) use efficiency (NUE) in rice even under unstressed conditions. Maize total chlorophyll contents, photosynthetic rate and ultimately yields increased following Si application, (Xie et al., 2014). Prakash et al. (2011) reported that foliar application of Si increased the percentage of Si and uptake in straw and grain, which led to improved rice yields.

Nitrogen is a major component of nucleic acids and proteins (Marschner, 1995). The element is involved in chlorophyll structure and plays an important role in plant photosynthetic capacity, as approximately 15% to 30% of total leaf N is partitioned in Rubisco, which is the most abundant leaf protein (Makino, 2003; Suzuki et al., 2009). The annual rate of inorganic N fertilizers applied to the soil worldwide is 85–90 million metric tons (Good et al., 2004), however, 50–70% is lost through the leaching of nitrate, gas emission of nitrous oxide (N_2O) and ammonia volatilization (Roshanravan et al., 2014; Yatim et al., 2015; Duran et al., 2016). Therefore, improving NUE is a worldwide concern, because the leaching of nitrate contributes to contamination of aquatic ecosystems and eutrophication (Duran et al., 2016; Piccini et al., 2016).

Nitrogen can be taken up actively from the soil in the form of nitrate, ammonium and amino acids through selective transporters in the root cells (Masdaux-Daubresse et al., 2010). In grain sorghum (*Sorghum bicolor*) and soybean (*Glycine max*), yield and N uptake increased significantly following N fertilization (Baker and Blamey, 1985). Abedi et al. (2011) reported that proper N rate and application timing increased protein content and yield in wheat. In maize N fertilization led to increased leaf size, light interception, and higher grain yield (Tajul et al., 2013). Higher chlorophyll content and increased activity of the Phenyl-Alanine-Lyase enzyme in three varieties of Kacip Fatimah (*Labisia pumila* Blume) plants were reported following N fertilization (Ibrahim et al., 2011). Nitrogen fertilization was shown to improve plant growth and grain yield in spring wheat (Pearman et al., 1977). Although N promotes plant health and productivity (Long et al., 2000), there are contradictory reports about the effect of N fertilization on disease development (Dordas, 2008; Veresoglou et al., 2013). Plant cultivars, pathogen types and N application timing are key factors in diseased plant responses to N fertilization (Long et al., 2000; Dordas, 2008; Hoffland et al., 2000).

A number of projects have focused on agronomical parameters of soil-applied Si under biotic stresses (Liang et al., 1994; Nanayakkara and Uddin, 2008; Ning et al., 2014). However, very little information has been provided to date related to foliar applications of Si on plant growth and yield. Thus, this study aimed to evaluate the effects of different Si sources (soil- and foliar-applied) on grain yield and Si uptake in wheat supplied with different N rates. Thus, this study aimed to evaluate the effects of different Si sources (soil- and foliar-applied) on grain yield and Si uptake in wheat supplied with different N rates.

2.2. Materials and Methods

2.2.1. Site Description, Treatment Structure, and Trial Establishment

This study was conducted from 2014-2016 with a total of four site-years in Louisiana. The first and second site-year were established in 2014 at the Macon Ridge Research Station near Winnsboro (32.1632° N, 91.7207° W), on a Gigger- Gilbert silt loam soil (Fine-silty, mixed, superactive, nonacid, thermic Fluvaquentic Endoaquepts) and at Ben Hur Research Station (Latitude 30°, 21', 40.4" N; Longitude 91°, 10', 01.9" W) near Louisiana State University campus in Baton Rouge on a Cancienne silt loam soil (Fine-silty, mixed, superactive, nonacid, hyperthermic Fluvaquentic Epiaquepts). The third and fourth site-years were established in 2015 at the Macon Ridge Research Station and at Ben Hur Research Station as well.

The treatment structure was a two-way factorial (two N rates x seven Si sources) in a randomized complete block design with four replications. The seeds were sown at Ben Hur and Winnsboro the rate of 106 and 84 kg ha^{-1}, respectively from mid to last week of November in 2014 and 2015. Winter wheat varieties, Terral 8525 (2014-2015) and Progeny 870 (2015-2016), were used in this study; both varieties are susceptible to leaf rust. Two N rates of 101 and 145 kg

N ha^{-1} were used for this study using urea (45% N) as the source. The 101 kg N ha^{-1} rate is the standard rate for wheat production in LA and 145 kg N ha^{-1} is considered as a high rate. For both N rates, there were seven Si treatments applied either as soil amendments or foliar application: wollastonite (280 kg Si ha^{-1}) and slag (280 kg Si ha^{-1}) as soil amendments or 1000, 2000 and 4000 ml Si L^{-1} respectively. Two control plots were included (without Si treatments and foliar treatment of carrier only). The plot size at Ben Hur was 14.4 m^2 and at Winnsboro was 6 m^2.

Phosphorus (triple superphosphate, 46% P) and potassium (KCl, 60% K) fertilizers were applied to fields as 67 kg P ha^{-1} and 112 kg K ha^{-1} respectively, according to the test results and recommendations of the LSU AgCenter Soil Testing Plant Analysis Laboratory. The solid forms of Si fertilizer, CaSiO$_3$ slag with the commercial name of Plant Tuff® (12% Si) and wollastonite (23% Si) were used for this study. Slag and wollastonite fertilizers were pre-weighed, and put into plastic bags, then spread by hand and incorporated into the soil in mid-November 2014 and 2015. Solution Si was diluted with water to attain a 701 L ha^{-1} foliar application rate. The first foliar application started at Feekes (F) growth stage 5 (Large, 1954) and was repeated three times at one week intervals. The two N treatments were top-dressed when plants reached (F) 5.

2.2.2 Soil Sampling

Soil samples were taken at F9 (late March) and F11 (late May). Each sample consisted of twelve soil cores (depth 0-15 cm): six cores taken from two inner rows adjacent to where biomass samples were taken. Soil samples were oven-dried (Despatch LBB series; model number LBB2-18-1) at 55°C for about a week. The Humboldt soil grinder was used to grind the soil to obtain finely ground, homogeneous soil samples. The soil samples were passed through a 2 mm sieve for later analysis.

2.2.3. Laboratory Soil Analysis

2.2.3.1. Silicon Extraction Procedure

Silicon content was determined by a 0.5 M acetic-acid extraction procedure followed by Molybdenum Blue Colorimetry (MBC) (Korndorfer et al., 2001). One g soil samples were weighed into polyethylene centrifuge tubes where ten (10) mL of 0.5 M acetic acid was added. All the tubes were placed on a reciprocal shaker (Eberbach; model number E6010.00) for 1 hour then filtered with Whatman No. 1 filter paper, and dispensed as 0.5 mL aliquots into 50 mL centrifuge tubes (Korndorfer et al., 1999). Ten (10) ml of deionized (DI) water, 0.5 mL of 1:1 HCl, and 1 mL of 10% ammonium molybdate (pH 7.5) were successively added to the samples. After 5 minutes, 1 mL of 20% tartaric acid was added and tubes were gently shaken by hand for 10 seconds then left to sit for 2 minutes. Afterwards, 1 ml of ANSA (0.5 mg 1-amino-2-naphthol-4-sulphonic acid + 1.0 g sodium sulfite + 30.0 g sodium bisulfite in DI water with a final volume of 250 mL) was added and the final solution was diluted to 25 mL with DI water. Absorbance was measured after 5 minutes at 630 nm using UV visible spectrophotometer (Hach DR 5000, Loveland, CO). A series of standard solutions were prepared with the same background (0.5 M acetic acid) at rates of 0, 0.2, 0.4, 0.8, 1.2, 1.6 and 2.0 ug mL^{-1} Si along with blanks and reference samples (Sharkey clay and Commerce silt loam).

2.2.3.2. Extractable Nutrients by Mehlich-3 Procedure (Mehlich, 1984)

Two g of soil was placed into 125 mL plastic bottles where 20 mL of Mehlich-3 solution (dilute acid-fluoride-EDTA solution corrected to pH 2.5) was added. Samples were shaken for 5 minutes and filtered using Whatman® No. 42 filter paper. Extracts were transferred to 10 mL plastic tubes and analyzed by Inductively Couple Plasma (ICP) atomic spectrometry (Spectro

Arcos AMETEK, Germany). Two blank reagents and two repetitions of each reference were included.

2.2.3.3. Soil pH (1:2 method)

Soil pH was measured using 1:2 soil to DI water. Five grams of soil was placed into 50 mL centrifuge tubes and 10 mL DI water was added. Tubes were placed on a reciprocal shaker for 1 hour, and pH was measured using an Oakton pH 5+ digital pH meter (Coleparmer Co. Vernon Hills, IL.

2.2.4. Biomass Sampling

Plant biomass was collected in late March (midseason) and late May (harvest) from 60 centimeter (cm) sections near the center of plots. The samples were placed in paper bags, dried in the oven (Despatch LBB series; model number 43 LBB2-18-1, Minneapolis, MN) at 65°C for five days, weighed, ground, and analyzed for Si and elemental composition.

2.2.5. Laboratory Plant Analysis

2.2.5.1. Silicon Analysis

Silicon content in plant tissue samples was determined by an Oven-Induced Digestion procedure (OID) (Kraska and Breitenbeck, 2010) followed by the MBC procedure (Hallmark et al., 1982). One hundred mg of plant tissue was weighed into 50 mL centrifuge tubes then dried at 60°C for 15 minutes in an oven (Yamato; DKN600, Santa Clara, CA). Five drops of octyl alcohol (approximately 250 microliter) and 2 mL of hydrogen peroxide (H_2O_2) were added to the tubes then placed in the oven at 95°C for 30 minutes. Samples were removed and 4 mL of 50% sodium hydroxide (NaOH) was added. During this 4 hour period, samples were gently mixed using a vortex mixer every 15 minutes. After 4 hours, 1 mL of ammonium fluoride (NH_4F) was

added to the digested samples, mixed, and diluted to 50 mL with DI water. Soybean and sugarcane Si reference samples and blanks also were digested.

The MBC procedure began by taking 2 mL aliquots from digested samples and pipetting into 30-mL centrifuge tube. Ten mL of 20% acetic acid and 4 mL of 0.26 M ammonium molybdate was added and samples were left alone for 5 minutes. Afterwards, 2 mL of 20% tartaric acid and 2 mL of the reducing agent (0.5 mg 1-amino-2-naphthol-4-sulphonic acid + 1.0 g sodium sulfite + 30.0 g in DI water with a final volume of 250 mL) were added. The final volume was adjusted to 30 mL using 20% acetic acid. Capped tubes were shaken for 30 minutes, and absorbance was measured using a Hach DR 5000 spectrophotometer at 630 nm. A series of standards were made at rates of 0, 0.4, 0.8, 1.6, 3.2, 4.8 and 6.4 ug mL^{-1} of Si. Silicon content (g kg^{-1}) of plants was determined using the following formula:

$$\text{Si content} = \frac{\left\lfloor (\text{Abs}_{\text{samp}} - \text{Abs}_{\text{blk}}) - \text{Cfi} \right\rfloor}{\text{Cfs}} * \left\lfloor \left(\frac{V_d}{S_{wt}} * \frac{V_c}{V_a} \right) \right\rfloor$$

Where:

Abs_{samp} = absorbance reading of sample

Abs_{blk} = absorbance reading of reagent blank

$\text{Cfi} = \mu g$ Si g^{-1} when absorbance is zero (intercept of the standard series curve)

$\text{Cfs} = \mu g$ Si g^{-1} per unit of absorbance (slope of the standard series curve)

V_d = final digest volume (mL)

S_{wt} = oven-dry equivalent weight of digested sample (g)

V_c = final colorimetric volume (mL)

V_a = volume of aliquot used for colorimetric analysis (mL)

<u>2.2.5.2. Silicon Uptake</u>

Silicon content of biomass, straw, and grain Si content was determined using the following formula:

$$\text{Plant Si Uptake} = (\%\text{Si in Straw}/100) \times \text{Straw Yield (kg/ha)}$$

$$\text{Grain Si Uptake} = (\%\text{Si in Grain}/100) \times \text{Grain Yield (kg/ha)}$$

<u>2.2.5.3. Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) Analysis</u>

Scanning Electron Microscopy (SEM) uses a high voltage electron beam to produce a variety of signals which translate information about fully hydrated specimen morphology, crystalline structure, and chemical composition (Echlin, 1971). The relative %Si from EDAX analysis indicates the frequency of Si bodies distributed within the sampling area relative to K, C, H and other nutrients. In the middle of the season (late March) at F 9, two replications of each treatment were randomly selected for sampling, where eight leaves were arbitrarily selected and three different random spots were analyzed per leaf. Leaves were thoroughly washed with DI water to eliminate remnants of Si foliar solution. Magnification was set at 400x at a voltage of 20 kV.

