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Study of Factors Affecting Growth and Development of Narrow Brown Leaf Spot of Rice Caused by Cercospora janseana (Racib.) O. Const.

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STUDY OF FACTORS AFFECTING GROWTH AND DEVELOPMENT OF NARROW BROWN LEAF SPOT OF RICE CAUSED BY CERCOSPORA JANSEANA (RACIB.) O. CONST.

A Dissertation

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Louisiana State University and
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in the partial fulfillment of the
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in

The Department of
Plant Pathology and Crop Physiology

by
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To my parents...

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ABSTRACT

Studies evaluated the effects of planting date, cultivar susceptibility, fungicide application timing, nitrogen management, and tillage practices on the severity of narrow brown leaf spot of rice (NBLS) caused by *Cercospora janseana.* All factors evaluated affected NBLS severity. The mid-April planted rice had less NBLS as compared to mid-May, late planted rice. Propiconazole fungicide (Tilt) application at panicle initiation or early boot stage was found equally effective in terms of disease reduction in mid-April planting while panicle initiation stage was the best time to apply fungicide in the late planting (mid-May). Early onset and higher severity of NBLS was observed on very susceptible cultivars (Cheniere and CL131) and delayed and least on resistant cultivars (Della and Presidio) at both the planting dates. The very susceptible to moderately susceptible cultivars produced similar yields under lower disease pressure (mid-April). Under, higher disease pressure, yields were lower, and variable responses to NBLS were detected among cultivars that did not relate to the susceptibility ratings based on disease severity. NBLS severity was higher when no nitrogen or excessive nitrogen was applied in susceptible and moderately susceptible cultivars, but no effect of nitrogen was observed in resistant hybrid LAH10. Split nitrogen applications (84/50 kg/ha) resulted in lower NBLS severity in susceptible cultivars compared to single nitrogen application. NBLS development was higher in a stale seed-bed as compared to conventional seed-bed system and increased at higher seeding densities. At a low seed density, NBLS severity was not significantly different between the two tillage systems. The demonstrated effects and interactions of host resistance, fungicide application, and cultural practices on NBLS severity indicate the potential for integrated disease management of this increasingly important rice disease.

CHAPTER 1
INTRODUCTION AND LITERATURE REVIEW

1.1. Introduction

The rice grain is the seed of the monocot plant *Oryza sativa* L. or *Oryza glaberrima* Steud. (Anonymous, 2000). As a cereal grain, it is the most important staple food for human nutrition and caloric intake, providing more than one fifth of the calories consumed worldwide by humans (Anonymous, 2004). Moreover, rice farms cover 11% of the world's arable land (IRRI, 2000). Worldwide, rice is planted on 155.62 million hectares with about 1.18 million hectares in the United States (FAS, 2013). As a result, rice research and its applications have the potential to affect the well-being of a large part of the world's population and will also have a substantial effect on the environment.

About 471.6 million metric tons (MMT) of rice is produced annually compared to 657.3 MMT for wheat, 474.4 MMT for oil seeds, 1138 MMT for coarse grains, and 2267 MMT total grains were produced annually on a global basis (FAS, 2013). Globally, rice is cultivated in more than 50 countries across Africa, Asia, Australia, Europe, North and South America and is the staple food for 17 countries (FAS, 2013). Of the 471.6 MMT produced, almost 60% is produced in Asian countries. The leading producers of rice are (in decreasing order) China, India, Indonesia, Bangladesh, Vietnam, Thailand, Burma, Japan, Philippines, Brazil, and the United States. The United States produces about 6.3 MMT (FAS, 2013), and its consumption, although increasing, is still approximately 11.3 kg per person annually, as compared with 90 to 180 kg per person in parts of Asia (USA, Rice Federation, 2013). Compared to the rest of the world, the United States accounts for only 1.5-2% of global production, but is the second largest exporter of rice after Thailand, representing 12 % of global rice exports (FAS, 2013). The United States exported 3,118 and 3,708 thousand tons all over the world in 2011 and 2012, respectively (Childs, 2013).

1.2. Morphology and biology of rice

Rice is a diploid grass (24 chromosomes) having a fibrous root system, long erect stems with jointed round culms, hairy sickle-shaped auricles, two ligules and long narrow leaves with parallel venation (Chang et al., 1965). Rice is normally grown as an annual plant, although in tropical areas, it can survive as a perennial, and can produce a ratoon crop for up to 30 years. The average height of the rice plant is 1–2.0 m tall, depending upon cultivar, nutrition and competition (Chang et al., 1965). The rice life cycle is dependent on the type of cultivar and climatic conditions. In warm and humid environments, the life cycle ranges from 100-120 days, and in cool and humid environments, it varies from 120-150 days. Its life cycle can be divided into three agronomic stages of development: vegetative, reproductive and grain filling. The small wind-pollinated flowers are produced in a 30-50 cm long branched, arching to pendulous inflorescence. The edible seed is a grain 5–12 mm long and 2–3 mm thick (Anonymous, 2011a). Rice cultivation is well-suited to countries and regions with low labor costs and high rainfall, as it is labor-intensive to cultivate and requires ample water. The ability to adapt and survive on diverse soil types makes this plant to achieve its staple food category. It can survive in a broad range of temperatures and latitudes, as far south as New South Wales (Latitude 35^0 S) to as far north as Czechoslovakia (Latitude 50^0 N), and from sea level to elevations of 3,000 m in the Himalayas (Webster and Gunnell, 1992). It can survive in a broad range of water regimes, upland, lowland, moderately low land and deep water.

1.2.1. Vegetative growth stages

The first stage of vegetative growth is seed germination, in which the seed coat imbibes water and the coleorhiza (sheath covering radicle) swells to release the emerging radicle by rupturing the seed coat (Moldenhaur et al., 2014). Coleoptile elongation to the leaf or root is determined by oxygen conditions of the soil. Under dry-seeded conditions, the radicle emerges before the coleoptile and vice versa in aerobic conditions. Duration of the germination stage is approximately 2 days depending upon the temperature (21 to 36 ^{o}C) (Moldenhaur et al., 2014). The coleoptile continues to grow, and the first internode, called the mesocotyl, emerges. At this point, seed germination is completed. Next, the pre-tillering phase begins with four to five leaves developing over the next 15-20 days (Chang et al., 1965). Also during this period, seminal roots develop. During tiller development, when the first tiller is at the sixth leaf stage, the second tiller forms from the axillary bud of the third leaf. At the same time, secondary roots start to grow. At the maximum tillering stage, it is hard to distinguish the main culm from tillers. This is a very critical stage for yield potential, as tillering and panicle potential is determined at this developmental stage (Moldenhaur et al., 2014). The vegetative lag phase is the stage when active tillering ends, height and stem diameter increases, and the reproductive stage starts.

1.2.2. Reproductive growth stages

The reproductive stage consists of culm elongation, booting, and emergence of the flag leaf (Anonymous, 2011a; Moldenhaur et al., 2014). The duration of the reproductive stage is almost 30 days and varies with cultivar and climatic conditions. It is divided into four sub-stages: panicle initiation, elongation, boot, and heading. The panicle initiation stage involves the panicle primordia initiating, production of the panicle in the first node of the culm (Anonymous, 2011a). It is also called the green ring stage. The panicle is not visible to the naked eye, but collection of chlorophyll above the first node indicates its presence. Below the green ring, hollow internodes are visible by cutting the stem vertically. Within a few days, the hollow internodes elongate initiating the panicle elongation stage. After 5-7 days of panicle elongation, the panicle is visible, and the panicle differentiation stage begins. It is approximately 2.5 to 5 cm of elongated internode. It is the second major factor that affects the yield, as the number of grains per panicle is determined at this stage. When the panicle is fully formed and elongated, it starts to swell, initiating the boot stage. Full boot stage is determined when the flag leaf is fully extended and elongated. All these stages, panicle initiation, elongation, early boot and late boot stages, are approximately 1 week apart. A week after the late boot stage, heading begins with the emergence of the panicle from the boot. Fifty percent emergence of the panicle is considered the agronomic heading stage (Webster and Gunnell, 1992). Anthesis occurs by opening or closing of the spikelet. Pollen are viable for 5 to 10 min after emerging from the anther. The stigma remains receptive for 1 week. As rice is self–pollinated, fertilization occurs before the opening of the lemma and palea. After the ovary is fertilized, grain filling stage begins. The last stages of rice development are milk, when kernels are soft and filled with white milk, soft dough, when the starch has not become firm, and hard dough when it is firm. Maturity is the harvest stage when moisture content is below 22% (Moldenhaur et al., 2014).

1.3 Rice diseases

Rice diseases result from infection by transmissible plant pathogenic biological agents like fungi, bacteria, viruses, and nematodes with the potential to reduce the yield or value of the rice crop directly or by their expression on any plant part (Ohlendorf and Neill, 2009). Non-infectious diseases are due to abiotic stresses or nutrient deficiencies (Scardaci, 1997). Rice is vulnerable to plant diseases at all phases of growth with resulting yield loss (Ohlendorf and Neill, 2009).

Severity of the losses depends upon the virulence of the pathogen, growth stage or susceptibility level of the host, and favorable environmental conditions. Among these, sheath blight caused by *Rhizoctonia solani*, blast (*Pyricularia grisea*), and bacterial panicle blight (*Burkholderia glumae*) are the major diseases of commercially cultivated rice in the United States. Over time, many other diseases also evolved and became serious threats to rice production. One of these diseases is narrow brown leaf spot of rice (NBLS) caused by *Cercospora janseana* (Racib.) O. Const. (Groth and Hollier, 2010)

1.3.1. History of *Cercospora janseana*

The fungal pathogen *Cercospora janseana* causes narrow brown leaf spot and other symptoms of the Cercospora complex on rice (Groth and Hollier, 2010). Raciborski in 1900 found this pathogen and named it *Napicladium janseanum* Racib. and in 1906, Metcelf mentioned the same disease in North America. It was described by the Japanese pathologist 'Miyake' in 1906 and named *Cercospora oryzae* Miyake. In 1982, Constantinescu gave credit to Raciborski and Miyake and renamed it *Cercospora janseana* (Racib.) O. Const. (USDA, ARS, Fungal database; Hollier, 1992). In 2000, Braun explained its phylogenetic relationship with the genus *Passalora* and named it *Passalora janseana* (Racib) U. (Braun, 2000). It belongs to the Division- Eumycota, Sub-division- Deuteromycotina, Class- Hyphomycetes, Order- Hyphomycetales, Family- Dematiaceae, Genus- *Passalora*, Species- *janseana*. The fungus produces white to pinkish mycelia, pale brown, geniculate, multiseptate conidiospores, singly or in fascicles. Conidia are hyaline, cylindrical, narrow clavate, three to ten septate, and 15-60 x 3.6-4.0 um (USDA, ARS, fungal database). *Cercospora janseana (*Racib).O. Const. or *Passalora janseana* Racib. U. is an anamorph stage while *Spherulina oryzina* K. Hara is the teleomorph stage of this fungus, as described by Hara in 1918 (USDA, ARS, fungal database). *Cercospora* *janseana* produces a red to maroon pigmented photo-activated perylenequinone toxin, cercosporin, which is a pathogenicity factor of this pathogen (Daub and Chung, 2007).

1.3.2. Narrow brown leaf spot symptoms and disease cycle

Cercospora janseana causes narrow brown leaf spot of rice (Groth and Hollier, 2010). Symptoms of narrow brown leaf spot (NBLS) disease include long cylindrical dark brown spots with dark margins and greyish centers with or without chlorosis. Lesions range from 1-10 mm x 1-1.5 mm on leaves and 15-45 x 1-2 mm on mid-ribs and leaf sheaths (Groth and Hollier, 2010). Morphology of symptoms varies with the susceptibility of the cultivar. On resistant cultivars, symptoms are long, narrow lesions that sometimes do not develop fully. In susceptible cultivars, spots are broad and necrotic (Groth and Hollier, 2010). Initially, dark spots develop on the leaf lamina and later on the leaf mid-vein, leaf sheath, panicle, seed coat and glumes. Symptoms appear late in the season on all leaves regardless of age. NBLS causes a premature ripening of the grains, reduces yield quantity, and grain milling quality (Groth and Hollier, 2010).

The disease cycle begins when *C. janseana* enters the plant tissues through stomata, establishes beneath the stomata in the parenchyma cells, and spreads longitudinally in intercellular spaces (Mew and Misra, 1994). Upon development, conidiophores emerge through the stomata. Preliminary studies have shown that 30 or more days are required to develop symptoms after inoculation (Chakraborti, 1964). This long latent period may be the probable reason of late appearance of symptoms during the season even though infection occurs at early plant developmental stages. The initial source of inoculum appears to be from *C. janseana* that has survived on residues of previous rice crops, infected seeds, and seasonal weeds (Hollier, 1992).

1.4. Cultivar resistance and plant disease development

Cultivar resistance plays a key role in reduction of rice diseases and *Cercospora* spp. associated diseases on other crops (Lemtur et al., 2013; Miah et al., 2013; Shaik and Ramakrishna, 2013). For example, rice blast and sheath blight development were more suppressed on resistant and moderately susceptible cultivars in comparison to susceptible ones (Lee and Rush, 1983; Bonman et al., 1991; Andi and Nur, 2013). Similar observations for other *Cercospora* spp. associated diseases were found to be impacted by the degree of host resistance For example, Cercospora leaf spot of sugarbeet caused by *C. beticola* and leaf spot in strawberry caused by *Mycosphaerella fragariae* were found to be less severe on resistant and moderately susceptible cultivars (Nathalie et al., 1995; Rossi, 1995; Kaiser et al., 2010). Resistant cultivars were found to delay the disease onset, hinder the development of *C. beticola* on sugarbeet, *C. carotae* on carrot, *C. apii* on celery and *C. arachidicola* on peanut and slowed down the progress of epidemics by low apparent infection rate of resistant cultivars (Berger, 1976; Waliyar et al., 1993; Lacy et al., 1996; Gaurilcikiene et al., 2006; Westerveld et al., 2008). Other studies conducted on rice diseases as well as *Cercospora* spp. diseases on other hosts showed that lower infection frequencies, smaller lesion diameter, lower number of lesions produced and longer incubation periods were the reasons for lower disease development on resistant cultivars (Berger, 1977; Nevill, 1981; Waliyar et al., 1993; Ringer and Grybausks, 1995; Parlevliet, 2002). Cantowine and his co-workers (2008) found the time of disease onset was delayed on less susceptible peanut cultivars to *Cercospora arachidicola* and *Cercosporidium personatum*. Likewise, epidemics of Cercospora leaf spot (CLS) in sugarbeet caused by *Cercospora beticola* started 2 weeks earlier on susceptible compared to resistant sugar beet cultivar (Gaurilcikiene et al., 2006), and the difference in CLS onset was explained by the difference in cultivar susceptibility levels, canopy closure and weather parameters (Wolf and Verreet, 2005). Some of the studies done on *C. arachidicola*, *Cercosporidium personatum* on peanut cultivars and *C. beticola* on sugarbeet had revealed a lack of immunity to disease in resistant cultivars that lead to disease development in their resistant hosts (Nevill, 1981; Weiland and Koch, 2004).