2.2.6. Tillers, Spikes, and Grain Yield

All tillers and heads were counted for each sampling location within plots, and then spikes were passed through a combine thresher (Almaco; SBT, Nevada, IA) where grain weight was determined. In 2016, plots were hand harvested at Ben Hur and combine harvested with a Massey Ferguson 8XP at Winnsboro. Grain subsamples collected from each site during harvesting were weighed and then analyzed for moisture content. Grain moisture content was adjusted to 12%, and yield was calculated as bushels per acre using the formula below:

$$\text{Grain Yield } \left(\frac{\text{bu}}{\text{ac}}\right) = \left[\frac{\text{Grain weight} \left(\frac{\text{lb}}{\text{plots}}\right)}{\text{Plot size (ft}^2)} \times \frac{43{,}560 \left(\frac{\text{ft}^2}{\text{ac}}\right)}{\text{ac}}\right] \times \left[\frac{\dfrac{[100 * (\text{ moisture content})]}{88}}{\text{test weight}}\right]$$

Grain yield was then converted to kg ha^{-1} using the formula:

$$\text{Grain Yield } \left(\frac{\text{kg}}{\text{ha}}\right) = \left[\frac{\text{Yield(bu)}}{\text{(ac)}}\right] \times \frac{60 \text{ (lb)}}{\text{(bu)}} \times \frac{1\text{kg}}{2.2\text{lb}} \times \frac{2.47\text{ac}}{\text{ha}}$$

2.2.7. Statistical Analysis

The ANOVA procedure on all measured variables was performed using SAS 9.4 (SAS Institute, 2012). PROC MIXED in SAS 9.4 was used to determine the significant effects of N, Si, and N x Si interactions on measured parameters. In the statistical model, N and Si treatments were fixed effects, while replications, site, and their interaction were considered random effects. Treatment means were compared using the least significant difference (LSD) test for any significant effect detected at $P<0.05$.

2.3. Results and Discussion

2.3.1. Effects of Different Sources of Si and Two Rates of Nitrogen Fertilizer on Yield Parameters and Si Uptake

The effects of Si amendments on soil Si content in 2015 and 2016 are reported in Table 2.1 and Table 2.2, respectively. Mean separation procedure (LSD, $P<0.05$) was performed in SAS to determine significant differences within Si amendments. The data show that wollastonite and slag significantly increased soil Si content at midseason and harvest in 2015 (Table 2.1) and 2016 (Table. 2.2) compared to the control which is consistent with our expectations. No significant changes were observed in soil Si content or soil pH across the Si foliar treatments in 2015 and 2016. Torlon et al. (2016) also showed that wollastonite and slag could significantly increase available soil Si. Furthermore, slag and wollastonite- treated soils showed significant

higher soil pH by 0.3 and 0.4 units, respectively compared to controls in 2015. Ning et al. (2016) reported that slag amendments could be an effective agricultural practice to correct soil acidity. This might be due to the property of slag which is applied as a liming material to neutralize soil acidity.

Table 2 1.Effect of Si amendments on soil 0.5 m acetic acid extractable-Si and pH in 2015.

Silicon Sources	Extractable-Si (mg kg^{-1})		Soil pH
	Midseason	Harvest	
Control	43 c	43 c	6.0 b
Carrier	38 c	49 c	6.0 b
Foliar 1000 ml Si L^{-1}	43 c	45 c	6.0 b
Foliar 2000 ml Si L^{-1}	37 c	39 c	6.0 b
Foliar 4000 ml Si L^{-1}	43 c	47 c	6.0 b
Slag, 280 kg Si ha^{-1}	141 b	144 b	6.2 a
Wollastonite, 280 kg Si ha^{-1}	251 a	260 a	6.3 a
P value	*P<0.05*	*P<0.05*	*P<0.05*

Means followed by the same letter within a column are not significantly different at 0.05 level of confidence.

No significant changes were observed in soil pH across the Si foliar treatments. However, slag and wollastonite applications resulted in significantly higher soil pH by 0.4 and 0.5 units, respectively in 2016 (Table 2.2).

Table 2 2. Effect of Si amendments on soil 0.5 m acetic acid extractable-Si and pH in 2016.

Silicon Sources	Extractable-Si (mg kg^{-1})		Soil pH
	Midseason	Harvest	
Control	43 c	43 c	6.0 b
Carrier	38 c	49 c	6.0 b
Foliar 1000 ml Si L^{-1}	43 c	45 c	6.0 b
Foliar 2000 ml Si L^{-1}	37 c	39 c	6.0 b
Foliar 4000 ml Si L^{-1}	43 c	47 c	6.0 b
Slag, 280 kg Si ha^{-1}	141 b	144 b	6.4 a
Wollastonite, 280 kg Si ha^{-1}	251 a	260 a	6.5 a
P value	*P<0.05*	*P<0.05*	*P<0.05*

Means followed by the same letter within a column are not significantly different at 0.05 level of confidence.

The main effects of N, Si, and N x Si interaction effects on tiller numbers, spike numbers, biomass, straw and grain yield in 2015 are summarized in Table 2.3. Tiller numbers, spike numbers, biomass, straw and grain yield were not significantly affected either by Si treatment or the interaction of N and Si fertilization. Plants receiving higher rate of N showing higher straw yield compared to lower rate of N.

Table 2 3. Analysis of variance for tiller and spike numbers, biomass, straw and grain yield of wheat under different Si sources and two nitrogen rates in 2015.

Si Sources	Tiller No.	Spike No.	Yield (kg ha^{-1})		
			Biomass	Straw	Grain
Control	535	507	6531	8291	2224
Carrier	568	549	6761	8434	2262
Foliar 1000 ml Si L^{-1}	566	551	6342	7717	2328
Foliar 2000 ml Si L^{-1}	494	474	6066	8608	2331
Foliar 4000 ml Si L^{-1}	540	528	6552	8448	2438
Slag, 280 kg Si ha^{-1}	562	537	6748	8363	2299
Wollastonite, 280 kg Si ha^{-1}	526	505	6398	8382	2333
Analysis of variance					
N Effect	NS	NS	NS	<0.05	NS
Si Effect	NS	NS	NS	NS	NS
N*Si Effect	NS	NS	NS	NS	NS

NS = non-significant. Means with the same letter within a column are not significantly different at 0.05 level of confidence.

The positive effects of N fertilization on crops growth and yield have been extensively studied. Consistent with this result, Babiker et al. (1995) found that N fertilization led to higher yield in faba bean (*Vicia faba* L.). Pearman et al. (1977) reported that N fertilization increased vegetative growth, leaf area and grain yield in spring wheat. Montemurro (2017) showed that N fertilization increased N uptake and grain yield in winter wheat.

In 2016, application of foliar Si at 1000 and 4000 ml Si L^{-1} significantly increased spike numbers by approximately 19% compared to the control (Table 2.4). Similarly, Prakash et al. (2011) reported that application of foliar Si at 4 ml Si L^{-1} increased numbers of tiller and panicle

length in rice. The positive effect of Si supplements on spike and spikelet numbers was shown in other studies (Ma et al., 1989; Meena et al., 2014). However, we observed no significant effect of other sources of Si treatments on spike numbers.

We observed a significant increase in biomass yield in Si treated plants. Wollastonite, slag and foliar application at 4000 ml Si L^{-1} increased biomass yield by 47, 41 and 28%, respectively (Table 2.4). However, there are no significant effects of Si treatments on either tiller numbers or grain yield in 2016 (Table 2.4). There have been numerous studies investigating the effects of Si supplementation on growth parameters and grain yield. In a similar study, Liang et al. (1994) reported that Si fertilization had little impact on the shoot dry matter in rice and wheat. Segalin et al. (2013) showed that foliar application of Si did not affect the yield and physiological quality of the seeds produced by different wheat cultivars. Mauad et al. (2003) reported that either Si fertilization alone or combined with N fertilizer did not result in yield improvement in rice.

Table 2 4. Effect of Si treatments on tiller number, spike number, biomass and grain yield in wheat grown under two N rates in 2016.

Si Sources	Tiller No.		Spike No.		Yield (kg ha^{-1})			
					Biomass		Straw	Grain
Control	196	b	186	b	2244	e	10540	1828
Carrier	227	ab	215	ab	2289	de	9029	1993
Foliar 1000 ml Si L^{-1}	236	a	229	a	2415	de	8988	2098
Foliar 2000 ml Si L^{-1}	215	ab	200	ab	2626	e	9868	2047
Foliar 4000 ml Si L^{-1}	232	ab	231	a	3120	c	8909	2211
Slag, 280 kg Si ha^{-1}	200	ab	183	b	3794	b	10224	2277
Wollastonite, 280 kg Si ha^{-1}	199	ab	192	b	4269	a	10298	2253
Analysis of variance								
N Effect	<0.05		<0.05		NS		NS	NS
Si Effect	<0.05		<0.05		<0.05		NS	NS
N*Si Effect	NS		NS		NS		NS	NS

NS = non-significant. Means with the same letter within a column are not significantly different at 0.05 level of confidence.

Wu et al. (2017) showed that the interaction between Si and N reduced accumulation of both N and Si in rice, decreased plant growth and made rice plants more susceptible to herbivores. However, several studies reported the positive effect of Si fertilization on crop yield. Pati et al. (2015) reported that Si fertilization increased rice straw and grain yields. Tamai and Ma (2008) reported that Si treatment significantly increased rice grain yield compared to Si-uptake defective mutants. In 2016, a study by Neu et al. found that biomass production and grain yield enhanced following Si supplementation in winter wheat. Plants treated with the higher N rate had significantly higher numbers of tillers and spikes than the lower N rate ($P<0.05$), (Table 2.5). However there were no significant effects of N fertilization on biomass, straw or grain yield. Nitrogen is a major component of most of the plant metabolic process and plays a key role in promoting cereals growth in particular at grain filling stage (Delogu et al. 1997). Consistent with our results, Ayoub et al. (1994) showed that wheat spike numbers increased with N level. Delogu et al. (1997) showed that N fertilization significantly increased N uptake and biomass production up to heading stage in winter wheat. Hussain et al. (2006) reported that N fertilization promoted increased number of tillers and spikes in wheat.

Table 2 5. Tiller and spike numbers, biomass, straw and grain yield of wheat under two nitrogen rates in 2016.

N rates (kg ha^{-1})	Tiller No.	Spike No.	Yield (kg ha^{-1})		
			Biomass	Straw	Grain
101	267 a	254 a	2996	2162	9873
145	163 b	157 b	2934	2040	9515
P value	<0.05	<0.05	NS	NS	NS

Means with the same letter within a column are not significantly different at 0.05 level of confidence.

Plant Si concentration at harvest remarkably increased in wheat receiving wollastonite at harvest and had a significant increase by approximately 25% in plant Si concentration compared

to control (Table 2.6). In midseason, only wollastonite- treated plants had a significant increase in plant Si concentration by approximately 34% compared to control. No significant changes were observed in plant Si concentration across foliar Si application compared to control in 2015. Grain Si concentration did not show any significant difference across all Si sources.

Table 2 6. The effect of Si treatments on biomass, straw and grain Si concentration in 2015.

Si Sources	Plant Si (g kg^{-1})		
	Biomass	Straw	Grain
Control	11.7 bc	18.6 bc	2.01
Carrier	11.2 bc	20.2 bc	2.59
Foliar 1000 ml Si L^{-1}	11.8 bc	19.6 bc	2.27
Foliar 2000 ml Si L^{-1}	10.4 c	19.0 bc	2.22
Foliar 4000 ml Si L^{-1}	10.8 c	20.5 b	2.20
Slag, 280 kg Si ha^{-1}	13.8 b	18.3 c	1.95
Wollastonite, 280 kg Si ha^{-1}	18.2 a	24.7 a	2.61
Analysis of variance			
N Effect	NS	NS	NS
Si Effect	<0.05	<0.05	NS
N*Si Effect	NS	NS	NS

NS = non-significant. Means with the same letter within a column are not significantly different at 0.05 level of confidence.

In 2016, plant Si concentration was significantly increased in wollastonite- treated plants by nearly 28% and 15% at midseason and harvest, respectively compared to control (P <0.05) (Table 2.7). Wollastonite and slag application increased grain Si concentration by around 20% compared to control. There was no significant interaction effect detected between N and Si on grain Si concentration (Table 2.7). Several studies have indicated that plant Si concentration increased followed by Si treatment. Torlon et al. (2016) reported that wollastonite- and slag- treated pumpkin plants showed higher level of Si concentration compared to control. Ning et al. (2014) showed that slag significantly increased Si concentration in rice tissue compared to control. Song et al. (2016) showed that in rice growing in a hydroponic system, both shoot and

root Si concentrations were significantly increased in Si treated plants than the control, however the difference in Si concentration between treated and control plants was not as remarkable compared to soil-cultured experiment.

Table 2 7. The effect of Si treatments on biomass, straw and grain Si concentration in 2016.

Si Sources	Plant Si (g kg^{-1})		
	Biomass	Straw	Grain
Control	10.5 b	20.5 bc	1.82
Carrier	9.8 c	21.9 bc	1.99
Foliar 1000 ml Si L^{-1}	10.6 bc	20.5 bc	2.09
Foliar 2000 ml Si L^{-1}	11.0 bc	18.9 bc	2.04
Foliar 4000 ml Si L^{-1}	10.7 bc	20.9 b	2.21
Slag, 280 kg Si ha^{-1}	11.8 b	23 c	2.27
Wollastonite, 280 kg Si ha^{-1}	14.6 a	24 a	2.25
Analysis of variance			
N Effect	NS	<0.05	NS
Si Effect	<0.05	<0.05	NS
N*Si Effect	NS	NS	NS

NS = non-significant. Means with the same letter within a column are not significantly different at 0.05 level of confidence.

Wollasotine- treated plants showed remarkable increase in Si uptake in midseason and harvest in 2015 by 34 and 23%, respectively compared to controls (Table 2.8). In 2016, wollastonite, slag and foliar 4000 ml Si L^{-1} significantly increased Si uptake by 65, 52 and 35% respectively compared to control in midseason. However, in 2016 harvest, the plant Si uptake across all Si treatments was significantly different. These results are consistent with several other studies that have been reported in this area. Prakash et al. (2011) reported that foliar application of Si increased the percent Si and its uptake in both straw as well as grain leading to improve yield in rice. Guével et al. (2007) reported that root applications of Si was more effective than the foliar application of Si in controlling the powdery mildew (*Magnaporthe grisea* (T.T.Hebert) M.E. Barr) on wheat plants and the concentration of 1.7 mM gave the best results in terms of

plant Si absorption. Torlon et al. (2016) demonstrated that among various Si sources, wollastonite treated pumpkin plants showed higher Si uptake and had more resistance against powdery mildew (*Podosphaera xanthii*) disease.

Table 2 8. The effect of Si treatments on biomass and straw Si uptake in 2015 and 2016.

Si Sources	Plant Si Uptake (kg ha^{-1})			
	Biomass	Straw	Biomass	Straw
Control	75 bc	172 b	22 de	217
Carrier	74 bc	188 b	24 e	201
Foliar 1000 ml Si L^{-1}	73 bc	165 b	26 de	223
Foliar 2000 ml Si L^{-1}	64 c	184 b	30 cd	241
Foliar 4000 ml Si L^{-1}	68 c	190 b	34 c	189
Slag, 280 kg Si ha^{-1}	93 ab	164 b	46 b	202
Wollastonite, 280 kg Si ha^{-1}	114 a	225 a	63 a	209
Analysis of variance				
N Effect	NS	<0.05	<0.05	NS
Si Effect	<0.05	<0.05	<0.05	NS
N*Si Effect	NS	NS	NS	NS

NS = non-significant. Means with the same letter within a column are not significantly different at 0.05 level of confidence.

Similarly, in a pot experiment, it was shown that Si application to soil in the form of silica (SiO_2) increased Si concentration in wheat shoot and root systems, however it did not have any significant effect on grain Si concentration (Neu et al., 2016). Mali and Aery (2008) showed that in a hydroponic system, addition of Si increased the Si concentration in both wheat shoots and roots.

Mehlich-3-extractable calcium (Ca), potassium (K), magnesium (Mg), sulfur (S), phosphorus (P) of soil samples are summarized in Tables 2.9. In 2015, the soil Si treatments did not result in a significant difference in the level of the mentioned extractable nutrients compared to control (Table 2.10).

Table 2 9. Effect of soil Si application on Mehlich-3 extractable nutrients in 2015.

Si Sources	Soil Extractable Maconutrients (mg kg^{-1})				
	Ca	Mg	K	S	P
Control	231	43	22	1.85	3.56
Carrier	259	47	23	1.99	4.02
Foliar 1000 ml Si L^{-1}	241	44	22	1.71	4.19
Foliar 2000 ml Si L^{-1}	226	41	21	1.63	3.93
Foliar 4000 ml Si L^{-1}	247	44	23	1.82	4.41
Slag, 280 kg Si ha^{-1}	216	39	20	1.65	3.82
Wollastonite, 280 kg Si ha^{-1}	246	45	22	1.64	3.83
Analysis of variance					
N Effect	NS	NS	NS	NS	NS
Si Effect	NS	NS	NS	NS	NS
N*Si Effect	NS	NS	NS	NS	NS

NS = non-significant. Means with the same letter within a column are not significantly different at 0.05 level of confidence.

In site-year 2016, wollastonite and slag significantly increase the amount of Ca in the soil ($P<0.05$) (Table. 2.10). Wollastonite and slag fertilizers contain Ca, thus the increase in soil Ca content was expected.

Table 2 10. Effect of soil Si application on Mehlich-3 extractable nutrients in 2016.

Si Sources	Soil Extractable Maconutrients (mg kg^{-1})				
	Ca	Mg	K	S	P
Control	203 d	33	20	1.4	3.3
Carrier	204 cd	33	20	1.3	3.2
Foliar 1000 ml Si L^{-1}	213 bc	32	21	1.2	3.7
Foliar 2000 ml Si L^{-1}	213 ab	32	21	1.3	3.8
Foliar 4000 ml Si L^{-1}	213 bc	33	22	1.3	3.4
Slag, 280 kg Si ha^{-1}	221 ab	34	21	1.4	3.2
Wollastonite, 280 kg Si ha^{-1}	222 a	32	21	1.2	3.1
Analysis of variance					
N Effect	<0.05	NS	<0.05	NS	NS
Si Effect	<0.05	NS	NS	NS	NS
N*Si Effect	NS	NS	NS	NS	NS

NS = non-significant. Means with the same letter within a column are not significantly different at 0.05 level of confidence.

It was reported that Si in soil solution increased P availability to plants (Meena et al., 2014). Pati et al. (2016) found that Si fertilization increased N, P, K availability in the soil. Nanayakkara et al. (2008) found that wollastonite fertilization increase soil Ca content.

2.3.2 Effects of Different Sources of Si on Distribution of Silica Bodies within Leaf Epidermis

The SEM images of silica body distribution at epidermal regions of plants treated with different Si sources are shown in Figure 2.1. Wollastonite, slag and foliar 4000 ml Si L^{-1} application significantly increased the percentage of Si body in the leaf epidermis compared to control (Figure 2.2). Although the highest relative %Si was associated with the wollastonite-fertilized plants, all Si treatments remarkably increased the %Si deposited in the leaves compared to control (Figure 2.2). Ning et al. (2014) also reported that application of slags resulted in higher cell biosilicification and more silica cells and larger papilla formation in treated rice leaves compared to control. Kanto et al. (2004) reported that silicate increased resistance against powdery mildew (*Sphaerotheca aphanis* Wallr.) in strawberry (*Fragaria vesca* L.) and through improving the physical hardness of leaves in strawberry grown in hydroponic system. Scanning electron microscopy and energy-dispersive X-ray spectroscopy analysis of dry ash samples showed the presence of phytoliths in both upper and lower epidermis in blueberry leaves (Morikawa and Saigusa, 2003). Consistent with our results, Agarie at al. (1996) found that the silica deposition and formation of silica bodies was significantly higher in rice leaves epidermis of Si-treated plants when compared to control. They reported the frequency of higher silica bodies in the leaves led to less light energy use efficiency and quantum yield in Si-treated leaves.

33
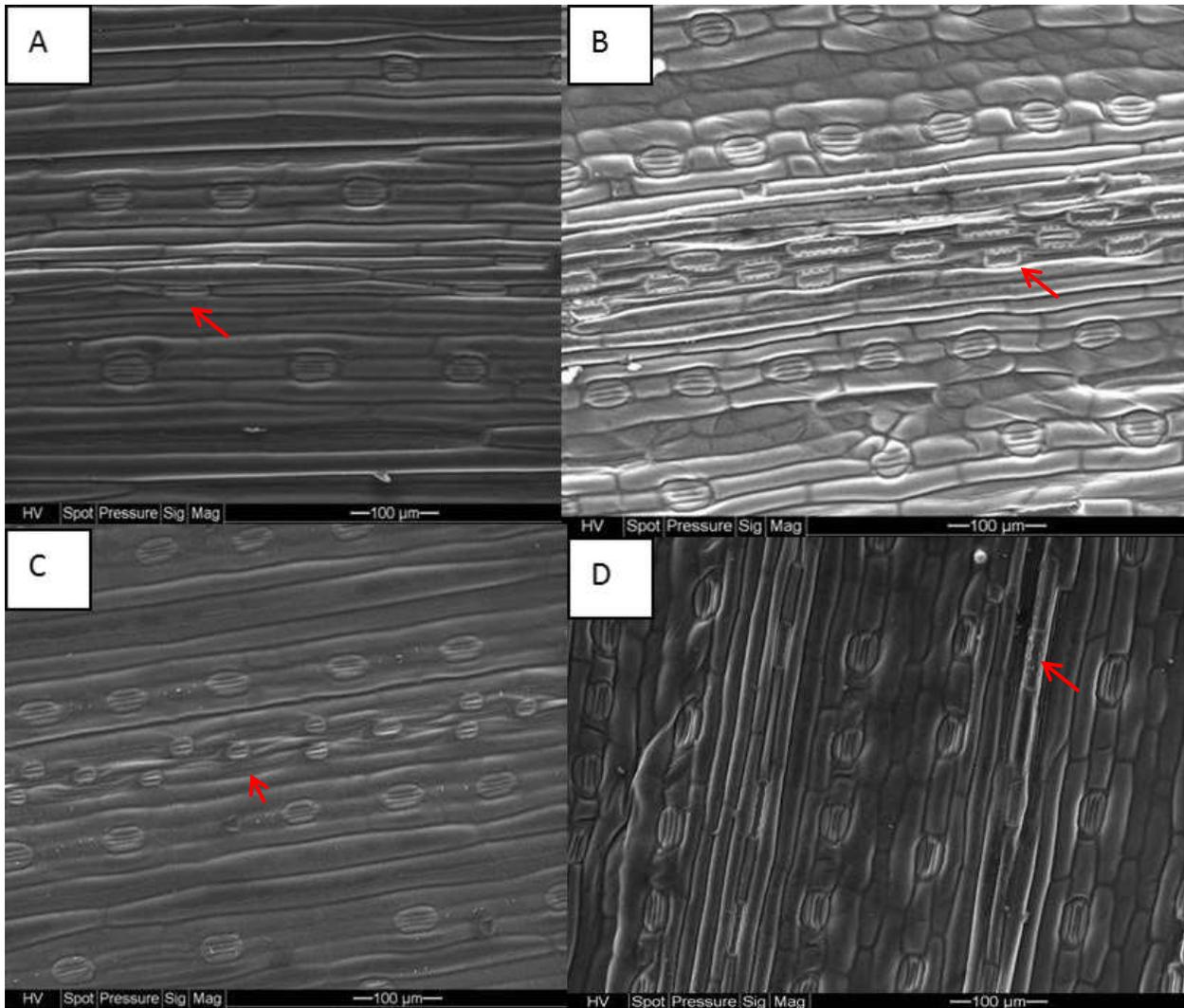
0. SEM and EDAX of leaf surface at 400x magnification. A-D. (A) control- 0.36% Si (B) wollastonite- 0.95% Si (C) slag- 0.78% Si (D) foliar application at 4000 ml Si L^{-1}- 0.50 %Si.