1.5. Planting date and plant disease development

Disease severity of rice blast was reduced by late planting time of susceptible cultivars (Andi and Nur, 2013: Atta et al., 2013). Incidence and severity of diseases caused by *Cercospora* spp. in sesame, pepper and maize were reduced by delayed planting time. Severity of *Cercospora zea-maydis* increased with increase in air inoculum in late planting due to more conducive conditions for disease development that lead to more secondary disease cycles (Bhatia and Munkvold, 2002). Similarly, Cercospora leaf spot of sesame increased by 13% in late planting due to prolonged exposure of plants to disease inoculum and humid conditions in late July increased the incidence of disease (Enikuomehin et al., 2002); Lemtur et al., 2013).

1.6. DMI fungicide application and plant disease development

The DMI (demethylation inhibitor) fungicide propiconazole (Tilt EC 250, Syngenta Corporation), has been used to manage different plant diseases (Jorgensen and Nielsen, 1994; Clarkson et al., 1997; Hossain, et al., 2011). Information regarding use of propiconazole on other rice diseases is known in the United States and other parts of world (Jones et al., 1987; Gupta et al., 2013). Applications of DMI fungicides decreased Cercosporidium leaf spot development in susceptible cultivars of groundnut while resistant cultivars did not need one (Moraes et al., 1994). Studies have also shown that the timing of fungicide applications may played an important role in disease management. Evidence of reduction in severity of Cercospora leaf spot in sugar beet and gray leaf spot of maize showed that early fungicide application, as the detectable level of disease show up, reduced the disease development (Ward et al., 1997; Biancardi et. al, 2005). However, it is possible to eliminate the fungicide applications if resistant or moderately susceptible cultivars were chosen (Kaiser et al., 2010). Similar results have been demonstrated that resistant cultivars did not need a fungicide application. This is the most effective way to control of sheath blight of rice (Groth and Bond, 2007).

1.7. Nitrogen application and plant disease development

There are numerous examples in the literature demonstrating that nitrogen influences the development of plant diseases. In some cases nitrogen applications increase the disease development and in others it hinder the disease development (Hoffland et al., 2000; Huber and Thompson, 2007). High nitrogen fertilizer rates and times have increased the severity of rice blast and sheath blight (Kurschner et al., 1992; Luong et al., 2003). Increase in nitrogen rate as well as high pre-flood nitrogen increased the blast, bacterial blight and sheath blight on susceptible cultivars, respectively (Reddy et. al., 1979; Savary et al., 1995; Long et al., 2000; Mukerjee et al., 2005; Slaton et al., 2005; Bhat et al., 2013). Likewise, Long and his co-workers (2000) found rice blast severity and incidence increased when more than the recommended rate of nitrogen was applied to susceptible cultivars except for the resistant ones. On the other hand, reports have been documented that showed less or no nitrogen application aggravated plant disease development. The severity of Cercospora leaf blight (*C. carotae*) in carrot was reduced when the recommended nitrogen rate (100%) and more than the recommended 150% and 200% nitrogen was applied as compared to no nitrogen application or 50% less nitrogen rates (Westerveld et. al., 2008). Okari and his co-workers (2004) found that nitrogen applied plots had less severity of gray leaf spot of maize caused by *C. zeae-maydis* in comparison to those that did not receive nitrogen. Caldwell and his colleagues (2002) showed that the severity of gray leaf spot and Cercospora leaf spot in hot pepper (Vos and Frinking, 1997) increased as the nitrogen rate increased for susceptible cultivars. Cercospora leaf spot, leaf spotting and defoliation were often lower at the highest rather than with lowest N rates (Hagan et al., 2008). Researchers have shown that time of nitrogen application impact disease development. For example, impact of late (30 days after sowing (DAS) and 60 (DAS) verses early (at the time of planting and 30 DAS) splits, single verses split nitrogen application for rice blast resulted in less rice blast development (Kuschner et al., 1992; Tajani et al., 1997; Kapoor and Sood, 2000).

1.8. Tillage practices and plant disease development

Reduced and no tillage were found to increase the level of sheath blight caused by *Rhizoctonia solani* as compared to conventional tillage practices (Cartwright et al., 1996; Cartwright et al., 1997). On the other hand, incidence and severity of blast increased in conventional tillage compared to zero tillage or direct drilling (Silva et al., 2003; Sester et al., 2014). Conventional tillage also lowered the incidence and severity of false smut but had no effect on kernel smut (Andres et al., 2008; Brooks et al., 2009, 2011). Rice yield was increased under conventional tillage while population and viability of sclerotia of *Sclerotium oryzae* causing stem rot of rice was completely lost (Hussain and Ghaffer, 1993).

Studies done on *Cercospora zeae-maydis* have shown higher numbers of conidia trapped from the no till versus tilled plots. Gray leaf spot lesions developed early in the season and doubled on the leaves at harvesting stage in no till plots (Peyne et al., 1987). Early development of gray leaf spot symptoms allowed for more secondary cycles and inoculum build-up over time. Researchers also found lower incidence of *Cercospora zeae-maydis* on leaf and sheath samples buried in fall than on the soil surface in spring due to survival of inoculum on the soil and crop debris in spring season (De Nazareno et al., 1992). Diseases caused by other *Cercospora* spp.

like frogeye leaf spot of soybeans and Cercospora leaf spot of soybean were reduced with conventional tillage by reducing inoculum on infested crop and soil debris as well as physical separation of the pathogen from plants by burying them deep in the soil (Yang, 2002). On the other hand, the severity of sugar beet leaf spot caused by *Cercospora beticola* was not affected by tillage practices like ploughing, mulching and direct drilling (Pringas and Marlander, 2004).

1.9. Seeding density and plant disease development

Seeding rates influence the level and intensity of plant disease For example, incidence of sheath blight and blast increased with increase in seed rate (Wu et al., 2014). Higher seed rates increased severity of rice diseases, including sheath blight, blast, brown spot, and false smut (Ottis et al., 2006; Mithrasena et al., 2007). On the other hand, some studies showed no impact of seeding rates on either sheath blight or rice kernel smut severity (Marchetti, 1983; Brooks et al., 2009).

Evidence of increase in *Cercospora* spp. with increase in seed rates have been reported for maize, sugar beets, peppers (Peyne et al., 1987; De Nazareno et al., 1992; Yang, 2002; Pringas and Marlander, 2004). A number of studies have demonstrated that higher seed rate will favor a higher plant density that will result in a more compact growth and closed plant canopy; consequently, these conditions are more favorable for enhancing severities of gray leaf spot of maize, Sclerotinia blight in peanut and peanut stem rot caused by *Sclerotium rolfsii* (De Nazareno et al., 1991; Andrea et al., 2006; Sconyers et al., 2007; Ahmed et al., 2011).

1.10. Rationale for the study and project objectives

Narrow brown leaf spot was first observed in the United States in 1937 (Ou, 1980). It was prevalent disease in all rice growing areas of Arkansas, Louisiana, Mississippi and Texas. Several outbreaks of NBLS have been reported in the past in rice growing areas of the world where it caused more than 40% yield losses (Anonymous, 2011b). NBLS was not considered as economically important diseases of rice in southern US rice growing states, but an epidemic in 2006 in southwestern parts of Louisiana caused economic significant yield losses (Smith, 2007; Groth, 2013). Susceptible cultivars, warm spring and wet summer aggravated the disease epidemic early in the season leading to a severe outbreak of NBLS (Groth, 2013). Since then, severe epidemics have not occurred, but most of the high yielding rice cultivars grown in Louisiana are either susceptible or very susceptible to NBLS. Currently available resistant cultivars are less popular among the producers due to their lower yield potential (Personal Communications, C. Hollier and D. Groth).

Frequent emergence of new races of *C. janseana*, sole dependence makes it not advisable to relay on resistance (Groth and Hollier, 2010, Groth, 2013). Physiological races of *C. janseana* were identified in 1940's and 1980's (Ryker, 1943, Sah and Rush, 1988) and evidence of resistance break down was reported in that era. However, after 1980's, no follow up studies were done on the potential development of new races even though resistant cultivars were reported to be susceptible (Communications with Dr. Hollier and Dr. Groth). It was speculated that new races probably evolved and this might explain why resistance broke down. Due to the erratic nature of NBLS and its potential threat to rice production, the development of integrated management strategies for NBLS is needed.

An important component to disease management is fungicide application. Information regarding the use of propiconazole on other rice diseases is well known in the United States and other parts of the world (Jones et al., 1987; Gupta et al., 2013), but no scientific data is available about its rate and optimum time of application for managing NBLS. The effects of planting date and propiconazole application on NBLS and rice yield of cultivars with different levels of susceptibility are unknown (Personal Communications, C. Hollier and D. Groth). Other aspects of disease management like nitrogen management and possible interactions with the susceptibility level of cultivars need to be included in an integrated management of NBLS.

Most of the rice production in the United States is done by a conventional tillage system (Saichuk, 2012). However, reduced or minimum tillage also has gained acceptance over time due to advantages of easy flood management and reduced sediment losses. The main issues of rice growing under reduced tillage are stand establishment and early season plant density (Saichuck, 2012). These issues eventually affect crop yield and are equally important in order to manage diseases under different tillage systems (Sharma et al., 2007; Brooks et al., 2009*)*. Currently, no information is available about the interaction of cultural practices like tillage preparation and seeding density on NBLS development.

Studies of NBLS have been limited by difficulties in isolation, sporulation, and slow growth of the fungus on artificial medium, as well as lesion development on the inoculated plants in the greenhouse. Since, NBLS has become a serious disease in rice, its etiology, biology, and management aspects need more in depth study. To develop an integrated approach to manage this disease, objectives were as follows:

1) to determine the effects of planting dates, relative susceptibility of cultivars, and fungicide timings on the development of NBLS and yield of rice,
2) to study NBLS progression on cultivars with different susceptibility at different planting dates,
3) to study the effects of nitrogen application on the development of NBLS,
4) to study the effects of seeding density and tillage system on the development of NBLS and yield of rice.

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CHAPTER 2
ROLE OF PLANTING DATE, FUNGICIDE TIMING AND CULTIVAR SUSCEPTIBILITY ON SEVERITY OF NARROW BROWN LEAF SPOT AND YIELD OF RICE

2.1. Introduction

Narrow brown leaf spot (NBLS), an erratic foliar disease of rice, caused by *Cercospora janseana* (Racib.) O. Const. (Groth and Hollier, 2010). The disease has been commonly found on rice in Australia, Asia, and Latin America (Mew and Misra, 1994). NBLS was first reported in 1906 by the Japanese pathologist (Hollier, 1992; Mew and Gonzales, 2002) and was reported as a serious problem in rice growing areas in the United States in the 1940's (Ryker, 1943). Severe epidemics have been reported over the last few decades in rice growing areas of the world causing more than 40% yield losses (Anonymous, 2011). Due to its erratic nature, it was not previously considered as a major consistent disease of rice in the United States Therefore, few studies have been done on its epidemiology, yield losses, and management. During The NBLS gained i2006, a severe its epidemic occurred in the southwestern rice production area (Groth and Hollier, 2010). Susceptible cultivars, a warm spring, and wet summer resulted in early initiation and severe NBLS development (Groth, 2013).

The DMI (demethylation inhibitor) fungicide propiconazole (Tilt EC250, Syngenta Corporation) has been used to manage rice other diseases the in the United States and other parts of world (Jones et al., 1987; Hossian et al., 2011; Gupta et al., 2013), but little is known about optimum rates and times of application for managing NBLS. Cultivar resistance plays a key role in NLBS development, and rice cultivars with different degree of susceptibility from resistant to very susceptible are available. In addition, the severity of diseases caused by *Cercospora* can be affected by planting date (Enikuomehin et al., 2002; Swamy et al., 2012; Lemtur et al., 2013). However, the effects of propiconazole application on NBLS and rice yield of cultivars with different susceptibility levels and crop planting times are unknown. Therefore, the objectives of this study were to determine the effects of cultivar susceptibility level, timing of fungicide application, and planting date on NBLS severity and yield of rice.

2.2. Materials and methods

2.2.1. Cultivar selection and planting dates

Field experiments were conducted at the LSU Agricultural Center Rice Research Station in Crowley, LA during 2011 and 2012. The cultivars for the study were selected based on NBLS natural infection severity ratings (Saichuk et al., 2010). very susceptible NBLS cultivars ‘Cheniere and CL131’, susceptible cultivar ‘CL111’, moderately susceptible cultivar ’CL151’, and resistant cultivars ‘Della’ (2011) and ‘Presidio’ (2012) were drill-seeded on 6 April (early planting date) and 17 May (late planting date) in 2011, and 15 April and 21 May in 2012, at 136 kg ha^{-1}. Plots were 1.2 x 4.9 m and consisted of seven rows with an 18-cm spacing. Soil type was silt loam (pH 6.0, clay 12%, silt 71% and 17% sand, CEC 9.4/kg). Fertilizer (N-P-K) was incorporated 1 day before planting at the rate of 24-67-67 kg ha^{-1}. Agronomic, weed, and insect management practices followed current standard recommendations (Saichuk, 2012). Nitrogen was applied prior to flooding at 133 kg N ha^{-1} as urea at the three to four leaf stage, and after flooding at the rate of 51 kg N ha^{-1} as urea at the beginning of stem internode elongation. Mid-April plantings were harvested on 30 July during 2011and 2 August, and 2012. Mid-May plantings were harvested on 15 and 18 September in years 2011 and 2012, respectively.