Ning et al., (2014) found that there were Si layers forming in Si-treated epidermal cell walls, and the Si layer appeared to be thicker following Si application in rice. Kim et al. (2002) showed that following Si treatment, Si was deposited in epidermal cell walls, middle lamellae, and intercellular spaces within sub-epidermal tissues rice leaves. Silica bodies in plant leaves may increase plant tolerance to biotic and abiotic stresses (Yamaji et al., 2008).
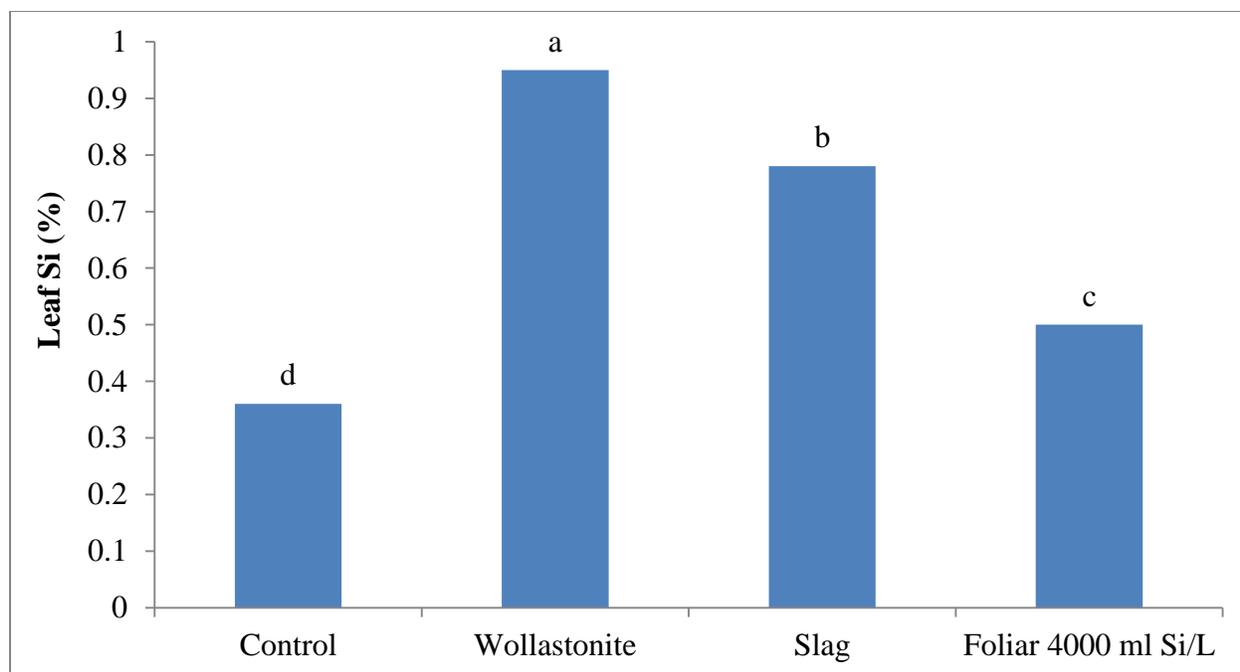
Figure 2 1. Effect of different sources of Si on relative %Si in leaf epidermis. Bars with the same letter are not significantly different according to LSD test at 0.05 level of confidence.

2.4. Conclusions

Our findings indicated that among several sources of Si fertilizers only wollastonite and slag application significantly increased Si concentration and uptake rate. In the context of Si amendments, wollastonite- treated wheat showed higher rates of plant Si concentration and uptake rates compared to slag-treated wheat. The Si concentration in foliar Si-treated plants did not show any significant difference compared to control. It was evident from our results that the Si treatments increased the distribution of silica bodies in the epidermal regions. The highest relative %Si in epidermis belonged to soil Si amendments and in particular wollastonite-treated plants. The impact of Si fertilization on grain yield was insignificant; nevertheless Si fertilization increased the biomass yield and spike numbers in 2016. Also, the results suggest that N and Si do not have any interactive effect on either wheat yield or Si uptake.

Our findings showed that soil Si application was consistently more effective than foliar treatments in plant Si uptake in wheat. This might be in accordance with the fact that Si transporters (*Lsi 1* and *Lsi2*) are mainly expressed in the root system.

The applications of $CaSiO_3$ slag and wollastonite increased the 0.5 M acetic acid extractable Si and soil pH; it could support the idea of using slag as a liming source to attain proper pH for plant growth.

The outcomes of this study suggested Si rates that were used in this study were not effective in increasing wheat yield. The efficacy of Si fertilization on crop's yield responses depends on several factors including soil Si initial level which is attributed to the soil texture and the extractants that are used to extract Si from the soil. There is lack of information on the critical soil Si levels for wheat plants in Louisiana. However knowing the fact that wheat accumulate less Si than rice, and based on our initial soil Si content at Ben Hur and Winnsboro in 2016 which were above 40 ug Si g^{-1} we can conclude that the initial Si level was high for wheat plants in 2016 and this might hinder obtaining positive yield responses. However, the initial soil Si concentration was around 10 ug Si g^{-1} in 2015, but the Si treatments did not make a significant difference in wheat yield responses. With a broad range of factors, encompassing insufficient vernalization, heavy precipitation, inadequate photoperiod, frequent flooding followed by long term hypoxia wheat plants were challenging over the course of this study and some of the severe, even transient alterations in environmental stimuli might adversely affect the yield and negate the positive effect of Si treatments on yield. Since that there are sources of environmental variability in field experiments further investigation will need to be performed to determine the optimal level of Si foliar and soil application which can lead to improve wheat yield.

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CHAPTER 3. EFFECTS OF DIFFERENT SILICON SOURCES ON LEAF RUST DISEASE DEVELOPMENT IN WHEAT PLANTS GROWN UNDER DIFFERENT NITROGEN RATE

3.1. Introduction

The requirement to double global food production by 2050 as concomitants of global population growth poses massive challenges in sustainable food production (Tilman, 2002). The global population growth and the world's food production need to increase from the current rough estimate 600 million tons to approximately 760 million tons by 2020 (CIMMYT, 2004). Plant diseases are considered major constraints on crop production and environmental health (Wild and Gong, 2010). Safety issues are among the biggest concerns in management practices using chemicals (Aktar et al., 2009). Sustainable agricultural approaches focus on agronomic practices to improve production with minimal detrimental impacts on the environment and reducing chemicals input (Tilman, 2002). Several reports indicate that nutrient management practices along with other cultural practices can successfully manage some of plant diseases (Dordas, 2008). Oerke et al. (1994) reported that the annual yield losses in wheat (*Triticum aestivum* L.) caused by diseases were about 12.4% worldwide. Also, several soil environmental factors such as nutrients, moisture, and pH can affect plant disease development (Dordas, 2008).

Among more than one hundred biotic and abiotic diseases that affect wheat yields, three wheat rusts (leaf, stem, and stripe) and Fusarium head blight are considered the major ones (Savary et al., 2012). According to historical records, severe wheat rust epidemics occurred in Egypt and causing significant yield losses centuries before the birth of Christ (Carefoot and Sprott, 1967). Wheat rust diseases are considered among the global constraints of wheat production and have posed a serious threat to global food security (Chaves et al., 2013). *Puccinia triticina* (Erikson) produces five spore types including teliospores, basidiospores, and

urediniospores on a primary host that is usually wheat, and pycniospores and aeciospores on the alternate hosts such as *Thalictrum speciosissimum* L. (Bolton et al., 2008). Urediniospores are produced on wheat hosts, and this asexual stage can cycle repeatedly. When the wheat ripens, the uredinial infections turns into teliospores that can oversummer in areas with mild climate condition. Leaf rust can overwinter as mycelial or uredinial infections on winter wheat and create a potential source of inoculum for subsequent infection (Kolmer, 2013). A film of moisture on leaves for several hours and a temperature of 15.5°C- 26.67°C is required for the growth and development of *Puccinia triticina* (Erikson) on susceptible wheat plants (Groth et al., 2013). Temperature and the presence of spores are the most important factors affecting leaf rust disease development on wheat plants (Cook and Veseth, 1991). Orczyk (2010) found 8 hours after inoculation, hydrogen peroxide (H_2O_2) accumulated in over 98% of guard cells of both susceptible and resistant cultivars and this phenomenon is considered to be the first phase of oxidative burst, one of the first plant reactions observed after the recognition of the pathogen. The second phase of the oxidative burst was observed in the epidermis of susceptible plants and the mesophyll part of resistant cultivars 4-5 days after inoculation. Wheat leaf rust disease occurs most often in northern parts of the Unites States. It is also prevalent on winter wheat in the Southeast, the southern Great Plains and the Pacific Northwest where wheat is grown between October and April or May (Cook and Veseth, 1991). Unlike other diseases such as stem rust or Fusarium head blight, the yield reduction in leaf rust diseased wheat is associated with reduced green leaf area and reduced photosynthetic rates of infected leaves. This is led to decreased numbers of grain per head, lower grain weights and lower grain quality (Robert et al., 2005; Bolton et al., 2008; Carretero et al., 2011). Pathogen infection leads to a decline in photosynthesis and other assimilatory metabolisms to increase respiration and provide the

required secondary metabolites for defense (Berger et al., 2007). Plant pathogens obtain soluble assimilates from their hosts and can cause plants to become nutrient deficient and increase their host's susceptibility to disease (Hancock and Huisman, 1981). The agent of wheat leaf rust, *Puccinia triticina* (Erikson), is an obligate, macrocyclic, heteroecious fungal pathogen which produces uredinospores on leaf surfaces, and wind dispersal of the spores contributes to the rapid outbreak of the disease (Bolton et al., 2008). Spore dispersal through wind and arising new races of pathogens due to constant breakdown of plant vertical resistance are critical attributes of this disease that make it a global wheat production concern (Chaves et al., 2013).

Current wheat leaf rust disease management includes the use of resistant varieties, fungicide sprays and cultural practices, such as controlling volunteer wheat plants (USDA- ARS, 2016). Production of genetically engineered resistant cultivars has been an effective strategy to manage wheat leaf rust (Dakouri et al., 2013). To date, more than 100 genes for leaf rust (Lr) resistance have been identified in wheat (Zhang et al., 2015). However, the wheat leaf rust pathogen has the ability to generate new races and overcome race-specific resistance genes (Kolmer et al., 2012).

Silicon (Si) is the second most abundant element present in the earth's crust, and it constitutes one of the major inorganic nutrient elements of many plants (Epstein, 1999; Ma et al., 2004). Great numbers of studies have shown that Si supplementation increases plant tolerance to biotic and abiotic stresses (Ma, 2004). Following an uptake of monosilicic acid (H_4SiO_4) by the roots, Si is transported from the roots to the shoots. It is then deposited into plant cell walls, intercellular spaces of cells and bracts in the form of amorphous silica gel ($SiO_2 \cdot nH_2O$) (Jones and Handreck, 1967, Epstein, 1999; Kim et al., 2002; Rodriguez and Datnoff, 2005). Silicon is taken up by root system through a NIP group of the aquaporin family transporters, called low Si

1 (*Lsi1*), which are involved in Si influx, whereas the efflux transport of Si is carried out by putative anion Si transporters called low Si 2 (*Lsi2*)(Ma et al., 2011). The Lsi1 is involved in passive transport of Si, however the *Lsi 2* considered an activate transporter of Si (Ma et al., 2007). Proteins homologous to the Lsi channel transports have been identified and characterized in some other plants such as barley (*Hordeum vulgare*) (HvLsi1) (Chiba et al., 2009), wheat (TaLsi1) (Montpetit et al., 2012), maize (*Zea mays*; ZmLsi1, ZmLsi6) (Mitani et al., 2009), and soybean (*Glycine max*; GmLsi1) (Deshmukh et al., 2013). The *Lsi2* channel homologous proteins in barley (HvLsi2), maize (ZmLsi2) (Mitani et al., 2009) and pumpkin (*Cucurbita pepo*) (CmLsi2) (Mitani-Ueno et al., 2011) has been identified. The various capabilities of plant species in Si uptake can be associated with Si transporters that differ in expression level and their localization in the cells (Chiba et al., 2009). If the concentration of silicic acid exceeds 2 mM, it starts polymerizing, leading to the precipitation of amorphous silica particles called phytoliths (Ma and Yamaji 2006). Kauss et al. (2003) reported that cucumber (*Cucumis sativus* L.) plants showed resistance to fungal infection by expression of a proline-rich protein together with the formation of silica bodies at the site of fungal penetration.

Silicon has been shown to contribute in cell wall reinforcement through silicifying epidermal cell walls and reducing plant fungal penetration (Yoshida et al., 1962; Hayasaka et al., 2008). Ning et al. (2014) found that there were Si layers forming in Si-treated epidermal cell walls, and the Si layers were seen to be thicker following Si application. They suggested that Si deposition in host cell walls and papillae sites acts as a physical barrier against fungal penetration. Kanto et al. (2004) suggested that silicate increased resistance against powdery mildew (*Sphaerotheca aphanis* Wallr) in strawberry (*Fragaria vesca* L.) plants through enhancing the physical hardness of leaves in strawberry plants grown in hydroponic system.

Silicon mediated cell wall fortification is not the only mechanism that is involved in the positive role of Si enhancing plant disease resistance. It is plausible that Si induces plant defense responses. Besides the cell wall fortification and induced systemic resistance, Si may reduce disease severity through other modes of actions. Several reports indicate that Si supplementation significantly decreased the level of malondialdehyde (MDA), an indicator of oxidative stress, in plant cells under biotic or abiotic stresses (Mohsenzadeh et al., 2011; Fortunato et al., 2012; Resende et al., 2012; Coskun et al., 2016). It has been reported that Si supplementation increased the production of phenolic compounds and phytoalexins (Rodrigues et al., 2003) and induced pathogenesis related genes such as PR-1 and peroxidase (Rodrigues et al., 2005). Cherif et al. (1994) indicated that less infection with *Pythium* spp. following Si treatment has been due to higher activity of defense enzymes such as peroxidase (POD), β-1,3 glucanase, and chitinase in cucumber plants. Han et al. (2016) showed that soil Si amendment resulted in increased numbers of silica cells in the leaves and also improvement of the antioxidant enzyme activities, which led to a significant reduction in rice (*Oryza sativa* L.) infestation by the herbivore (*Cnaphalocrocis medinalis* Guenée) Fawe et al (1998) reported that Si application increased the accumulation of plant defense metabolites leading to Si-induced resistance which has similarities with the systemic acquired resistance (SAR), however, SAR lasts for a longer period of time. A difference between known SAR and Si induced resistance is following the withdrawal of the Si supplement, Si starts to polymerize in the cells and cannot play a role as an inducer of defense responses (Fauteux, et al., 2005). It was reported that Si treatment increased phytoalexins in cucumber (*Cucumis sativus* L.) plants infected by powdery mildew (*Podosphaera xanthii,* Castagne) (Fawe et al., 1998). Another study showed that Si increased production of momilactones A and B and boosted Si-induced resistance to rice blast (*Magnaporthe grisea*) ([T.

49

T. Hebert] M. E. Barr) (Rodrigues et al., 2004). Bélanger et al., (2003) reported that Si treated plants showed papilla formation, production of callose and production of glycosilated phenolics following infection with *Blumeria. Graminis* DC f. sp. *Tritici*. It was shown that Si inhibits the production of fungal ethylene, which keeps the fungus *Cochliobolus miyabeanus* Ito & Kurib from decreasing rice's innate immune system (Van Bockhaven et al., 2015). In an extensive microarray study in the Arabidopsis–powdery mildew pathosystem, Fauteux et al. (2006), showed that Si treated plants showed a higher magnitude of the upregulation of defense genes and a lower magnitude of downregulation of genes involved in primary plant metabolism, a phenomenon that confirms the protective role of Si in improving host disease resistance. Dallagnol et al. (2009) demonstrated that rice mutants deficient in active Si uptake showed a significantly lower disease resistance against brown spot (*Bipolaris oryzae* Breda de Haan (Shoemaker). The efficacy of Si enrichment in managing turf grass diseases in Florida was investigated by Datnoff and Rutherford (2003). The gray leaf spot (*Magnaporthe grisea* (T.T.Hebert) M.E. Barr) on St. Augustine grass (*Stenotaphrum secundatum* (Walter) Kuntze.) was controlled by Si applications; the combined effect of calcium silicate and the fungicide (chlorothalonil) was remarkably more effective at reducing the disease development.

With awareness of the beneficial role of Si in alleviating biotic and abiotic stress, the application of Si to wheat fields to manage wheat leaf rust could be a sustainable approach in particular soils with low initial levels of Si. In this study, we aimed to evaluate difference sources of foliar and soil applied Si in leaf rust disease development in wheat. Plant nutrient management can be an effective and a more sustainable approach to manage plant disease.

50

3.2. Materials and Methods

3.2.1. Site Description, Treatment Structure, and Trial Establishment

This study was conducted from 2014-2016 with a total of four site-years in Louisiana. The first and second site-year were established in 2014 at the Macon Ridge Research Station near Winnsboro (32.1632° N, 91.7207° W), on a Gigger- Gilbert silt loam soil (Fine-silty, mixed, superactive, nonacid, thermic Fluvaquentic Endoaquepts) and at Ben Hur Research Station (Latitude 30°, 21', 40.4" N; Longitude 91°, 10', 01.9" W) near Louisiana State University campus in Baton Rouge on a Cancienne silt loam soil (Fine-silty, mixed, superactive, nonacid, hyperthermic Fluvaquentic Epiaquepts). The third and fourth site-years were established in 2015 at the Macon Ridge Research Station and at Ben Hur Research Station as well.

The treatment structure was a two-way factorial (two N rates x seven Si sources) in a randomized complete block design with four replications. The seeds were sown at Ben Hur and Winnsboro at the rate of 106 and 84 kg ha^{-1}, respectively from mid to last week of November 2014 and in 2015. Winter wheat varieties, Terral 8525 (2014-2015) and Progeny 870 (2015-2016), were used in this study; both varieties are susceptible to leaf rust. Two N rates of 101 and 145 kg N ha^{-1} were used for this study using urea (45% N) as the source. The 101 kg N ha^{-1} rate is the standard rate for wheat production in LA and 145 kg N ha^{-1} is considered as a high rate. For both N rates, there were seven Si sources applied either as soil amendments or foliar application: wollastonite (280 kg Si ha^{-1}) and slag (280 kg Si ha^{-1}) as soil amendments or1000, 2000 and 4000 ml Si L^{-1} respectively, two control plots were included (without Si treatments and carrier). The plot size at Ben Hur was 14.4 m^2 and at Winnsboro was 6 m^2.

Phosphorus (triple superphosphate, 46% P) and potassium (KCl, 60% K) fertilizers were applied to fields as 67 kg P ha^{-1} and 112 kg K ha^{-1} respectively, according to the test results and recommendations of the LSU AgCenter Soil Testing Plant Analysis Laboratory. The solid forms of Si fertilizer, $CaSiO_3$ slag with the commercial name of Plant Tuff® (12% Si) and wollastonite (23% Si) were used for this study. Slag and wollastonite fertilizers were pre-weighed and put into plastic bags, then spread by hand and were then incorporated into the soil to a depth of 7.5 cm in mid-November 2014. Solution Si was diluted with water to attain a 701 L ha^{-1} foliar application rate. The first foliar application started at Feekes (F) growth stage 5 (Large, 1954) and was repeated three times at one week intervals. The two N treatments were top-dressed when plants reached (F) 5.

3.2.2. Leaf Rust Detecting and Rating

Leaf rust was first detected in late April when plants growth stage (GS) was around Feekes 10.5 or 11. Disease ratings were recorded at 1-week intervals starting after rust pustules appeared on wheat leaves. Data are based on four evaluations from the end of April through the end of May 2016. The modified Cobb scale is used to estimate the rust intensity on leaves (Peterson et al., 1948) and the disease severity expressed as mean percentage of diseased leaf areas. Disease ratings were used to create disease progress curves for each treatment. Treatment effectiveness was determined by calculating individual area under the disease progress curves (AUDPCs), with the following formula (Shaner and Finney, 1977):

$$AUPDC = \sum_{i=1}^{n} \left(X_{i+1} + Xi \right)(t_{i+1} - ti)/2$$

where Xi = disease proportion at the ith observation,

ti = time (in days)

 n = total number of evaluations.

3.2.3 Soil Sampling

Soil samples were taken at F9 (late March) and F11 (late May). Each sample consisted of twelve soil cores (depth 0-15 cm): six cores taken from two inner rows adjacent to where biomass samples were taken. Soil samples were oven-dried (Despatch LBB series; model number LBB2-18-1) at 55°C for about a week. The Humboldt soil grinder was used to grind the soil to obtain finely ground, homogeneous soil samples. The soil samples were passed through a 2 mm sieve for later analysis.

3.2.4. Silicon Extraction Procedure

Silicon content was determined by 0.5 M acetic-acid extraction procedure followed by Molybdenum Blue Colorimetry (MBC) procedure (Korndorfer et al., 2001). One (1) g soil sample was weighed into a polyethylene centrifuge tube and ten (10) mL of 0.5 M acetic acid was added to them. All the tubes were placed on a reciprocal shaker (Eberbach; model number E6010.00) for 1 hour. The soil suspension samples were filtered with Whatman No. 1 filter paper into 50 mL centrifuge tubes (Korndorfer et al., 1999). A 0.5 mL aliquot was added into 50 mL centrifuge tubes. Ten (10) ml of DI water, 0.5 mL of 1:1 HCl:water, and 1 mL of 10% ammonium molybdate (pH 7.5) were successively added to the samples.. After 5 minutes, 1 mL of 20% tartaric acid was added to tubes and they were gently shaken by hand for 10 seconds and then left to sit for 2 minutes. Afterwards, 1 ml of ANSA (0.5 mg 1-amino-2-naphthol-4-sulphonic acid + 1.0 g sodium sulfite + 30.0 g sodium bisulfite in deionized (DI) water with a final volume of 250 mL) was added and then the solution was diluted to the final volume of 25 mL with DI water. Absorbance reading was measured after 5 minutes at 630 nm using UV

visible spectrophotometer (Hach DR 5000). A series of standard solutions were prepared with the same background (0.5 M acetic acid) at rates of 0, 0.2, 0.4, 0.8, 1.2, 1.6 and 2.0 ug mL^{-1} of Si along with blanks and reference samples (Sharkey clay and Commerce silt loam soil) were also prepared.

3.2.5. Soil pH (1:2 method)

Soil pH was measured using 1:2 soil to DI water. Five grams of soil was placed into 50 mL centrifuge tubes and 10 mL DI water was added. Tubes were placed on a reciprocal shaker for 1 hour at X RPMS, and pH was measured using an Oakton pH 5+ digital pH meter (Coleparmer Co. Vernon Hills, IL).

3.2.6. Silicon Analysis

Silicon content in plant tissue samples was determined by an Oven-Induced Digestion procedure (OID) (Kraska and Breitenbeck, 2010) followed by the MBC procedure (Hallmark et al., 1982). One hundred mg of plant tissue was weighed into 50 mL centrifuge tubes then dried at 60°C for 15 minutes in an oven (Yamato; DKN600, Santa Clara, CA). Five drops of octyl alcohol and 2 mL of hydrogen peroxide (H_2O_2) were added to the tubes then placed in the oven at 95°C for 30 minutes. Samples were removed and 4 mL of 50% sodium hydroxide (NaOH) was added. During this 4 hour period, samples were gently mixed using a vortex mixer every 15 minutes. After 4 hours, 1 mL of ammonium fluoride (NH_4F) was added to the digested samples, mixed, and diluted to 50 mL with DI water. Soybean and sugarcane Si reference samples and blanks also were digested.