2.2.2. Fungicide application

Propiconazole (Tilt 250 EC, Syngenta, Raleigh, NC) was applied at the rate of 0.17 kg a.i. ha^{-1}, at either panicle initiation (PI, 3 to 5 mm panicle in the boot), early boot (EB, 5- to 10-cm panicle in the boot), or late boot (LB, just before heads emerge from the boot) rice growth stages (Table 2.1). Treatments were applied at a delivery rate of 140 L ha^{-1} with a CO_2-

Table 2.1. Fungicide application time schedule for mid-April and mid-May plantings during 2011 and 2012.

		Growth stage and dates for fungicide application[a]		
Year	Planting date	Panicle initiation	Early boot	Late boot
2011	Mid-April	15 June (70)	30 June (85)	6 July (91)
	Mid-May	22 July (60)	29 July (67)	4 Aug. (72)
2012	Mid-April	21 June (67)	3 July (79)	10 July (86)
	Mid-May	18 July (58)	26 July (66)	2 Aug. (73)

[a] Numbers in the parentheses are days after sowing.

pressurized backpack sprayer, using 8002 flat fan tips.

2.2.3. Disease assessment and yield determination

To follow the NBLS epidemics, five arbitrarily selected plants in the central two rows of the experimental unit were selected and their lower, middle and flag leaves were tagged as they appeared. Lower and middle leaves were tagged 45 days after sowing (DAS), and flag leaves were tagged 64 DAS. At 109 DAS, final disease severity at harvest was rated for each leaf using a 0-9 scale where 0 = no disease, 1 = 1%, 2 = 3%, 3 = 5%, 4 = 12%, 5 = 25%, 6 = 40%, 7 = 65%, 8 = 75% and 9 = more than 75% leaf area diseased (Groth et al., 1993). NBLS severity on lower, middle and flag leaves was averaged across leaves and used as percentage of diseased leaf area for the entire plant. The center four rows of each experimental unit were harvested with a small-plot combine (Mitsubishi, VM13). Grain yield and moisture were determined, and rice yield was adjusted to 12 g kg^{-1} moisture content.

2.2.4. Data analysis

Treatments were replicated four times in a randomized complete block design. Factors included cultivars and fungicide timings for the two planting dates. Box plots and Kolmogorov-Smirnov (KS) tests were used as a test for data normality. Final NBLS disease severity values were used to analyze the effect of treatments. Final NBLS percentage was logit transformed as ln x/ (1-x) where x is proportion of disease (Campbell and Madden, 1990; Arneson, 2001). Transformed data were analyzed using the SAS mixed procedure and factorial design with repeated measures (SAS 9.3 Institute, Cary, NC). Replications were taken as a random factor, and year, planting date, cultivar, and fungicide application timing including a non-sprayed control were taken as fixed factors. Tukey's Kramer method of means comparison was used to compare least square means. Significant differences among the treatments were determined using SAS Macro function. After analysis, logits were back transformed to percentage using the formula {Exp. (logit)/1+Exp. logit}*100 (Arneson, 2001) for presentation in tables and figures.

2.3. Results

2.3.1. Analysis of variance

Test of normality KS (D = 0.07) showed that disease severity data were not statistically normal. NBLS was affected by cultivar, planting date, fungicide application timing, and their interactions (P-value ≤ 0.05) (Table 2.2). Yield was affected by cultivar, planting date, and fungicide timing and interactions planting date x cultivar and planting date x fungicide timing. Years and replications were not significantly different.

2.3.2. Effects of planting date, cultivar and fungicide application time on NBLS severity

NBLS severity was higher in the mid-May planting in all the treatments, cultivars and fungicide timings, as compared to mid-April (Table 2.3). The very susceptible cultivars (CL131 and Cheniere) had the highest and the resistant cultivars (Della and Presidio) had the lowest disease severity in both the planting dates. In the mid-April planting with lower severity, the resistance ratings in plots without fungicide application were reflected with decreasing severity

Table 2.2. Analysis of variance of factors affecting final NBLS severity and yield of rice.

Factors	Final disease severity (P-value)	Yield of rice (P-value)
Planting date (PD)	0.001	0.001
Cultivar (VAR)	0.001	0.001
Fungicide treatment (FT)	0.001	0.002
PD x VAR	0.038	0.014
PD x FT	0.042	0.041
VAR x FT	0.013	0.165
PD x VAR x FT	0.049	0.518
Year (Y)	0.053	0.214
Y x PD	0.329	0.584
Y x VAR	0.983	0.545
Y x PD x VAR	0.129	0.614
Y x FT	0.911	0.092
Y x PD x FT	0.556	0.214
Y x VAR x FT	0.42	0.522
Y x PD x VAR x FT	0.690	0.965
Replication (R)	0.605	0.369
R x PD	0.062	0.094
R x VAR	0.643	0.999
R x PD x VAR	0.536	0.856
R x FT	0.717	0.344
R x PD x FT	0.623	0.454
R x VAR x FT	0.486	0.635
R x PD x VAR x FT	0.728	0.093
Y x R	0.208	0.985
Y x R x PD	0.435	0.779
Y x R x VAR	0.774	0.946
Y x R x PD x VAR	0.899	0.667
Y x R x FT	0.599	0.1798
Y x R x PD x FT	0.164	0.5481
Y x R x VAR x FT	0.523	0.8412

as the level of resistance increased. Very susceptible cultivars CL131 and Cheniere had the highest disease followed by susceptible CL111, moderately susceptible CL151 and least in resistant Della and Presidio with more disease in Della. In the mid-May planting with higher disease levels, susceptible (CL111) and moderately susceptible (CL151) cultivars had similar disease severity.

Fungicide application reduced disease severity in the very susceptible, susceptible, and moderately susceptible cultivars in both planting dates (Table 2.3). Fungicide treatment had variable lesser effects on the resistant cultivars. Untreated plots of resistant cultivars had less disease than the treated plots of very susceptible and susceptible cultivars at both planting dates. Fungicide application at panicle initiation was the most effective time for very susceptible and susceptible cultivars at both planting dates. For the moderately susceptible cultivar, fungicide application at panicle initiation or early boot stage were equally effective in the mid-April planting, but panicle initiation was the most effective application time in mid-May. In the mid-May planting, differences in fungicide efficacy were evident between application timings for all susceptible cultivar types with the greatest disease reduction resulting from application at panicle initiation (the earliest treatment time) and the least reduction from the application at late boot

2.3.3. Effects of planting date and cultivar on yield

Yield was influenced by time of planting and cultivar NBLS susceptibility level; however, the effect of cultivar was not the same for both planting dates (Figure 2.1). In the mid-April planting under lower disease pressure, very susceptible Cheniere and CL131, susceptible CL111, and moderately susceptible CL151 had similar yield while resistant Presidio yielded the lowest. Yields were lower in the mid-May planting under higher disease pressure for the very susceptible and susceptible cultivars while yields of the moderately susceptible and resistant cultivars were similar to the mid-April planting. The very susceptible cultivar, Cheniere, had higher yield in the mid-May planting as compared to the other very susceptible cultivar CL131, susceptible CL111, and the resistant cultivars Della and Presidio.

Table 2.3. Final NBLS severity at crop harvest for mid-April and mid-May plantings in cultivars with different NBLS susceptibility levels and fungicide application timings.

Mid-April				Mid-May			
Cultivar	Susceptibility level of the cultivars[a]	Fungicide treatment[b]	Final disease percentage	Cultivar	Susceptibility level of the cultivars	Fungicide treatment	Final disease percentage
CL131	VS	UN	19.5 ef	CL131	VS	UN	31.1 a
Cheniere	VS	UN	19.0 fg	Cheniere	VS	UN	30.8 a
CL111	S	UN	18.2 h	CL111	S	UN	25.7 b
CL151	MS	UN	13.0 l	CL151	MS	UN	25.7 b
CL131	VS	LB	11.7 j	Cheniere	VS	LB	25.6 b
Cheniere	VS	LB	10.2 jk	CL131	VS	LB	24.7 c
CL111	S	EB	10.0 kl	CL131	MS	EB	24.4 c
CL111	S	LB	9.9 kl	Cheniere	VS	EB	22.5 d
CL131	VS	EB	9.4 lm	CL111	S	LB	20.2 de
CL151	MS	LB	8.9 mn	CL131	VS	PI	18.5 g
Cheniere	VS	EB	8.6 n	CL151	MS	LB	18.4 gh
Cheniere	VS	PI	8.7 op	CL111	S	EB	18.1 h
CL151	MS	EB	7.1 q	Cheniere	VS	PI	17.1 i
CL151	MS	PI	7.0 q	CL111	S	PI	15.7 j
CL111	S	PI	6.2 r	CL151	MS	EB	15.2 k
Della	R	UN	5.7 rs	CL151	MS	PI	12.8 l
CL131	VS	PI	5.6 s	Della	R	UN	9.0 mn
Della	R	PI	4.8 t	Della	R	EB	8.4 n
Della	R	EB	4.8 t	Presidio	R	UN	8.2 n
Della	R	LB	4.4 t	Della	R	LB	8 n
Presidio	R	UN	3.8 u	Della	R	PI	7.4 o
Presidio	R	LB	3.7 u	Presidio	R	EB	5.7 s
Presidio	R	EB	3.6 u	Presidio	R	LB	5.7 s
Presidio	R	PI	3.6 u	Presidio	R	PI	5.6 s

[a] Susceptibility level of cultivars. VS- Very susceptible; S- Susceptible; MS- Moderately susceptible; R- Resistant

[b] UN- Untreated, PI- Panicle initiation stage, EB- Early boot stage, LB- Late boot stage of crop growth are the timings of fungicide applications.

Treatment means were compared by Tukey's test with P=0.05.

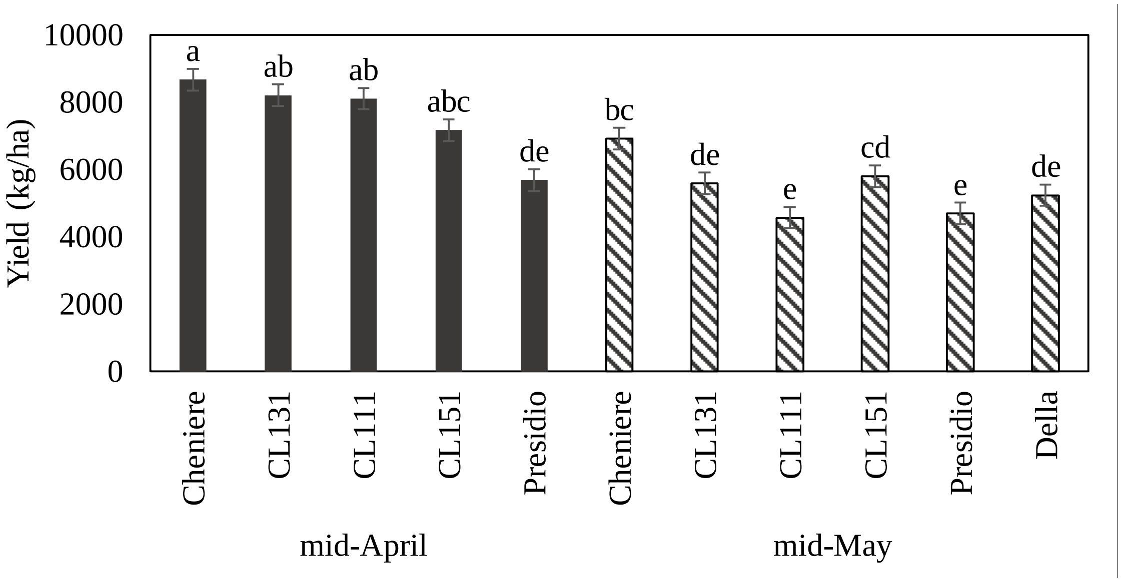

Figure 2.1. Effect of planting dates on yield of different cultivars with different NBLS susceptibility levels in mid-April and mid-May plantings. Cultivars Cheniere and CL131 were rated very susceptible, CL111 was rated susceptible, CL151 was rated moderately susceptible, and Della and Presidio were rated resistant. Means were compared with a Tukey's test, with mean standard errors indicated. Means with the same letters were not significantly different ($P = 0.05$). Black bars represent the yield of cultivars in the mid-April planting, and patterned bars represent yield of different cultivars in the mid-May planting.

2.3.4. Effects of planting date and fungicide application timing on yield

All fungicide treatments increased yield across cultivars regardless of planting date compared to the untreated control; however, the effect of fungicide application timing was not the same for both planting dates (Figure 2.2). For the mid-April planting date, no differences in yield were found among the fungicide application timings. For the mid-May planting, yield was higher with early application at panicle initiation than at early and late boot growth stages. Yield increases resulting from fungicide application were greater in the mid-May as compared to mid-April planting. Yield increases of 8.3, 9.4 and 6.1% were recorded in the mid-April planting

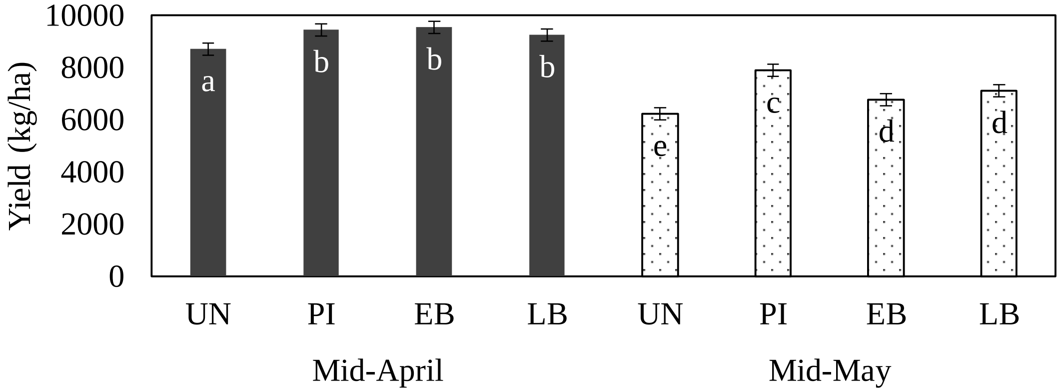

Figure 2.2. Effect of fungicide application timing on yield in mid-April and mid-May plantings. Means were compared with a Tukey’s test with mean standard errors indicated. Different letters represent significant differences between means (P = 0.05). Numeric values above the bars represent percent difference in yield for fungicide application as compared to the untreated control in the respective planting dates. UN-untreated; PI-panicle initiation fungicide application timing; EB-early boot stage application; LB-late boot stage application.

when fungicide was applied at panicle initiation, early boot, and late boot, respectively, as compared to the untreated plots. In the mid-May planting, fungicide application at panicle initiation stage had 34.2% higher yield as compared to the untreated. Application at early boot or late boot stages had 28.7% and 30.2 % higher yield, respectively, as compared to the untreated.

2.4. Discussion

Severe epidemics of NBLS in Louisiana prompted a research project to evaluate the effects of planting date, cultivar susceptibility level, and fungicide application timing on NBLS severity and yield. In the southern states of the United States, rice plantings are done from mid-March to mid-May. In the current study, NBLS severity was higher in rice planted in mid-May (late planted) than mid-April regardless of cultivar susceptibility level. Studies done on Cercospora leaf spot of sesame (Enikuomehin et al., 2002; Lemtur et al., 2013), Cercospora leaf spot and frogeye leaf spot of soybean (Dube et al., 2003) and Cercospora leaf spot of pepper

(Swamy et al., 2012) demonstrated increases in disease severity in late plantings. In addition, rice blast was increased in late plantings (Andy and Nur, 2013).

The cultivars rated as very susceptible had the highest levels of disease, and the cultivars rated as resistant had the least disease for both planting dates with variable levels of disease pressure. The resistant cultivars, Della and Presidio, had minimum disease development even under high disease pressure. The cultivars rated as susceptible and moderately susceptible could be distinguished only under lower disease pressure. The results suggest a quantitative expression of resistance to NBLS occurs within susceptible cultivars that is probably affected by inoculum pressure.

A single fungicide application at panicle initiation or early boot stage was found most effective to suppress NBLS in mid-April, but in the late planting (mid-May) under higher disease pressure, application at panicle initiation was the most effective time to control NBLS for all the susceptible cultivars. NBLS was found earlier in the growth cycle of mid-May planted crop compared to mid-April. NBLS was first observed 72 days after sowing in the lower plant canopy in the mid-April planting and 58 days after sowing in the mid-May planting (personal observations, data not shown). Plants were exposed to *C. janseana* spores for a longer time when planted late. Therefore, an early application of a fungicide (PI stage) gave the best protection from NBLS infection. The current study revealed that application at panicle initiation is the most effective time of fungicide application to control NBLS. However, the effect of multiple applications needs to be investigated. The effect of fungicide application timing on NBLS varied with cultivar susceptibility and disease pressure. NBLS reductions resulting from fungicide application were slight and variable for the two resistant cultivars. The results suggested that a fungicide application may not be cost effective for resistant cultivars. Reductions in NBLS severity resulting from fungicide application were greater under higher disease pressure, and cultivar susceptibility level affected final disease severity in the fungicide treatments. The results suggest partial resistance in combination with well-timed fungicide application could provide an effective NBLS management strategy under heavy disease pressure. Development of leaf spot of groundnut caused by *Cercosporidium personatum* was suppressed in resistant and susceptible cultivars by a fungicide application (Moraes et al., 1994). Severity of Cercospora leaf spot in sugar beet and gray leaf spot in maize was reduced by early fungicide application (Ward et al., 1997; Biancardi et. al, 2005) and by selection of resistant or moderately susceptible cultivars (Kaiser et al., 2010).

Yield was comparatively less in mid-May (late) planting compared to mid-April probably due to higher disease pressure and environmental conditions. The lower yields for susceptible but not resistant cultivars in the late planting, the higher disease severities, and the greater responses to fungicide application all suggest that lower yields in the mid-May plantings were associated with NBLS. Along with this, early maturity of rice crop due to warm and humid conditions may have affected the yield (Saichuk, 2012). In spite of having resistant reactions to NBLS, the yield potential of Della and Presidio is lower than for the susceptible cultivars. Under low disease pressure, no differences in yield were observed among the very susceptible, susceptible and moderately susceptible cultivars, whereas yield differences were detected under higher disease pressure. These differences suggest variable responses to NBLS among the susceptible cultivars. There is a need to develop cultivars with good yield parameters and disease resistance.

The results of this study have implications for NBLS management. Late planting resulted in higher disease pressure and hence a need to plant cultivars with resistance and/or fungicide application. Cultivar resistance can effectively control NBLS. Early planting will result in lower severity in susceptible cultivars, but fungicide application may still be needed. Further research is needed to determine if fungicide application on resistant cultivars is economical or not. Application of propiconazole, reduced NBLS. Therefore, disease in very susceptible and susceptible cultivars can be managed by applying fungicides, particularly in the current situation where the yield potential of NBLS susceptible cultivars is greater than for resistant cultivars.

2.5. Conclusions

The study results have demonstrated that susceptible cultivars had the highest and resistant ones had the lowest NBLS in both mid-April and mid-May planting. Fungicide application at panicle initiation stage or early boot stage was an effective time of fungicide application in the very susceptible, susceptible and moderately susceptible cultivars in mid-April, whereas panicle initiation was found to be the best in late planting. NBLS reductions resulting from fungicide application were slight and variable for the two resistant cultivars. Resistant cultivars need a fungicide application in late planting or under higher NBLS pressure but do not need one under low NBLS pressure. Yield was found to be higher in mid-April than mid-May. Yield was determined to be similar for all the cultivars at mid-April planting, except for the resistant cultivars. On the other hand, at late planting, yield was highest in very susceptible, Cheniere, and followed by moderately susceptible, CL151. For all the fungicide treatments, yield was found to be the same but higher than the untreated cultivars in mid-April planting but application at panicle initiation gave the highest increase in yield during late planting with variable results among cultivars.

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CHAPTER 3
EFFECT OF CULTIVAR SUSCEPTIBILITY AND PLANTING DATE ON NARROW BROWN LEAF SPOT PROGRESSION IN RICE

3.1. Introduction

Narrow brown leaf spot of rice (NBLS) is caused by the fungal pathogen *Cercospora janseana* Racib. O. Const. also known as *Cercospora oryzae* Miyake or *Passalora janseana* (Racib.) U. Braun (Braun, 2000). NBLS is an erratic foliar disease in the rice growing states of the United States, including Arkansas, Mississippi, Louisiana and Texas, as well as other rice growing regions of the world (Hollier, 1992; Mew and Misra, 1994). Several outbreaks of NBLS were reported in the past in rice growing areas of the world where it caused more than 40% yield loss (Anonymous, 2011). NBLS was not in the category of economically important diseases of rice in southern rice growing states of the United States, but an epidemic in 2006 in south-western parts of Louisiana caused economic yield losses (Smith, 2007; Groth, 2013). Susceptible cultivars, a warm spring, and early summer onset resulted in an early start for the epidemic leading to a severe outbreak (Groth, 2013). Since then, severe epidemics have not occurred, but most of the high yielding rice cultivars grown in Louisiana are either susceptible or very susceptible to NBLS. Currently, available resistant cultivars are less popular among producers due to their lower yield potential. Although resistant cultivars are available, sole dependence on resistance is not advisable due to the frequent emergence of new races of *C. janseana* (Groth and Hollier, 2010a; Groth, 2013). Due to the erratic nature of NBLS and its potential threat to rice production, better understanding of factors affecting epidemic severity is needed.

Susceptible cultivars and late planting had been reported to affect disease development caused by *Cercospora* spp. (Elmer et al., 1996; Enikuomehin et. al., 2002; Dube et al., 2003). Resistant cultivars delayed the appearance and hindered the development of *C. beticola* on sugarbeet, *C. apii* on celery and *C. arachidicola* on peanut cultivars and slowed down the progress of the epidemic due to a low apparent infection rate (Berger, 1976; Waliyar et al., 1993; Gaurilcikiene et al., 2006). Disease severity of rice blast (*Pyricularia grisea*) and gray leaf spot (*Cercospora zea-maydis*) in maize were also reduced by changing the planting time of susceptible cultivars (Bhatia and Munkvold, 2002; Andi and Nur, 2013; Atta et al., 2013).

This study was conducted to evaluate the effects of cultivar susceptibility and time of planting on NLBS epidemic development. The objectives of this study were to determine and compare the temporal increase of NBLS epidemics as determined by apparent infection rate and area under the disease progress curve in cultivars with different susceptibility levels in mid-April and mid-May plantings and evaluate those parameters and final disease severity to find a suitable method of NBLS assessment that can be used for selection of cultivars for resistance. Information generated from this study will help to develop effective disease management strategies for NBLS.

3.2. Materials and methods

3.2.1. Cultivars and planting dates

Field experiments were conducted at the Louisiana State University Agricultural Center Rice Research Station in Crowley, LA. Two cultivars rated very susceptible, ‘Cheniere and CL131’, one susceptible cultivar, ‘CL111’, one moderately susceptible cultivar, ‘CL151’, and two resistant cultivars, ‘Della’ (2011) and ‘Presidio’(2012) (Saichuk et al., 2012) were drill-seeded on, 6 April and 17 May in 2011, and 15 April and 21 May in 2012, at a seeding rate of 136 kg ha^{-1}. Experimental units were 1.2 x 4.9 m and consisted of seven rows with 18 cm spacing. Soil type was a Crowley silt loam (pH 6.0, clay 12%, silt 71%, 17% sand, and CEC 9.4/kg). Fertilizer (N-P-K) was incorporated 1 day before planting at the rate of 24-67-67 kg ha^{-1}. Agronomic, weed, and insect management practices followed current standard recommendations

(Saichuk, 2012). Nitrogen was applied prior to flooding at 133 kg N ha^{-1} as urea at the 3 to 4 leaf stage, and after flooding at the rate of 51 kg N ha^{-1} as urea at the beginning of stem internode elongation.

3.2.2. Disease assessment and parameters

To assess NBLS, five arbitrarily selected plants in the central two rows of each plot were selected and their lower, middle and flag leaves were tagged as they appeared. Lower and middle leaves were tagged 45 days after sowing (DAS) and flag leaves tagged after 64 DAS. At each sampling date, the selected leaves were visually evaluated for NBLS to determine progression over time. Onset of the disease was noted as a pinhead sized lesion observed on the leaf lamina. Disease severity was rated weekly using a 0-9 scale, where 0 = no disease, 1 = 1%, 2 = 3%, 3 = 5 %, 4 = 12%, 5 = 25%, 6 = 40%, 7 = 65%, 8 = 75% and 9 = more than 75% leaf area diseased (Groth et al., 1993). Weekly disease assessment began at 45 days after sowing (DAS) and the last or final disease assessment was done at 109 DAS. NBLS severity ratings for lower, middle, and flag leaves of selected plants were converted to the rating percentage interval midpoint, averaged, and used as one datum for the observation. NBLS percentage data were transformed using the logit transformation method for polycyclic diseases, $\ln x/(1-x)$, where x is proportion of disease (Campbell and Maddan, 1990; Arneson, 2011). In addition, the apparent infection rate was calculated for each epidemic by regressing logits over time. A second method for measuring disease increase was area under the disease progress curve (AUDPC) (Shaner and Finney, 1977). Disease assessment ratings from 52 to 109 DAS were used to calculate AUDPC. No disease was observed at 45 DAS for both the planting dates and years.

3.2.3. Data analysis

Cultivars were replicated four times in a randomized complete block design for each planting date. Della was selected as resistant cultivar in 2011, but it was completely lodged at the time of harvesting. Therefore, in 2012, another resistant cultivar, Presidio, was used for the study. Logits were analyzed using the SAS mixed procedure with a factorial design (SAS 9.3, Cary, NC). Years, planting dates, and cultivars were fixed factors, and replications were a random factor in the mixed procedure. Tukey's Kramer method of means comparison was used to compare least square means for final disease severity, AUDPC, and apparent infection rate.

3.3. Results

3.3.1. Factors affecting disease progress

Cultivar susceptibility level affected all three disease parameters, AUDPC, apparent infection rate, and final disease severity (Table 3.1). Planting date significantly affected AUDPC and final disease but not apparent infection rate. Significant interactions were detected between cultivar and planting date for AUDPC and final disease but were non-significant for apparent infection rate. Interaction of fixed factor, cultivars and planting dates, with year and replications was found to be non-significant ($P > 0.05$).

3.3.2. Disease progress over time for different cultivars and planting dates

NLBS increased with time in all cultivars at both planting dates (Figure 3.1). Onset of disease varied with cultivar susceptibility level and planting date. Earlier onset of NBLS was observed for the mid-May planting date as compared to mid-April planting in all susceptible cultivars. In mid-April planting, the average epidemic onset was 64 DAS in the very susceptible Cheniere and CL131 and susceptible CL111, and 72 DAS in moderately susceptible CL151 and resistant Della and Presidio cultivars. In comparison, for the mid-May planting, disease onset in

Table 3.1. Analysis of variance of factors affecting NBLS parameters, area under the disease progress curve, apparent infection rate, and final disease severity of NBLS.