The MBC procedure began by taking 2 mL aliquots from digested samples and pipetting into 30-mL centrifuge tube. Ten mL of 20% acetic acid and 4 mL of 0.26 M ammonium molybdate was added and samples were left alone for 5 minutes. Afterwards, 2 mL of 20%

tartaric acid and 2 mL of the reducing agent (0.5 mg 1-amino-2-naphthol-4-sulphonic acid + 1.0 g sodium sulfite + 30.0 g in DI water with a final volume of 250 mL) were added. The final volume was adjusted to 30 mL using 20% acetic acid. Capped tubes were shaken for 30 minutes, and absorbance was measured using a Hach DR 5000 spectrophotometer at 630 nm. A series of standards were made at rates of 0, 0.4, 0.8, 1.6, 3.2, 4.8 and 6.4 ug mL^{-1} of Si. Silicon content (g kg^{-1}) of plants was determined using the following formula:

$$Si\ content\ =\ \frac{\left[(Abs_{samp} - Abs_{blk}) -\ Cfi\right]}{Cfs}\ *\ \left[\left(\frac{V_d}{S_{wt}} * \frac{V_c}{V_a}\right)\right]$$

Where:

Abs_{samp} = absorbance reading of sample

Abs_{blk} = absorbance reading of reagent blank

Cfi = μg Si g^{-1} when absorbance is zero (intercept of standard series curve)

Cfs = μg Si g^{-1} per unit of absorbance (slope standard series curve)

V_d = final digest volume (mL)

S_{wt} = oven-dry equivalent weight of digested sample (g)

V_c = final colorimetric volume (mL)

V_a = volume of aliquot used for colorimetric analysis (mL)

3.2.7. Silicon Uptake

The biomass, straw and grain Si content was determined using the following formula:

$$Plant\ Si\ Uptake\ =\ (\%Si\ in\ Straw/100) \times Straw\ Yield\ (kg/ha)$$

$$Grain\ Si\ Uptake\ =\ (\%Si\ in\ Grain/100) \times Grain\ Yield\ (kg/ha)$$

3.2.8. Scanning Electron Microscopy (SEM) and Energy Dispersive X-ray (EDX) Analysis

Scanning Electron Microscopy (SEM) uses a high voltage electron beam to produce a variety of signals which translate information about fully hydrated specimen morphology, crystalline structure, and chemical composition (Echlin, 1971). The relative %Si from EDAX analysis determined the frequency of Si bodies distributed within the sampling area relative to K, C, H and other nutrients. At midseason harvest (late March) two replications of each treatment were randomly selected for sampling, where eight leaves were arbitrarily selected and three different random spots were analyzed per leaf. Leaves were thoroughly washed with DI water to eliminate remnants of Si foliar solution. Magnification was set at 400x at a voltage of 20 kV.

3.2.9. Statistical Analysis

The ANOVA procedure on all measured variables was performed using SAS 9.4 (SAS Institute, 2012). PROC MIXED in SAS 9.4 was used to determine the significant effects of N, Si, and N x Si interactions on measured parameters. In the statistical model, N and Si treatments were fixed effects, while replication, site and their interaction were considered random effects. Treatment means were compared using the least significant difference (LSD) test for any significant effect detected at $P<0.05$.

3.3. Results and Discussion

In 2015, the first disease severity rating was done at mid-April and the second rating was accomplished at the end of April and the statistical analysis of data is summarized in Table 3.1. According to the results, Si fertilizers did not have a significant effect on reducing the disease severity in 2015 (Table 3.1).

The leaf rust disease progress in 2016, when it started al low level until its incidence and severity increased over a period of four weeks are shown in both sites in Figure 3.1 and Figure 3.2.

Table 3 1. Disease severity rating in April 2015.

Silicon Sources	Leaf Rust Disease Severity (%)	
	Mid- April	Late- April
Control	25	36
Carrier	22	39
Foliar 1000 ml Si L^{-1}	16	36
Foliar 2000 ml Si L^{-1}	17	41
Foliar 4000 ml Si L^{-1}	17	35
Slag, 280 kg Si ha^{-1}	20	39
Wollastonite, 280 kg Si ha^{-1}	14	29
Analysis of Variance		
N	NS	NS
Si	NS	NS
N x Si	NS	NS

NS = non-significant. Means followed by the same letter within a column not differ significantly according to LSD at 0.05 level of confidence.
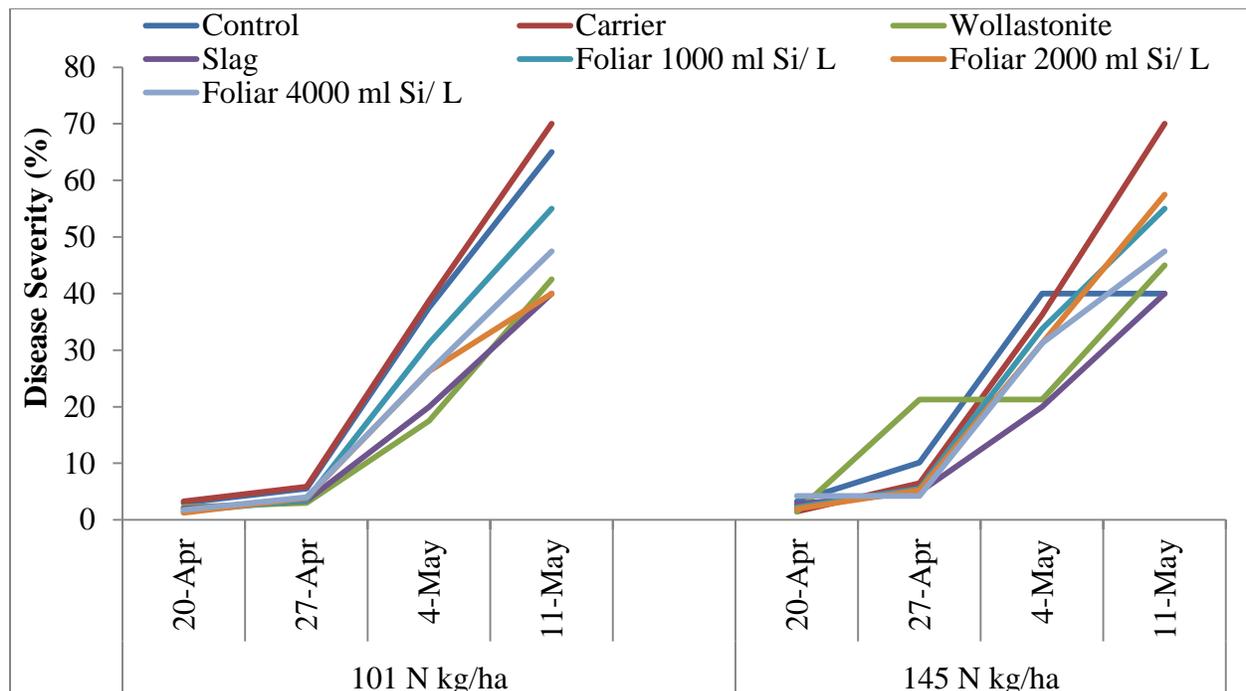

Figure 3 1. Effect of Si application on leaf rust disease severity in Winnsboro in 2016.
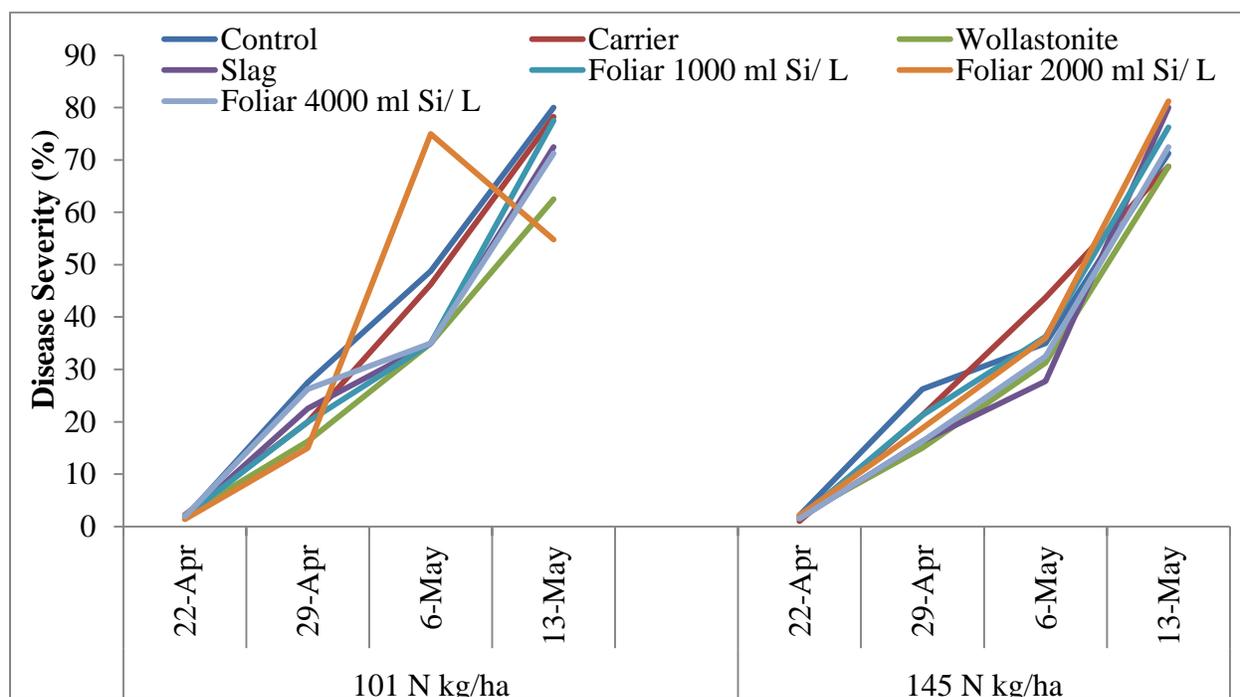
57

Figure 3 2. Effect of Si application on leaf rust disease severity in Ben Hur in 2016.

Based on the AUDPC values obtaining from the wollastonite- and slag- treated plants, leaf rust severity were significantly reduced by 34 and 30% respectively in 2016. Foliar application of Si at the three rates reduced the disease severity by over 20% and there was no significant difference among the three rates of Si foliar application (P <0.05) (Figure 3.3). Analysis of variance (ANOVA) at P <0.05 showed that there was no significant effect of Si and N interaction on leaf rust disease severity.