	Probability values for factors affecting NBLS parameter[c]		
Factors	AUDPC[a, c]	Apparent infection Rate[c]	Final disease severity [b, c]
Year (Y)	0.123	0.896	0.351
Replication (R)	0.167	0.158	0.174
Cultivars (C)	0.001	0.059	0.001
Planting date (PD)	0.001	0.204	0.001
C x PD	0.001	0.122	0.001
Y x C	0.761	0.142	0.059
Y x PD	0.193	0.985	0.901
Y x PD x C	0.080	0.548	0.224
R x C	0.116	0.315	0.238
R x PD	0.325	0.402	0.336
R x PD x C	0.962	0.513	0.523
Y x R	0.938	0.841	0.128
Y x R x C	0.999	0.242	0.206
Y x R x PD	0.690	0.204	0.245

[a] AUDPC= Area under disease progress curve

[b] Final disease severity in percentage at 109 days after sowing.

[c] Probability values were significant at P-value ≤ 0.05.

very susceptible and susceptible cultivars averaged 58 DAS, but onset was not until 64 DAS in moderately susceptible and resistant cultivars. Differences in disease progress with time were detected among cultivars and planting dates (Figure 3.1). Resistant cultivars Della and Presidio had the lowest disease increase over the season at all the observation times for both planting dates. Even with similar later onset of disease in resistant and moderately susceptible cultivars, the resistance of Della and Presidio resulted in a slower disease progression.

AUDPC was highest in the very susceptible cultivars, Cheniere and CL131 and lowest in resistant cultivars for both mid-April and mid-May plantings (Table 3.2). The AUDPC for mid-May plantings were higher for all cultivars. AUDPC increased with increasing susceptibility rating in the mid-April planting. It was similar in moderately susceptible (CL151) and

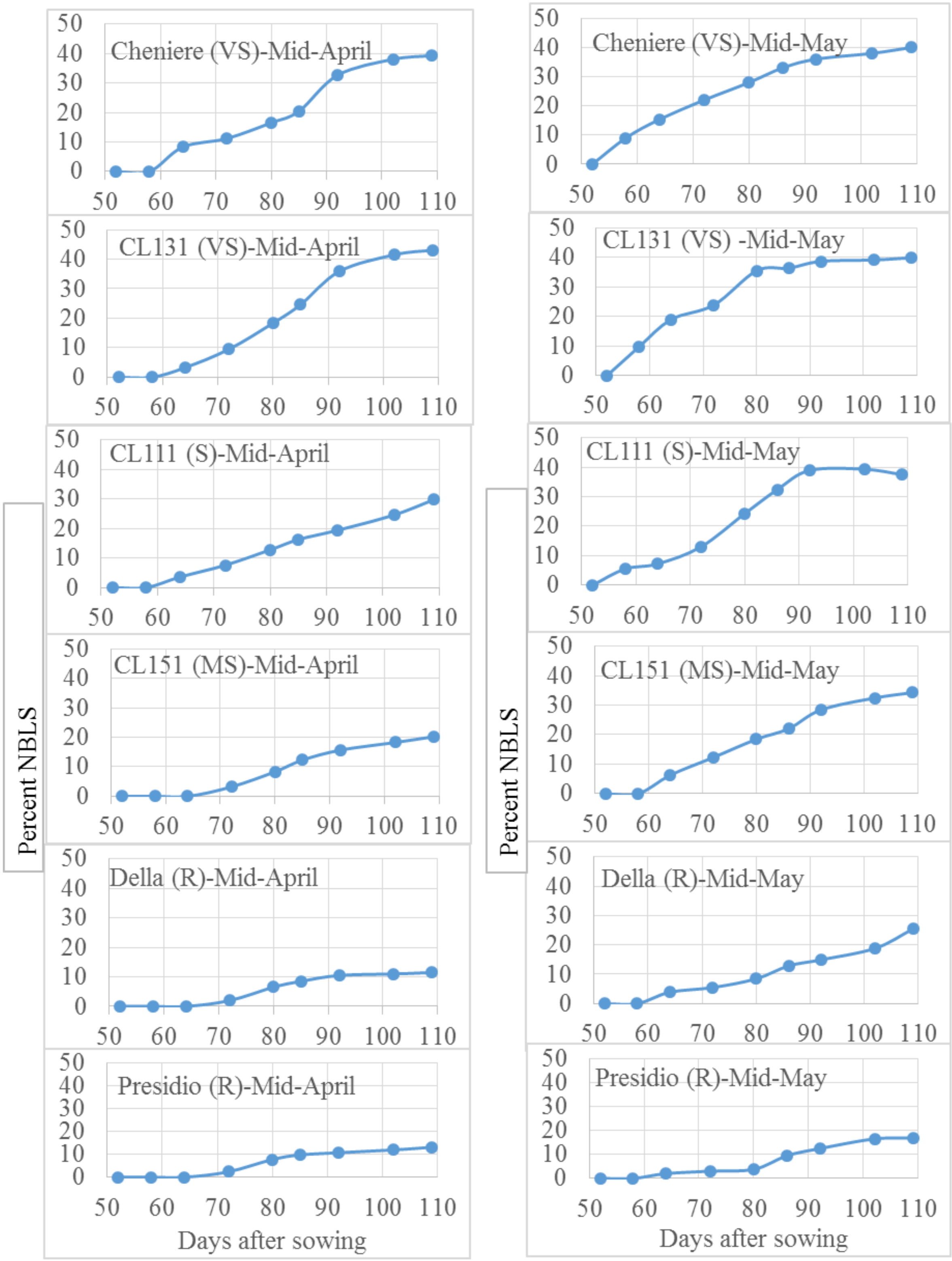

Figure 3.1 Disease progress curves of NBLS in six cultivars with different susceptibility to NBLS in mid-April and mid-May plantings. VS-very susceptible, S-susceptible, MS-moderately susceptible, R-resistant cultivars to NBLS.

susceptible (CL111) in the mid-May planting, but in the early planting, CL151 had less AUDPC than CL111. Final NBLS severity exhibited similar patterns to AUDPC for cultivars and planting dates (Table 3.2). It was higher in the mid-May planting than mid-April for cultivars with all the different susceptibility ratings. The very susceptible cultivars had the highest final NBLS for both planting dates. Similar NBLS severity was observed in the moderately susceptible and susceptible cultivars in mid-April but not in mid-May. Resistant Presidio had the least NBLS in the mid-May planting.

Apparent infection rate of NBLS was greatest in highly susceptible Cheniere and CL131 in the mid-May planting (Table 3.2). The mid-May planting date apparent infection rate was only higher than the mid-April planting for the very susceptible cultivars. Apparent infection rate was less in the susceptible and moderately susceptible cultivars and least for the resistant cultivars in both plantings.

All three disease parameters were highly correlated for the mid-April planting date (Table 3.3). For the mid-May planting, the correlation between apparent infection rate and AUDPC was moderate and non-significant, but apparent infection rate was significantly correlated with final disease severity. However, final disease percentage and AUDPC were highly correlated (r = 0.98). AUDPC and final disease severity were highly correlated between the planting dates, whereas correlations with apparent infection rate were lower.

3.4. Discussion

NBLS severity increased with time in all the cultivars. Cultivar susceptibility level affected disease progression and severity. Higher AUDPCs and final disease severities of NBLS were observed in the very susceptible and susceptible cultivars as compared to moderately susceptible and resistant ones. NBLS epidemics were delayed and slower for cultivars with

Table 3.2. Area under disease progress curve (AUDPC), apparent infection rate, and final disease severity of NBLS in cultivars with different levels of susceptibility to NBLS at two planting dates.

Planting date	Cultivar	Susceptibility level[a]	AUDPC[b,e]	Apparent infection rate[c,e]	Final NBLS Severity[d,e]
mid-April	Cheniere	VS	167.57 c	0.12 b	23.13 c
	CL131	VS	171.37 c	0.12 b	24.18 c
	CL111	S	114.53 d	0.10 b	15.64 d
	CL151	MS	82.04 e	0.10 b	12.26 de
	Della	R	58.83 f	0.08 c	4.32 g
	Presidio	R	53.22 f	0.08 c	3.21 g
mid-May	Cheniere	VS	226.00 a	0.13 a	31.50 a
	CL131	VS	242.85 a	0.15 a	33.20 a
	CL111	S	194.62 b	0.11 b	27.20 b
	CL151	MS	174.21 bc	0.10 b	24.40 c
	Della	R	89.01 e	0.09 c	10.10 e
	Presidio	R	85.07 e	0.09 c	9.40 f

[a] Susceptibility levels corresponding cultivars are presented, as VS- very susceptible, S- susceptible, MS- moderately susceptible, and R-resistant to NBLS

[b] Least square means of Proc. Mixed for area under disease progress curve (AUDPC) calculated from disease assessment of 52 to 109 days after sowing.

[c] Apparent infection rate was estimated by linear regression of logit transformed data over time.

[d] Final NBLS disease severity in percentage at 109 days after sowing.

[e] Means followed by the same letters were not significantly different based on Tukey's test ($P \leq 0.05$).

Table 3.3. Regression coefficients of disease parameters: area under the disease progress curve (AUDPC), final severity and apparent infection rate of NBLS at mid-April and mid-May plantings.

Planting date		Mid-April			Mid-May		
	Disease parameters	AUDPC[a]	Final disease severity[b]	Apparent infection rate	AUDPC	Final disease severity	Apparent infection rate
Mid-April	AUDPC	1					
	Final disease percentage	0.92 (0.001)	1				
	Apparent infection rate	0.87 (0.001)	0.83 (0.001)	1			
Mid-May	AUDPC	0.87 (0.001)	0.90 (0.001)	0.53 (0.001)	1		
	Final disease percentage	0.84 (0.001)	0.88 (0.001)	0.71 (0.0139)	0.98 (0.001)	1	
	Apparent infection rate	0.43 (0.001)	0.48 (0.01)	0.35 (0.001)	0.63 (0.099)	0.73 (0.001)	1

[a] AUDPC = Area under disease progress curve

[b] Final NBLS severity in percentage at 109 days after sowing.

Total number of observations used (n=80). Numbers in parentheses are the probability values of regression coefficients significant at P≤0.05.

moderate susceptibility and resistance. The partial or quantitative expression of resistance to NBLS was effective. The two resistant cultivars had the lowest AUDPC and final disease, but each had some level of NBLS that increased with time. Similar findings of some levels of disease on resistant cultivars have been reported in studies done on *C. arachidicola*, *Cercosporidium personatum* in peanut and *C. beticola* in sugarbeet (Nevill, 1981; Weiland and Koch, 2004). Other studies conducted on rice diseases and *Cercospora* associated diseases on peanut, sesame, maize, and sugarbeet showed lower infection frequencies, smaller lesions, and longer incubation periods on resistant cultivars (Berger, 1977; Nevill, 1981; Waliyar et al., 1993; Ringer and Grybausks, 1995; Rossi, 1995; Parlevliet, 2002). Lesions of NBLS were observed to be smaller on the resistant cultivars in this study, but it was not quantified. Similar observations were made in other field studies on NBLS (Smith, 2007; Groth 2013). However, currently no information is available regarding latent period and sporulation efficiency and infection cycle frequency of NBLS on cultivars with different susceptibility levels. The reasons for lower NBLS severity on resistant cultivars need further investigation.

Delayed disease onset shortened the duration of NLBS epidemics in moderately susceptible and resistant cultivars. Similarly, disease onset was delayed in peanut cultivars having more resistance to *Cercospora arachidicola* and *Cercosporidium personatum* (Cantowine et al., 2008). Epidemics of *Cercospora beticola* started 2 weeks earlier on susceptible compared to resistant sugarbeet cultivars (Gaurilcikiene et al., 2006).

Planting date affected NBLS development. Disease onset was earlier in the late planting (mid-May planting) compared to early planting (mid-April) in all the cultivars regardless of the susceptibility level. Very susceptible cultivars had the most disease and resistant cultivars had the lowest disease for both planting dates. However, AUDPC and final disease percentage were higher for all cultivars in the late planting compared to early planting. The results suggest early planting of very susceptible cultivars might result in lower NBLS severity.

The reasons for more disease severity in the late plantings are uncertain. Late planted rice plants probably experience earlier infections, as well as more infection cycles. These results are consistent with studies done on epidemics of gray leaf spot of maize, Cercospora leaf spot in sugarbeet, carrot, sesame and peanuts where late plantings increased disease development in their hosts (Lipps, 1995; Bhatia and Munkvold, 2002; Lemtur et a l., 2013). *C. janseana* overwinters on crop debris. Weeds and fully established early planted crops could act as an inoculum source for a late planted crop. Incidence and severity of other rice diseases, including rice blast and sheath blight were reported to be greater in late plantings (Wrather and Sweet, 2009; Groth and Hollier, 2010b). More severe rice blast and *Cercospora* associated diseases in other hosts in late plantings were due to favorable micro- and macro-climatic conditions (Pandey and Pandey, 2002; Wolf and Verreet, 2005).

One objective of the study was to determine the most appropriate method to evaluate and compare NBLS progression and severity for use in cultivar selection for rice breeding programs. Resistance inhibited NBLS development but not completely, so some measure of disease progress or severity is needed. A single observation earlier in the season may underestimate the disease development, and one observation late in the season might lead to error due to natural death or senescence of the leaves. Observations in sequential manner over the season may provide a better assessment of NBLS development. Three methods to determine disease progress, apparent infection rate, AUDPC, and final disease severity, were compared. Apparent infection rate could not separate the treatments as clearly as compared to AUDPC and final disease severity. AUDPC and final disease severity detected differences among the cultivars for both planting dates, whereas apparent infection rate did not distinguish the susceptible cultivars, particularly for the early planting. Calculation of AUDPC used all the data available over time determining disease development. However, it is time consuming to calculate AUDPC as compared to single time assessment method. In this study, final disease severity and AUDPC were highly correlated for both planting dates. Therefore, final disease severity could be used to study NBLS resistance.

3.5. Conclusions

NBLS severity was affected by cultivar susceptibility level and planting date. NBLS development was found to be less in mid-April planting as compared to mid-May. Onset of NBLS epidemic was observed 64 DAS in very susceptible and susceptible and 72 DAS in moderately susceptible and resistant cultivars in mid-April planting. NBLS was found under lower rice canopy at 52 DAS on very susceptible and susceptible, and 64 DAS on moderately susceptible and resistant cultivars. Susceptible cultivars demonstrated high AUDPC and final NBLS severity at both the planting dates in comparison to resistant and moderately susceptible cultivars. AUDPC was similar in moderately susceptible (CL151) and susceptible (CL111) in the mid-May planting, but in the early planting, CL151 had less AUDPC than CL111. Apparent infection rate of NBLS was greatest in highly susceptible Cheniere and CL131 in the mid-May planting. Final disease severity and AUDPC were equally powerful to compare cultivars based on disease development. Final disease severity can be used to differentiate the cultivars having partial or quantitative resistance.

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CHAPTER 4
EFFECTS OF NITROGEN APPLICATION ON SEVERITY OF NARROW BROWN LEAF SPOT OF RICE

4.1. Introduction

Narrow brown leaf spot (NBLS) of rice, caused by *Cercospora janseana* (Racib.) O. Const., is a disease increasing in importance in rice growing regions of the world (Braun, 2000; Mew and Gonzales, 2002). NBLS was not economically important in southern rice growing states of the United States until a severe outbreak in 2006 in Louisiana. Reports indicated that NBLS can cause more than 40% yield loss under favorable environmental conditions (Anonymous, 2011). Very limited information exists in the literature about the pathogen, disease development, and its management.

NBLS is an erratic disease affecting rice development at late maturity similar to rice blast caused by *Pyricularia oryzae* and sheath blight caused by *Rhizoctonia solani*. Higher nitrogen (N) fertilizer rate and early application increase the severity of rice blast and sheath blight (Kurschner et al., 1992; Luong et al., 2003). An increase in rice blast severity in susceptible cultivars with increase N application also has been reported (Long et al., 2000; Mukerjee et al., 2005; Bhat et al., 2013).

Cercospora zea-maydis in maize and *Cercospora capcisi* in capsicum increased in severity with an increase in N rate (Vos and Frinking, 1997; Caldwell et al., 2002). However, some studies revealed a decrease in *Cercospora* spp. incidence and disease severity in carrot and crape myrtle with an increase in N rate and its time of application (Hagan et al., 2008; Westerveld et al., 2008; Hagan, 2009).

Many high yielding rice cultivars in Louisiana are susceptible to NBLS (Personal communications, C. Hollier and D. Groth). Resistant cultivars to NBLS are available but prevalence of new races of NBLS may be a limiting factor (Groth and Hollier, 2010).

Applications of propiconazole fungicides provided effective control, but sole dependence on one chemical is not advisable due to a history of adaptability in *C. janseana* (Groth and Hollier, 2010). Other aspects of disease control like nitrogen management and interactions with the susceptibility level of cultivars need to be investigated for possible inclusion in integrated management of NBLS. So far, no information is available regarding interaction of susceptibility levels of cultivars and N rate and its time of application. The objective of this study were to determine the effect of time and rate of N application on NBLS severity in rice cultivars differing in their susceptibility to NBLS.

4.2. Materials and methods

4.2.1. Experimental details

Two experiments were conducted at the Louisiana State University Rice Research Station in Crowley. In the first, the effect of N fertilizer (urea) rate on NBLS in seven cultivars was studied. The seven cultivars were: LAH10 (resistant hybrid), Caffey (moderately resistant cultivar), CL152 and CL261 (moderately susceptible cultivar), CL181 (susceptible cultivar), CL111 (susceptible) and Jazzman-2 (susceptible) cultivars. N (urea) was applied at 0, 100, and 168 kg N/ha rates to cultivars at the 4-5 leaf stage (pre-flooding) of the crop using regular agronomic practices (Saichuk, 2009). Experimental plots were planted 15 March, 2011 and 18 March, 2012 and harvested on 2 August, 2011 and 1 August, 2012. Experimental plots were flooded on 21 April, 2011 and 27 July, 2012 and drained on 19 July 2011 and 27 April 2012.

In the second study, the effect of rate and time of application of N fertilizer (urea) was studied on one moderately susceptible (CL152) and one susceptible cultivar (CL111). N was applied as single application at the 4-5 leaf stage (pre-flooding) at 0, 34, 67, 100, 134, 168, 200 and 235 kg N/ha or as a split application of 50/50, 84/50,118/50, 150/50 kg N/ha at 4-5 leaf stage (pre-flooding) and panicle differentiation stage (post-flooding). Experimental plots were planted on 15 March, 2011 and 18 March, 2012 as per the guidelines of the Rice Production Handbook (Saichuk, 2009). Seeds were drilled at depth of 1.87 cm with 136 kg N/ha in fall stale seed bed and experimental plot size was 1.2 x 4.9 m. Soil type was silt loam. Seed treatments, integrated insect and pest management practices were practiced according to rice production practices (Saichuck, 2009).

4.2.2. Disease assessment and data collection

To determine NBLS severity, 10 plants were arbitrarily selected per experimental unit in the central two rows for assessment. Data were collected on lower, middle and flag leaves of each selected plant at 109 days after sowing. Disease severity was rated using 0-9, where 0= no disease, 1 = 1%, 2 = 3%, 3 = 5 %, 4 = 12%, 5 = 25%, 6 = 40%, 7 = 65%, 8 = 75% and 9 = more than 75% leaf area diseased (Groth et al., 1993).

4.2.3. Experimental details and Data analysis

The selected seven cultivars and three N rates were replicated four times in a randomized complete block design for 2011 and 2012. Disease severity on lower, middle and flag leaves was converted to percentage leaf area diseased, averaged and used as percentage of diseased leaf area for the whole plant. NBLS percentage data were transformed using the logit transformation method for polycyclic diseases, ln x/ (1-x), where x is proportion of disease (Campbell and Madden, 1990, Arneson, 2011). Logits were analyzed using the SAS mixed procedure and factorial design (SAS 9.3, Cary, NC). Years, planting dates and cultivars were fixed factors and replications were a random factor in the mixed procedure. Box plots and the Kolmogorov-Smirnov test were used as test for normality. Tukey's Kramer method was used to compare least square means of logits. Significant differences among the treatments (logits) were determined using the SAS Macro function. After the analysis, logits were back transformed to percentage using the formula {Exp.logit/1+Exp.logit}*100. Tables and figures were prepared using back transformed disease percentage data.

4.3. Results

4.3.1. Study 1: Effect of N (Urea) rate on NLBS severity in seven cultivars having different susceptibility to NBLS

Cultivar susceptibility level, N rate, and their interaction affected NBLS severity ($P \leq 0.05$) (Table 4.1). NBLS was similar in the both years of the study 2011- 2012 and among the replications ($P > 0.05$), and interactions of fixed factors, cultivars and N rates, with year and replications were non-significant (Table 4.1).

Table 4.1 Analysis of variance of effects of N rate and cultivar susceptibility on final NBLS severity.

Factors and interactions	DF	Final NBLS severity (F value)	Final NBLS severity (P value)
Year (Y)	1	0.91	0.34
Replications (R)	3	2.43	0.08
Cultivar (C)	6	3.29	0.01
N rate (N)	2	3.25	0.05
C*N	12	2.05	0.04
Y*C	6	1.04	0.41
Y*N	2	1.06	0.37
Y*R	3	0.16	0.91
Y*C*N	12	0.31	0.98
R*C	18	1.70	0.08
R*N	6	0.84	0.54
R*C*N	36	0.62	0.91

Variation in NBLS severity was found among the cultivars with different susceptibility levels (Table 4.2). Significant differences in NBLS severity were observed among the susceptible cultivars at all the N rates. Among the susceptible cultivars, in the no N application, NBLS was highest in CL281 while at 100 kg N/ha Jazzman-2 and at 168 kg N/ha CL111 had the maximum level of NBLS. For the moderately susceptible cultivars, NBLS severity was higher in

CL261 than CL151 for all the N rates. The moderately resistant cultivar, Caffey, had the maximum level of NBLS at 0 kg N/ha while no difference in NBLS severity was detected at 100 and 168 kg N/ha. The NBLS severity was not affected by N rates for the resistant hybrid, LAH10. In the no N application, susceptible cultivars had maximum levels of NBLS followed by moderately susceptible, moderately resistant and resistant hybrid (Table 4.2). When 100 kg N/ha was applied, NBLS decreased in all the cultivars in comparison to 0 and 168 kg N/ha except for LAH10 hybrid. Comparison of cultivars at 100 kg N/ha revealed that susceptible cultivars, had the highest levels of NBLS as compared to all other studied cultivars, and the moderately susceptible CL261 had equivalent NBLS to susceptible cultivars CL281 and CL111. Another moderately susceptible CL151 and the moderately resistant Caffey showed some lower resistance to NBLS, and had statistically lower NBLS than the susceptible cultivars and another moderately susceptible cultivar CL261. LAH10 hybrid had the lowest level of NBLS among all the cultivars at 100 kg N/ha. No clear differences in NBLS severity were seen between susceptible and moderately susceptible cultivars at 168 kg N/ha. Moderately susceptible CL261, susceptible CL111, and another moderately susceptible, CL151 and susceptible CL181 had similar levels of NBLS. Caffey and the hybrid LAH10 had lowest level of NBLS among all the cultivars at 168 kg N/ha. N applied at 168 kg N/ha rate resulted in increased NBLS in two of the three susceptible and both moderately susceptible cultivars in comparison to 100 kg N/ha. The moderately resistant Caffey had higher NBLS at 0 kg N/ha compared to 100 and 168 kg N/ha. However, in resistant hybrid LAH10, no effect of N application rate on NBLS was observed.

Table 4.2. Effects of N (urea) rate on NBLS severity in seven rice cultivars with variable disease resistance reactions.

N rate (kg N/ha)	Cultivar/ Hybrid	Susceptibility level of cultivar[a]	Final NBLS severity (%)[b]
0	Jazzman-2	Susceptible	24.6 c
0	CL281	Susceptible	28.4 a
0	CL111	Susceptible	26.8 b
0	CL151	Mod. susceptible	20.5 fg
0	CL261	Mod. susceptible	22.8 d
0	Caffey	Mod. resistant	13.7 i
0	LAH10	Resistant	2.1 k
100	Jazzman-2	Susceptible	19.5 g
100	CL281	Susceptible	15.5 h
100	CL111	Susceptible	15.7 h
100	CL151	Mod. susceptible	12.6 i
100	CL261	Mod. susceptible	15.9 h
100	Caffey	Mod. resistant	9.9 j
100	LAH10	Resistant	2.5 k
168	Jazzman-2	Susceptible	21.7ef
168	CL281	Susceptible	19.52 g
168	CL111	Susceptible	23.34 de
168	CL151	Mod. susceptible	19.81 g
168	CL261	Mod. susceptible	21.82 de
168	Caffey	Mod. resistant	10.10 j
168	LAH10	Resistant	3.01 k

[a] Susceptibility levels of the cultivars; Mod. = moderately.

[b] Final NBLS severity percentage on the rice leaves at 109 days after sowing. Means followed by the same letter were not significantly different based on Tukey's test ($P \leq 0.05$).

4.3.2. Study 2: Effect of N application on the severity of NBLS in CL152 (moderately susceptible) and CL111 (susceptible) cultivars

NBLS severity was affected by susceptibility level of the cultivars and time of N application (Table 4.3). NBLS severity was statistically the same over both years of study and among the replications.

NBLS severity was highest at the single N rate of 235 kg N/ha for both cultivars with 31.6% for susceptible CL111 and 25.9% for moderately susceptible CL152 (Table 4.4). The next highest NBLS severity was observed for the 200 kg N/ha single rate and no N application and the lower N application rates, 34 and 67 kg N/ha (Table 4.4). The susceptible cultivar CL111

Table 4.3 Analysis of variance of effects of N applications on final NBLS severity.

Effect	DF	Final NBLS severity (F value)	Final NBLS severity (P value)
Cultivar (C)	1	1292.09	0.0001
N application (N)	11	1249.29	0.0084
C x N	11	128.40	0.0014
Year (Y)	1	2.63	0.1054
Replication (R)	3	2.93	0.0501
Y x R	3	0.66	0.5783
Y x C	1	0.001	0.9972
Y x N	11	1.43	0.2021
Y x C x N	11	1.77	0.0991
R x C	3	2.56	0.0714
R x N	33	1.61	0.0874
R x C x N	33	0.75	0.7845

had more NBLS than the moderately susceptible cultivar CL152 with all the N rates and application timings. Single applications of 235, 200, 168, 34 and 0 kg N/ha enhanced NBLS severity as compared to 100, 134, and 67 kg N/ha application for the susceptible cultivar, CL111. In contrast, more NBLS was detected for the 67 kg N/ha rate and less for the no N application rate for the moderately susceptible CL152. Among the single application N rates, minimum NBLS was observed at 100 kg N/ha for CL152 and similar observations were made at 100 and 134 kg N/ha for CL111. Comparing split applications to single applications of 100, 168 and 200 kg N/ha, all split applications except for the lowest (50/50) resulted in less severe NBLS for the susceptible CL111. The results were similar for the moderately susceptible CL152, except that NBLS severity was observed to be the same for the single 200 and 150/50 kg N/ha applications. Severity was lowest for CL111 at the 84/50 kg N/ha split application and lowest in CL152 for the 100 and 84/50 rates.

4.4. Discussion

The results indicate that the rate and timing of N application affects the severity of NLBS rice. In the first study, seven cultivars with different susceptibility levels had different in levels of NBLS,

Table 4.4 Effects of rate and timing of N (urea) application in susceptible cultivar CL111 and moderately susceptible cultivar CL152 on final NBLS severity.