Consistent with our results, numerous studies have shown the positive effect of Si application or reducing the plant disease severity. Foliar applications of Si reduced powdery mildew (*Podosphaera xanthii* Castagne) Braun & Shishkoff) disease development in melon (*Cucumis melo* L.) through the cell wall fortification and promoting the antioxidant defense system (Dallagnol et al., 2012). It was reported that foliar application of Si decreased bacterial speck symptoms by impacting directly on *Pseudomonas syringae* pv. *tomato* without any effects

on host defense enzymes (Anderade et al., 2013). Liang et al. (2005) concluded that root applied Si enhanced cucumber plant disease resistance to powdery mildew (*Podosphaera xanthii* (Castagne) Braun & Shishkoff), however foliar-applied did not affect the disease resistance and it could effectively control infections by either the formation of physical barrier of Si deposited on leaf surfaces, and/or osmotic effect. Bowen et al (1992) observed that foliar sprays at 17 mM Si significantly reduced powdery mildew (*Vitis vinifera* L.) infection on grape leaves while root application was not effective in reducing the disease severity and their scanning electron micrographs showed that fungal hyphal growth was suppressed on Si-sprayed leaves due to thick Si deposits. Their investigation showed that conidia germination and germ tube development were impaired on agar media supplemented with Si. Guével et al. (2007) reported that root applications of Si was more effective than the foliar application of Si in controlling the powdery mildew on wheat plants and the concentration of 1.7 mM gave the best results in terms of plant Si absorption. Liang et al. (1994) reported that Si fertilization enhanced the resistance of rice and wheat against pathogen attacks and lodging although Si treatment had little impact on either wheat or rice yield response. Nanayakkara et al. (2008) reported that application of silicon fertilizers such as calcium silicate slag and wollastonite reduced gray leaf spot (*Magnaporthe oryzae* B.C. Couch) incidences and severity in perennial ryegrass (*Lolium perenne* L.) suggesting that the material may be considered in integrated management programs.
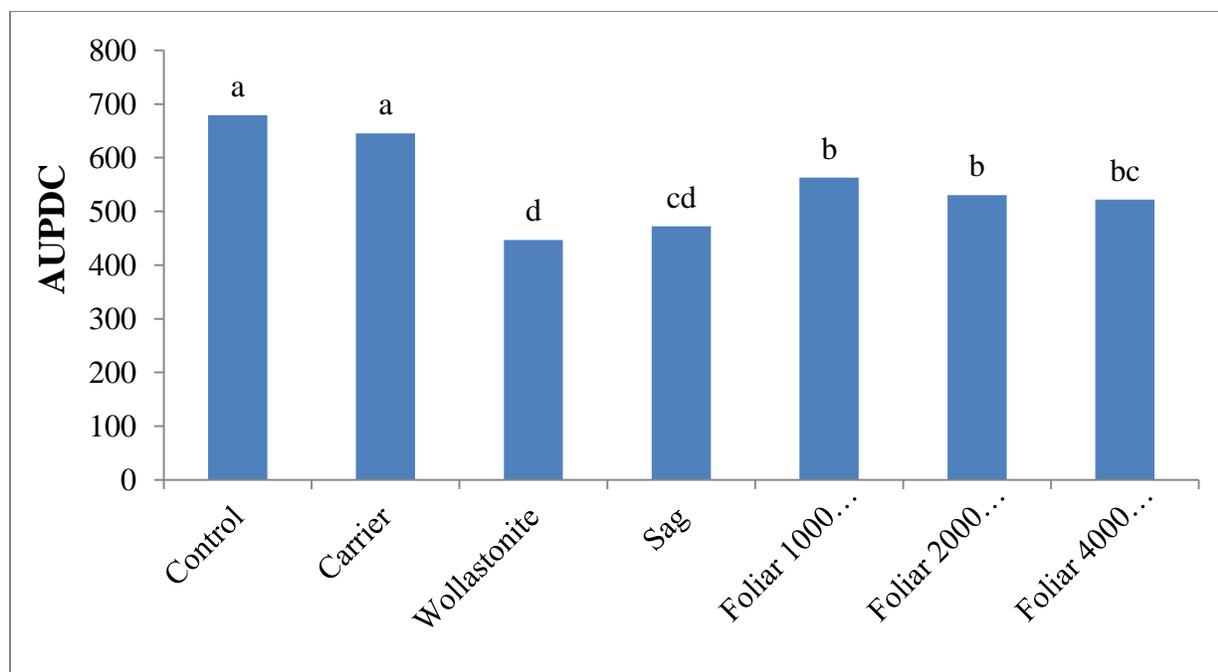
Figure 3 3. The effects of different sources of Si on AUPDC in 2016. Bars with the same letter are not significantly different according to LSD at 0.05 level of confidence.

The effects of Si amendments on soil Si content in 2015 and 2016 are reported in Table 3.2 and Table 3.3, respectively. A mean separation procedure (LSD, $P<0.05$) was performed in SAS to determine significant differences within Si amendments. The data show that wollastonite and slag application significantly increased soil Si content at midseason and harvest in 2015 (Table 3.2) and 2016 (Table. 3.3) compared to the control. Torlon et al. (2016) showed that wollastonite and slag could significantly increase available soil Si. Also, wollastonite- and slag-treated soils showed a significant difference in soil pH by 0.4 and 0.3 units increase, respectively compared to control in 2015. Wollastonite and slag application significantly increased soil pH by approximately 0.4 unit compared to control in 2016 (Table 3.3). No significant changes were observed in soil Si content and soil pH across the Si foliar treatments.

Table 3 2. Effect of Si amendments on soil 0.5 m acetic acid extractable-Si and pH in 2015.

| Silicon Sources | Extractable-Si (mg kg^{-1}) | | Soil pH |
	Midseason	Harvest	
Control	43 c	43 c	6.0 b
Carrier	38 c	49 c	6.0 b
Foliar 1000 ml Si L^{-1}	43 c	45 c	6.0 b
Foliar 2000 ml Si L^{-1}	37 c	39 c	6.0 b
Foliar 4000 ml Si L^{-1}	43 c	47 c	6.0 b
Slag, 280 kg Si ha^{-1}	141 b	144 b	6.2 a
Wollastonite, 280 kg Si ha^{-1}	251 a	260 a	6.3 a
P value	$P<0.05$	$P<0.05$	$P<0.05$

NS = non-significant. Means followed by the same letter within a column not differ significantly according to LSD at 0.05 level of confidence.

The increase in pH following Wollastonite and slag application might be due to the intrinsic property of slag which is applied as a liming material to neutralize soil acidity. Ning et al. (2016) reported that slag amendments could be an effective agricultural practice to correct soil acidity.

Table 3 3. Effect of Si amendments on soil 0.5 m acetic acid extractable-Si and pH in 2016.

| Silicon Sources | Extractable-Si (mg kg^{-1}) | | Soil pH |
	Midseason	Harvest	
Control	43 c	43 c	6.0 b
Carrier	38 c	49 c	6.0 b
Foliar 1000 ml Si L^{-1}	43 c	45 c	6.0 b
Foliar 2000 ml Si L^{-1}	37 c	39 c	6.0 b
Foliar 4000 ml Si L^{-1}	43 c	47 c	6.0 b
Slag, 280 kg Si ha^{-1}	141 b	144 b	6.4 a
Wollastonite, 280 kg Si ha^{-1}	251 a	260 a	6.5 a
P value	$P<0.05$	$P<0.05$	$P<0.05$

NS = non-significant. Means followed by the same letter within a column not differ significantly according to LSD at 0.05 level of confidence.

In 2016, wollastonite, slag and foliar 4000 ml Si L^{-1} significantly increased Si uptake by 65, 52, and 35% respectively compared to control (Figure 3.4). Torlon et al. (2016) demonstrated that among various Si sources, wollastonite treated pumpkin plants showed higher Si uptake and more resistance against powdery mildew (*Podosphaera xanthii*) disease.

Wollastonite, slag and foliar 4000 ml Si L^{-1} significantly increased the percentage of Si body distribution in epidermis compared to control (Figure 3.5). Silica bodies not only act as a mechanical barrier against fungal pathogens (Datnoff et al., 2007), but also function as a cooling system in the leaves by increasing mid- infrared thermal emission (Wang et al., 2005). Ning et al (2014) found that there were Si layers forming in Si-treated epidermal cell walls, and the Si layer was seen to be thicker following Si application in rice. Kim et al. (2002) showed that following Si treatment, Si was deposited in epidermal cell walls, middle lamellae, and intercellular spaces within sub-epidermal tissues rice leaves.
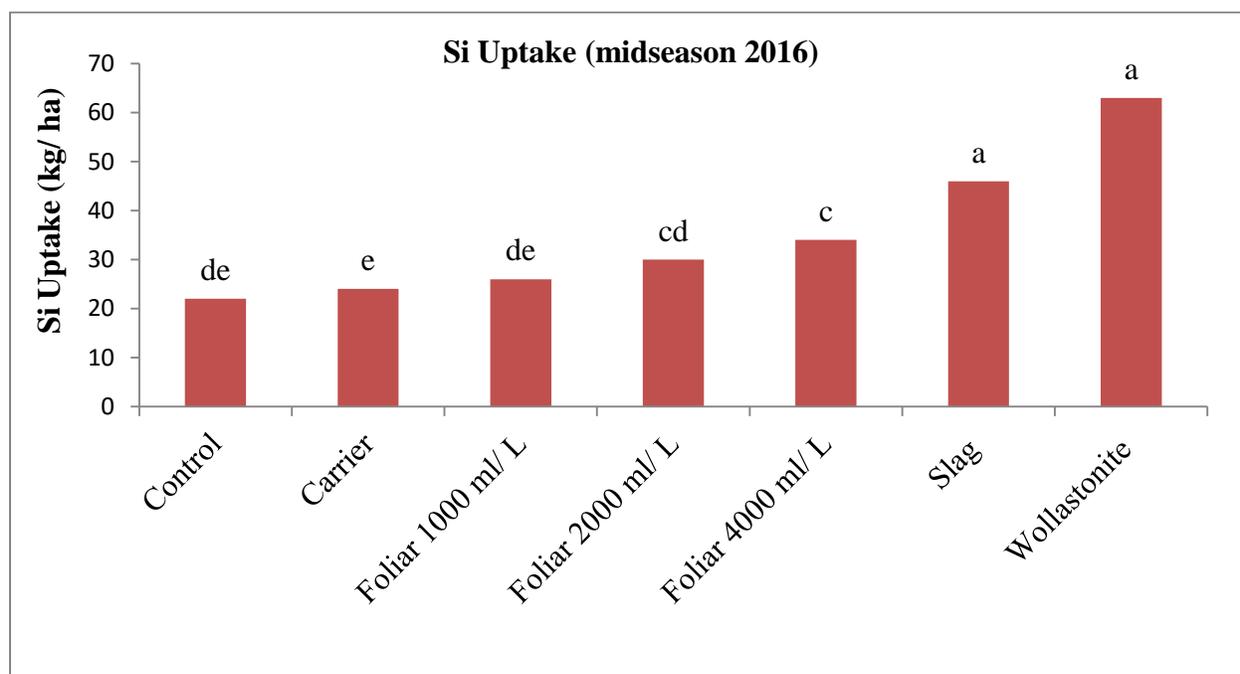

Figure 3 4. The effects of different sources of Si on plant Si uptake in 2016. Bars with the same letter are not significantly different according to LSD at 0.05 level of confidence.

There was a negative relationship between the %Si in leaves epidermis and the AUPDC. Perhaps the higher count of Si bodies in leaf epidermis led to lower disease development (Figure 3.6). Also, there was a negative relationship between the plant Si uptake and the AUPDC; the higher Si uptake resulted in lower disease development (Figure 3.7).
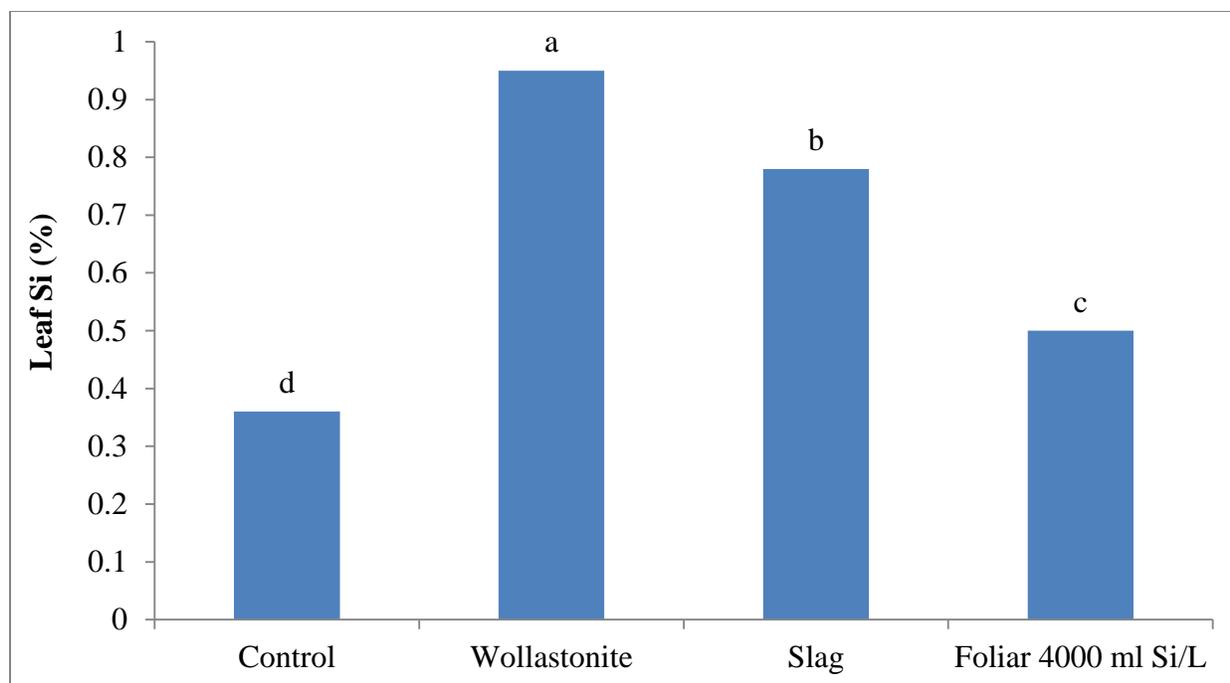
Figure 3 5. Effect of different sources of Si on relative %Si in leaf epidermis. Bars with the same letter are not significantly different (LSD's test) based on LSD at 0.05 level of confidence.