Cultivar	Susceptibility level of cultivar [a]	Rate of N application (kg N/ha)[b]	Time of N application [c]	Final NBLS severity (%)[d]
CL111	Susceptible	0	4-5 leaf stage	29.0 b
		34	4-5 leaf stage	27.0 c
		67	4-5 leaf stage	23.6 ef
		100	4-5 leaf stage	20.5 i
		134	4-5 leaf stage	20.9 hi
		168	4-5 leaf stage	26.2 cd
		200	4-5 leaf stage	29.0 b
		235	4-5 leaf stage	31.6 a
		50/50 (100)	4-5 leaf stage/ PD stage	23.7 ef
		84/50 (134)	4-5 leaf stage/ PD stage	18.3 k
		118/50 (168)	4-5 leaf stage/ PD stage	21.2 hi
		150/50 (200)	4-5 leaf stage/ PD stage	24.2 e
CL152	Mod. susceptible	0	4-5 leaf stage	19.5 j
		34	4-5 leaf stage	21.3 h
		67	4-5 leaf stage	22.7 g
		100	4-5 leaf stage	14.1 n
		134	4-5 leaf stage	15.6 m
		168	4-5 leaf stage	19.2 j
		200	4-5 leaf stage	23.3 fg
		235	4-5 leaf stage	25.8 d
		50/50	4-5 leaf stage/ PD stage	21.4 h
		84/50	4-5 leaf stage/ PD stage	14.3 n
		118/50	4-5 leaf stage/ PD stage	16.3 l
		150/50	4-5 leaf stage/ PD stage	23.2 fg

[a] Susceptibility levels of the two cultivars; Mod.= moderately susceptible to NBLS.

[b] N applied as single and split applications at different rates. Numbers in parentheses show total N rates.

[c] N was applied as single time application at 4 -5 leaf stage of crop, and second applications were made at panicle differentiation stage (PD).

[d] Final disease severity in percentage on rice leaves. Means followed by the same letter were not significantly different at P=0.05.

and severity was affected by N rate. Susceptible and moderately susceptible cultivars could not always be distinguished by severity. Moderately resistant Caffey had more severe NBLS when no N was applied, and the resistant hybrid LAH10 had the lowest NBLS severity among all the cultivars regardless of the N rate. Susceptible and moderately susceptible cultivars also were more prone to NBLS when no N was applied. Current recommended N rates for the studied cultivars is 134-180 kg N/ha except for CL151 in which is recommended at 100-145 kg N/ha (Saichuk et al., 2009). Results showed N sufficient plants had less NBLS. This finding is consistent with the hypotheses that plants perform better when grown under an appropriate N level, and its availability improves resistance to diseases (Huber and Watson, 1974; Hoffland et al., 2000; Huber and Thompson, 2007). Severity of Cercospora leaf blight (*C. carotae*) in carrot was reduced when the recommended and more than the recommended N rate was applied as compared to no N application (Westerveld et. al., 2003). Okari and his colleagues (2004) found lower severity of gray leaf spot of maize caused by *C. zeae-maydis* when plants received N as oppose to no N. A significant increase of bacterial leaf blight was observed in susceptible compared to resistant cultivars at high nitrogen rates (Reddy et. al., 1977). Caldwell and his colleagues (2002) showed severity of gray leaf spot caused by *C. zeae-maydis* and Cercospora leaf spot caused by *C. capsici* in hot pepper (Vos and Frinking, 1997) increased with an increase in N rate in susceptible cultivars. Ihejirka and co-workers (2006) found reduced foliar diseases, such as Cercospora leaf spot, rust and seedling blight, in groundnut with recommended NPK fertilizer application versus no or low NPK. Other plant disease severities were reduced when optimal N was applied. Huber and McCay-Buis (1993) found lower disease severity of *G. graminis* in optimum N in comparison to N deficient wheat plants. Elevated CO_2 and N supply decreased the leaf spot disease incidence and severity by increasing photosynthetically active leaf area in *Solidago rigida* (Strengbom and Reich, 2006).

In the current study, NBLS severity was lowest at the optimal N level and increased in susceptible and moderately susceptible cultivars when N rate increased from 100 to 168 kg N/ha. However, no change was detected for the moderately resistant and resistant cultivars. Sheath blight of rice increased in severity when a high dose of pre-flood N was applied (Savary et al., 1995; Slaton et al., 2005). Differential behavior of cultivars to rice blast was influenced by N application (Long et al., 2000) with higher severity when more than the recommended N was applied to susceptible but not resistant cultivars. Evidence of increase in disease incidence and severity of rice diseases such as blast, false smut and red stripped disease of rice with increase in N rate had been reported (Luong et al., 2003).

Findings of the study done by Murithee and his co-workers (2010) showed more rice blast severity in the no N applied, more than 120 kg N/ha and below 40 kg N/ha applied plots. They also concluded when N was applied below 80 kg N/ha, cultivar resistance to rice blast decreased due to insufficient N available to perform normal physiological functions of the rice plant. These findings strengthened the results of the current study that more NBLS occurred in no, low or higher N applied rates. The current study detected minimum NBLS in 100 kg N/ha as single N application.

Results of the second study confirmed that the rate of N application affected NLBS severity and the time of N application impacted severity. NBLS was more severe in the susceptible (CL111) than moderately susceptible cultivar (CL152) for all the N rates and times of application. NBLS was again found to be more severe for the no N and the higher rates of N at 200 and 235 kg N/ha in both cultivars. An optimal single N application rate of 100 kg N/ha was confirmed to be associated with lower NBLS severity and at the 134 kg N/ha rate too. NBLS severity was reduced for some rates by applying N in split applications. The 84/50 and 118/50 kg N/ha split applications had lower NBLS severity than the corresponding 134 and 168 kg N/ha single applications. However, the 50/50 kg N/ha split rate had higher NBLS severity than the optimal 100 kg N/ha single rate. The lowest NBLS severity was observed for the 84/50 (134) kg

N/ha split N application. These results suggest that higher N rates should be applied as split applications in order to reduce NLBS severity. Similar effects of split versus single N applications have been observed for other rice diseases, including rice blast and sheath blight (Slaton et al., 2005). In pathosystems other than rice, severity of plant diseases have been demonstrated to be reduced by split applications of N. Area under the disease progress curve was lower for powdery mildew and Septoria leaf spot with a split N application as compared to single N application (Olesen et al, 2003). A split application of N showed an 18% reduction in stem rust severity in rye grass as compared to a single N application (Koeritz et al., 2013).

4.5. Conclusions

NBLS severity was affected by rate and time of N application in susceptible rice cultivars. No or low N application and high N application increased NBLS severity. NBLS severity was lowest when 100 kg N/ha was applied as single application. However, split applications of 100 kg N/ha enhanced the NBLS development. Lower NBLS severities were observed for split applications of 134 kg N/ha and 168 kg N/ha compared to single applications. NBLS severity was less affected by the N rate in a resistant hybrid. Hence, selection of cultivars and N applications are both important factors and should be included as part of integrated management of NBLS.

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CHAPTER 5
EFFECTS OF SEEDING DENSITY AND TILLAGE PRACTICES ON DEVELOPMENT OF NARROW BROWN LEAF SPOT AND YIELD OF RICE

5.1. Introduction

Narrow brown leaf spot (NBLS) of rice is an erratic disease caused by *Cercospora janseana* (Racib.) O. Const. (Braun, 2000). It is commonly found in Arkansas, Mississippi, Louisiana, and Texas, as well as other rice growing parts of the world (Hollier, 1992; Mew and Misra, 1994). This disease can cause more than 40% yield loss under conducive conditions (Anonymous, 2011). The pathogen is wind-borne but can also be dispersed by splashing water and physical contact by leaf rubbing. It affects rice at various crop growth stages, from seedling to panicle formation but is most severe at heading (Groth and Hollier, 2010). Susceptible host, a warm spring, and a wet summer increased an epidemic early in the season leading to a severe outbreak of NBLS in 2006 (Groth, 2013).

Most of the high yielding rice cultivars produced in the southern rice growing states of the United States are susceptible to NBLS, and some of the resistant and moderately susceptible cultivars are becoming more susceptible over time due to the probable emergence of new pathogenic races (Groth and Hollier, 2010; Groth, 2013). For the last 8 years, consistent NBLS development was observed in fields located in southwestern Louisiana, but environmental conditions were not as favorable as they were for the epidemic of 2006 (Groth, unpublished). Currently, NBLS is managed with fungicides such as propiconazole (Groth and Hollier, 2010), but *Cercospora* spp. are known to develop fungicide resistance over time (Reshi et al., 2001; Kirk et al., 2012; Paul et al., 2014). Therefore, knowledge of the potential for cultural practices to mitigate NBLS development would be desirable.

Most rice production in the United States utilizes a conventional tillage system (Saichuck, 2009). However, reduced or minimum tillage has gained acceptance over time due to advantages of flood management and reduced sediment losses. The main issues of farming rice under reduced tillage is stand establishment and early season plant density (Saichuck, 2009). These issues eventually affect yield of the crop, and interaction with rice diseases is an important factor (Sharma et al., 2007; Brooks et al., 2009).

The effects of tillage on development of rice blast and false smut, kernel smut and sheath blight have been evaluated. Conventional tillage reduced the incidence and severity of false smut but had no effect on kernel smut (Brooks et al., 2009, 2011). Reduced tillage and no tillage increased the level of *Rhizoctonia solani* inoculum and incidence of sheath blight of rice as compared to conventional tillage (Cartwright et al., 1996 Cartwright et al., 1997). However, incidence and severity of blast increased in conventional tillage compared to zero tillage or direct drilling (Silva et al., 2003; Sester et al., 2014). Incidence of sheath blight and blast increased with an increase in seed rate (Wu et al., 2014). Evidence of increased incidence of gray leaf spot of maize caused by *Cercospora zeae-maydis* and sugarbeet leaf spot caused by *Cercospora beticola* under no tillage have been reported (Peyne et al., 1987; De Nazareno et al., 1992; De Nazareno et al., 1993; Yang, 2002; Pringas and Marlander, 2004). No information about the interaction of tillage and seeding density on NBLS is available. Therefore, a study was designed to improve our understanding of NBLS development under different tillage systems and seeding density or plant density. The objectives of the study were to determine the effects of seeding density and tillage practices (fall stale tillage and conventional) on plant populations, severity and incidence of NBLS, and yield loss in rice.

5.2. Materials and methods

5.2.1. Experimental details

Experiments were planted and maintained at the Louisiana State University Rice Research Station, Crowley, Louisiana. The cultivar ‘CL152’ was seeded at 2 cm depth at nine different rates of 56, 111, 167, 222, 278, 333, 389, 444, and 500 seeds/m^2 with conventional and fall stale tillage systems in experimental units of 1.37 m x 4.68 m. Planting was done on 16 March and 18 March and harvested on 2 August and 1 August in 2011 and 2012, respectively. In fall stale seed-bed, beds were completely prepared in the fall after the crop and left as such for the entire winter. In spring, winter and spring vegetation on the bed was destroyed using herbicides prior to planting. Under conventional tillage, beds are prepared thoroughly by ploughing, leveling and pulverization of soil. Seed treatments, fertilization, water management, insect and pest management was practiced according to guidelines of rice production handbook (Saichuk, 2009).

5.2.2. Disease assessment and data collection

To follow the NBLS epidemics, 10 plants were arbitrarily selected per experimental units in the central two rows for NBLS assessment. At each sampling date, NBLS progression on lower, middle and flag leaves of the selected plants was visually evaluated. Onset of the disease was noted as a pinhead sized lesion observed on the leaf lamina. Disease severity was rated using 0-9, where 0 = no disease, 1 = 1%, 2 = 3%, 3 = 5 %, 4 = 12%, 5 = 25%, 6 = 40%, 7 = 65%, 8 = 75% and 9 = more than 75% leaf area diseased (Groth et al., 1993). Disease assessment was started at 45 days after sowing (DAS), and the last or final disease assessment was done 109 DAS. Plant populations per m^2 at the 2-3 leaf stage and yield data were obtained

5.2.3. Data analysis

Treatments were replicated four times in a randomized complete block design with a factorial arrangement of tillage methods and seeding density. Disease severity on lower, middle and flag leaves was converted to percentage leaf area diseased, averaged and used as percentage of diseased leaf area for the whole plant. Final NBLS severity at 109 DAS was used to study the effect of tillage methods and seeding densities on NBLS. Box plots and the Kolmogorov-Smirnov test were used to test for normality. NBLS percentage data were transformed using the logit transformation method for polycyclic diseases, ln x/ (1-x), where x is proportion of disease (Campbell and Madden, 1990, Arneson, 2001). Logits were analyzed using the SAS mixed procedure (SAS 9.3, Cary, NC). Years, tillage methods, and seeding densities were fixed factors, and replications were a random factor in the mixed procedure. Tukey's Kramer method was used to compare least square means of logits. Significant differences among the treatments (logits) were determined using the SAS Macro function. After the analysis, logits were back-transformed to percentage using the formula {Exp. logit/1+Exp.logit}*100. Tables were prepared using back-transformed disease percentage data. Yield data were transformed using log transformation, log Y, and final disease percentage was used to analyze NBLS development and its effect on yield of rice. After analysis, logit Y was back transformed to mean yield using the formula {Power (logit), 10} (Arneson, 2001). Pearson coefficients were determined for the variables plant population, NBLS incidence, severity and rice yield. Separate correlations were determined for conventional and stale seed bed methods but no significant difference were observed between the factors in two stillage methods. Therefore, combined data were used to determine correlation between the variables.

5.3. Results

5.3.1. Analysis of variance

NBLS incidence and severity data were not normal (KS, D=0.16, 0.091 > 0.05, respectively). Tillage system, seeding density and their interaction had significant effects on severity and incidence of NBLS (Table 5.1). Plant populations were affected by seeding densities but not by tillage methods. Tillage method did not affect rice yield but seeding density did.

NBLS incidence, severity, plant populations and rice yield were not affected by year and replications (Table 5.1). Interactions of fixed factors, tillage and seeding density, with replications and year were found non-significant in terms of NBLS development, plant population, and yield.

Table 5.1. Analysis of variance of factors affecting NBLS final severity and incidence.