Consistent with our result, Song et al. (2015) showed that soil Si amendment significantly reduced disease severity of bacterial blight caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) in rice. Electron microscopy and X-ray microanalysis showed that Si layers were deposited beneath the cuticle in epidermal cell walls of Si treated plants and this structure significantly increased leaf thickness resulting in a remarkable reduction in rice blast disease severity (*Magnaporthe grisea* (T.T.Hebert) M.E. Barr) (Kim et al., 2002). Dallagonal et al. (2012) reported that the AUPDC of powdery mildew (*Podosphaera xanthii*) was significantly reduced in both foliar and root Si treated melon plants. It was shown that wollastonite was effective in reducing the AUPDC in pumpkin infected with powdery mildew. Further, the AUDPC of soybean plants receiving Si treatments was significantly lower than the AUDPC of control plants infected with *Phakopsora pachyrhizi,* the agent of soybean rust (Lemes et al., 2011).
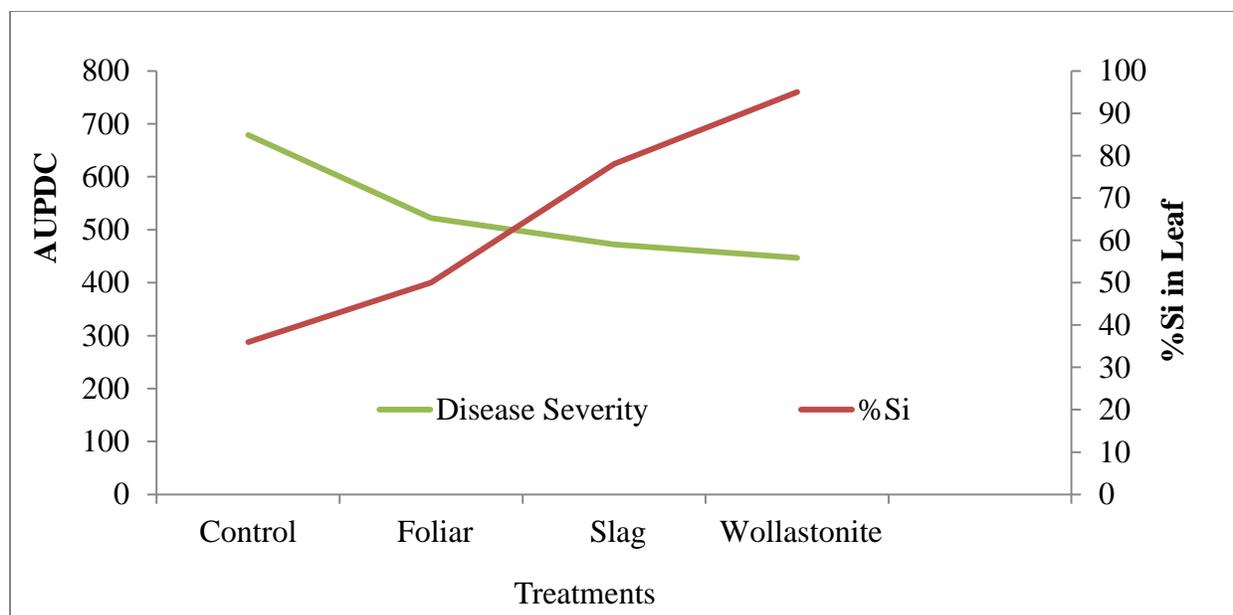
Figure 3 6. The pattern of %Si in leaf and leaf rust disease severity of wheat treated with silicate slag and wollastonite rate at 280 kg Si ha^{-1}, and foliar application of Si at 4000 ml Si L^{-1} in 2016.
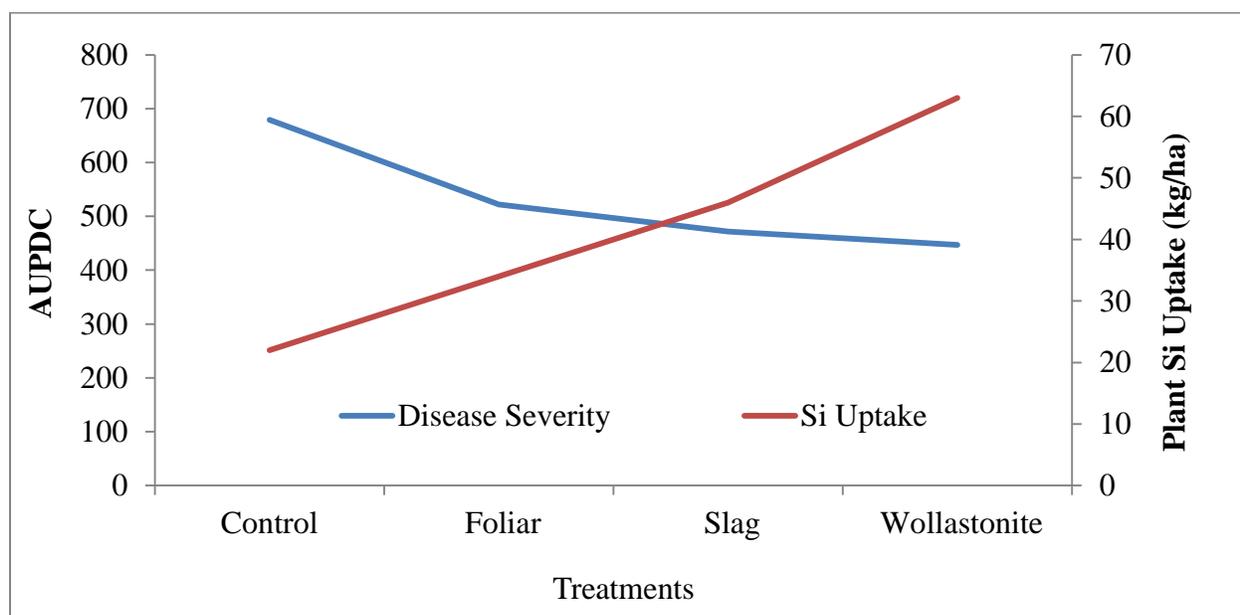

Figure 3 7. The pattern of plant Si uptake and leaf rust disease severity of wheat treated with silicate slag and wollastonite rate at 280 kg Si ha^{-1}, and foliar application of Si at 4000 ml Si L^{-1} in 2016.

3.4. Conclusions

While significant decrease in leaf rust disease severity was observed across all Si treatments compared to control, the highest level was associated with soil Si applications. In the context of Si amendments, wollastonite- treated plants showed a significant decrease in leaf rust diseases development compared to slag- treated plants. Present investigation indicated that there was a negative relationship between Si uptake in midseason and the leaf rust disease severity reduction. Also, the results from scanning electron microscopy on the distribution of silica bodies in leaf epidermis were consistent with the reduced disease development in Si treated plants. Although the Si uptake or silica body distribution are not significant across lower rates of Si foliar applications (1000 and 2000 ml Si ha^{-1}), the reduction in leaf rust disease severity was significantly different in those treatments compared to control. These results may imply the positive role of Si in the induction of plant defense responses and plant pathogen resistance. The in-depth molecular investigation on a wide variety of plant species will be helpful to elucidate the minimum concentration of Si that can trigger defense responses in plant cells. In vitro cell culture studies could be a promising approach for studying physiological and biochemical plant responses reflecting the Si mode of action at molecular level. This, to our knowledge, is the first study to investigate the effect of Si uptake on leaf rust disease management. These results may provide practical information for further investigation on determining the exact mechanisms behind Si signaling pathways triggering defense responses.

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CHAPTER 4. CONCLUSIONS

Our study shows promising efficacy of Si fertilizers on reducing leaf rust disease development in Louisiana. Silicon applied as soil amendments were consistently more effective than foliar application in enhancing plant Si concentration, increasing the biomass, and reducing disease development. This might be in agreement with the fact that Si transporters are mainly expressed in the plant root system. All things considered, the type and the rate of Si amendments significantly contributed to plant responses to Si treatments. Silicon application did not have a significant effect on wheat yield in this study. This might be due to the fact that initial Si levels for wheat were high in 2016. With a broad range of factors, encompassing insufficient vernalization, heavy precipitation, inadequate photoperiod, frequent flooding followed by long term hypoxia, wheat plants were challenged over the course of this study and some of the severe, even transient, alterations in environmental stimuli might adversely affect the yield and negate the positive effect of Si treatments on yield. The results showed that N and Si did not have any interactive effect on wheat yield, Si uptake, or leaf rust disease development. Silicon fertilization alone or combined with N may have a significant role in improving yield and plant stress tolerance only in areas with highly weathered soils that contain low levels of Si.

Foliar application of Si significantly decreased the leaf rust disease and further testing of the effects of varying frequencies and rates might provide more consistent results. Although the Si uptake or silica body distribution were not significantly different across lower rates of Si foliar applications, decreases in leaf rust disease severity were significant in those treatments compared to control. These results supported the emerging theory that the mechanisms by which Si exerts its protective role against plant fungal penetration are not completely dependent on the role of Si in cell wall fortification. Rather, the induction of plant defense responses may contribute to Si

mediated plant pathogen resistance. A substantial number of studies reported the protective role of Si against plant fungal penetration through cell wall fortification and triggering of induced systemic resistance (ISR) pathways. In the context of foliar application of Si and plant leaf rust disease resistance, although there is no report confirming that the influx of Si is carried out through the epidermal transporters, the potential role of Si as signaling molecules to stimulate pathogen recognition receptors (PRRs) and thus, the improvement of crop protection against diseases certainly needs to be investigated. Since there are some reports indicated that Si reduced a disease development through a direct effect on the pathogen and not contributing in plant defense responses, the effect of Si on the growth and development of the pathogen *Puccinia triticina* is worth further investigation. It is feasible that Si and/or the carrier, used for better Si uptake, have an effect on pathogens by changing their optimal pH or osmotic condition. The results indicate that further prospective studies are needed to define the exact rate of Si fertilization that is required to trigger plant defense responses in wheat, and our studies will open avenues for doing research on the impact of Si in plants and pathogens.

Since it has been reported that Si and selenium (Se), the essential trace element for human, animal and microorganism, have a number of common uptake transporters in rice (Ma et al., 2008; Zhao et al., 2010), further research is required to see if Si fertilization will maintain Se in the required dietary concentration range in edible parts of the plants. The results indicated that further prospective studies are needed to define the exact rate of Si fertilization that is required to trigger plant defense responses in wheat, and our studies will suggest potential molecular research on the impact of Si in plants and pathogens.

VITA

Maryam Shahrtash was born in Shiraz, Iran, in November of 1981. She attended Shiraz University and received her Bachelor of Science in Plant Pathology in February of 2004 and Master of Science in Plant Physiology in February of 2010. After graduating, she took a couple of years off from her studies to teach plant sciences in colleges and was later accepted in to the School of Plant, Environmental, and Soil Sciences at LSU in August of 2013. Since then, she has worked under the guidance of Dr. Brenda Tubana on the effects of N and Si fertilization on wheat production and the alleviation of biotic and abiotic stresses in wheat in the State of Louisiana.