Factors	Plant population (P value)	Final NBLS incidence (P value)	Final NBLS severity (P value)	Rice yield (P value)
Tillage system (T)	0.0519	0.0001	0.0223	0.9226
Seeding density (S)	0.0443	0.0001	0.0136	0.0001
S x T	0.6172	0.0195	0.0278	0.5419
Year (Y)	0.9513	0.5978	0.3248	0.8445
Y x S	0.0763	0.0578	0.6891	0.2146
Y x T	0.8417	0.0523	0.0564	0.9852
Y x S x T	0.0743	0.9941	0.0996	0.1423
Replications (R)	0.5984	0.2184	0.1752	0.8012
R x S	0.9874	0.9801	0.2149	0.0651
R x T	0.2147	0.0849	0.0547	0.1023
R x Y	0.9324	0.2494	0.0644	0.9512
R x S	0.3971	0.8746	0.6478	0.2341
R x T	0.2951	0.2185	0.9713	0.5123
R x S x T	0.3214	0.1450	0.9123	0.5413
Y x S x R	0.8413	0.5489	0.1078	0.0214
Y x T x R	0.3697	0.7413	0.9972	0.8412

5.3.2. NBLS incidence in different tillage systems and seeding density

Final NBLS incidence was highest at the highest seeding density for both tillage methods (Table 5.2). Incidence then generally decreased as seeding density decreased for both methods.

However, incidence was higher under stale tillage as compared to a conventional tillage system for each seeding density (Table 5.2). Seeding density from 389 to 500 seeds/m^2 under stale tillage resulted in more than 50% of the plants infected. In contrast, in a conventional tillage, incidence did not exceed 30% even at 500 seeds/m^2.

5.3.3. NBLS severity in different tillage systems and seeding density

Final disease severity was higher under stale tillage compared to conventional tillage at the higher seeding density of 389 and 500 seeds/m^2 (Table 5.2). Maximum NBLS severity was found in 389-500 seeds/m^2 in stale seed bed followed by next highest at 500 seeds/m^2 under conventional seed bed. Stale seed bed with seeding densities of 278 to 388 seeds/m^2 and conventional with 444 seeds/m^2 had similar levels of NBLS. Lower seeding densities, 56 to 222 seeds/m^2, had similar levels of disease regardless of the tillage system.

5.3.4. Plant population in different tillage systems and seeding density

Plant population increased with increasing seeding density for both tillage methods (Table 5.2). Plant populations were similar for both tillage methods at seeding densities from 56-333 seeds/m^2. At higher seed densities, 389-500 seeds/m^2, high number of plants were observed in a conventional seed bed in comparison to a stale seed bed.

5.3.5. Relationship between NBLS and plant population

Disease incidence and severity generally increased as the plant population increased, and stale tillage at the highest seeding density of 500 seeds/m^2 had the highest disease severity (18.6%). At similar plant populations for both tillage methods at seeding densities ranging from 56-222 seeds/m^2 no noticeable differences in NBLS severity were generally detected. However, the incidence of NBLS was significantly different at comparable seeding densities.

Table 5.2. Effects of seeding density and tillage systems on development of NBLS in the susceptible cultivar CL152.

Tillage system[a]	Seed density (seeds/m^2)	Plant population (plants/m^2)[b, c]	Final NBLS incidence (%)[b, d]	Final NBLS severity (%)[b, e]	Yield (kg/ha)[b]
Con	56	33.3h	3.5 li	8.7 e	7,626 e
Con	111	66.6 g	9.7 k	8.6 e	9,237 cd
Con	167	99.9 f	12.6 k	8.6 e	9,422 bcd
Con	222	133.3 e	15.3 j	8.6 e	10,124 abcd
Con	278	145.4 e	17.9 ij	7.8 e	10,601 ab
Con	333	177.7 d	22.5 hi	8.6 e	10,444 abc
Con	389	224.1 b	26.1 fg	9.6 e	10,479 abc
Con.	444	252.5 a	28.3 ef	11.6 d	10,427 abc
Con	500	264.6 a	30.5 e	14.6 c	10,745 ab
Stale	56	33.3 h	26.0 fg	9.6 e	7,256 e
Stale	111	66.6 g	23.8 fg	8.6 e	9,049 d
Stale	167	99.9 f	24.7 gh	7.6 e	9,498 bcd
Stale	222	123.2e	31.3 e	7.7 e	10,502 abcd
Stale	278	144.4 e	37.0 d	10.6 d	10,106 abcd
Stale	333	165.6 d	41.4 d	10.6 d	10,386 abcd
Stale	389	198.9 c	50.8 c	11.6 d	10,636 ab
Stale	444	188.8 c	54.1 b	16.6 b	10,858 a
Stale	500	233.3 b	59.4 a	18.6 a	10,716 ab

[a] Con = Conventional tillage. Stale = Fall stale tillage.

[b] Means followed by the same letter were not significantly different based on Tukey's test ($P \leq 0.05$).

[c] Plant population was recorded from the central two rows of the experimental units in one square meter area.

[d] Final disease incidence based on percentage of plants infected per experiment unit.

[e] Final mean disease severity on rice leaves.

5.3.6. Rice yield

Rice yield was similar in conventional and stale seed bed systems (P=0.9226), but with an increase in seeding density, yield increased (P<0.0001). Yield was similar for seeding densities at 222-500 seeds/m^2 under conventional and stale seed bed tillage systems. Rice yield was positively correlated with increases in plant population (r=0.8407, P<0.0001).

5.4. Discussion

The present study determined that both seeding density and tillage systems affect NBLS incidence and severity. The highest levels of NBLS occurred at the highest seeding density for both tillage systems. Stale seed bed as a method of tillage is gaining popularity among the rice farmers as it lowers the cost of production and is efficient in terms of time and labor (Saichuk, 2009). To obtain a crop stand of 222 plants/m^2 (ideal for rice), LSU AgCenter recommended seeding density of 444 seeds/m^2 (45 kg/ha seed rate) for CL152. To overcome potential disadvantages of the stale seed bed system, such as lower germination and crop stand density, higher seeding rates are recommended (Saichuk, 2009). However, NBLS incidence was higher at all seeding densities and severity was increased at higher seeding density for the stale seed bed compared to conventional tillage.

The lower NBLS incidence and severity observed in conventional tillage was similar to lower levels of sheath blight in conventional tillage (Cartwright et al., 1997). False smut of rice and rice blast was found less in no tillage verses traditional tillage or conventional, but severity of kernel smut was similar in both traditional and no tillage systems (Andres et al., 2008; Brooks et al., 2009; Sester et al., 2014). Many diseases caused by *Cercospora* spp., including, gray leaf spot of maize, Cercospora leaf spot of sugarbeet were found to be reduced in conventional tillage as compared to no or stale seed bed tillage method due to reduction of *Cercospora* inoculum maintained in crop residue by burying of residue to decompose in the soil (Peyne et al., 1987; De Nazareno et al., 1992; Yang, 2002; Pringas and Marlander, 2004).

NBLS increased with an increase in seeding density due to increasing plant populations with higher populations under conventional tillage than a stale seed bed at seeding density of 389 seeds/m^2 and above. However, seeding densities from 389-500 seeds/m^2 in a stale seed bed resulted in more than 50% of the plants infected, whereas under conventional tillage, incidence did not exceed 30%. NBLS incidence was higher in a stale seed bed at seeding density from 56-333 seeds/m^2 even though plant populations were similar for both tillage methods at the lower seeding densities. Stale seed beds have more crop residues that may act as reservoirs and overwintering sources for *C. janseana*, and this could result in higher NBLS incidence due to a greater initial inoculum levels. This observation is supported by evidence of higher inoculum of *Rhizoctonia solani* under no till plots (Cartwright et al., 1997) and higher numbers of *Cercospora zeae-maydis* conidia (gray leaf spot of maize) trapped from no till versus tilled plots of maize (Peyne et al., 1987). Gray leaf spot lesions developed early in the season and doubled on the leaves by harvest in no-till plots. Early development of gray leaf spot symptoms allowed for more secondary cycles and inoculum build up over time. Similarly, higher incidence of gray leaf spot of maize was detected on leaf and sheath samples found on the soil surface in spring than on the samples buried in fall and removed the following spring (De Nazareno et al., 1992). Diseases caused by other *Cercospora* spp. like frogeye leaf spot of soybeans and Cercospora leaf spot of soybean have reportedly been reduced with conventional tillage by reducing propagules on infested crop and soil debris through burying them deep in soil (Yang, 2002). However, *Cercospora beticola* severity was not affected by ploughing, mulching and direct drilling (Pringas and Marlander, 2004).

Higher plant populations may have affected microclimate within the crop canopy resulting in more NBLS development. However, that incidence was more affected by seeding densities than severity. Ottis and his co-workers (2006) found minor differences in the incidence and severity of sheath blight at 166, 333.3 and 666.6 seeds/m^2. Rice diseases like panicle blight and sheath blight were increased with selection of susceptible cultivars and planting dates rather than seeding densities (Bennet, 2006; Wamishee et al., 2013). On the other hand, rice blast incidence and severity was reported to be increased with seeding densities (Hai et al., 2007).

NBLS incidence and severity increased with seeding density with both tillage methods, but yield was similar for all the seed densities except for the lowest levels of 56 and 111 seeds/m^2. The tillage effects on diseases and yield in field crops has been studied extensively. For example, incidence of ear rot of maize caused by *Stenocarpella maydis* were found higher in no-tilled plots as compared to reduced and conventional tillage, but yield was similar in all the tillage methods for 13 seasons (Lawrence et al., 1999). Their study showed yield was more impacted by environmental conditions, such as rainfall and moisture, ambient soil temperature, and plant density which had a compensation effect on yield. Findings of study done on effect of rotation, tillage and variety on rice yield have shown more nitrogen uptake in no tilled verses tilled plots of rice (Anders et al., 2004). Evidence of compensation effects in yield in wheat, rice, corn and barley has been reported (Lawrence et al., 1999; Bavec et al., 2002; Ramadhan, 2013). Yields of rice were similar in stale seed bed, conventional and no-till beds despite higher levels of *Rhizoctonia solani* in stale and no-till beds (Cartwright et al., 1996). In contrast, reduced yield with a higher seed rate was due to more sheath blight and empty heads of infected plants (Ottis et al., 2006; Mithrasena et al., 2007). In another study, semi-dwarf lines of rice showed no impact of seeding rates on sheath blight disease index and yield of rice (Marchetti, 1983). Yield and plant stand for two rice tillage systems, conventional tillage and stale seed bed, were similar in the absence of the rice diseases (Wrather et al., 2005). Rice yield increased with conventional tillage, and the population and viability of sclerotia of *Sclerotium oryzae* causing stem rot of rice was completely lost (Hussain and Ghaffer, 1993).

In this study with one susceptible cultivar CL152, increasing levels of NBLS did not result in demonstrable yield losses. Yields obtained from different seeding rates showed little variation with a similar pattern for both tillage methods. Seeding density at the recommended rate (444 seeds/ m^2) and above produced equivalent yields for both tillage methods. However, increasing disease intensity in higher plant densities could have had an impact on yield. This shifting relationship might have resulted in equivalent yields across the wide range of seeding densities. To be certain, the effect of increasing seeding density in the absence of NBLS should be determined.

5.5. Conclusions

This study demonstrated that tillage and seeding densities affected plant populations, NBLS incidence and severity. Plant population was reduced under stale seed bed systems at higher seeding densities from 389-500 seeds/m^2. At lower seeding density, disease severity was not much different between the tillage systems. *Cercospora janseana*, a pathogen that survives in crop debris, can be affected by seeding density and tillage system. However, there is a complex interplay between the disease and cultural practices that probably affects pathogen life cycle factors and, in turn, the impact of disease on yield. The agronomic effects of seeding density and tillage can be confound in the presence of NBLS, and the effect of disease on yield can be confused by the interaction of cultural practices. These results suggest that, in the absence of other control measures, there is no benefit to increasing the seeding density above the recommended range (444 seeds/m^2) and that NBLS incidence will be lower under conventional tillage.

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CHAPTER 6
SUMMARY

Studies evaluated the effects of planting date, cultivar susceptibility, fungicide application timing, nitrogen management, and tillage practices on the severity of narrow brown leaf spot of rice (NBLS) caused by *Cercospora janseana.* The study results have demonstrated that susceptible cultivars had the highest and resistant ones had the lowest NBLS in both mid-April and mid-May planting. Fungicide application at panicle initiation stage or early boot stage was an effective time of fungicide application in the very susceptible, susceptible and moderately susceptible cultivars in mid-April, whereas panicle initiation was found to be the best in late planting. NBLS reductions resulting from fungicide application were slight and variable for the two resistant cultivars. Resistant cultivars need a fungicide application in late planting or under higher NBLS pressure but do not need under low NBLS pressure. Yield was found to be higher in mid-April planting than mid-May. Yield was determined to be similar for all the cultivars at the mid-April planting, except for the resistant cultivars. On the other hand, at late planting, yield was highest in very susceptible, Cheniere, and followed by moderately susceptible, CL151 cultivar. For all the fungicide treatments, yield was found to be same but higher than the untreated cultivars in mid-April but application at panicle initiation gave the highest increase in yield during late planting with variable results among cultivars.

NBLS severity is affected by cultivar susceptibility level and planting date. NBLS development was found to be less in mid-April planting as compared to mid-May. Onset of NBLS epidemic was observed 64 DAS in very susceptible and susceptible and 72 DAS in moderately susceptible and resistant cultivars in mid-April planting. NBLS was found under lower rice canopy at 52 DAS on very susceptible and susceptible, and 64 DAS on moderately susceptible and resistant cultivars. Susceptible cultivars demonstrated high AUDPC and final

NBLS severity at both the planting dates in comparison to resistant and moderately susceptible cultivars. AUDPC was similar in moderately susceptible (CL151) and susceptible (CL111) in the mid-May planting, but in the early planting, CL151 had less AUDPC than CL111. Apparent infection rate of NBLS was greatest in highly susceptible Cheniere and CL131 in the mid-May planting. Final disease severity and AUDPC are equally powerful to compare cultivars based on disease development. Final disease severity can be used to differentiate the cultivars having partial or quantitative resistance.

NBLS severity was affected by rate and time of N application in susceptible rice cultivars. No or low N application and high N application increased NBLS severity. NBLS severity was lowest when 100 kg N/ha was applied as single application. Lower NBLS severities were observed for split applications of 134 kg N/ha and 168 kg N/ha compared to single applications. However, split applications of 100 kg N/ha enhanced the NBLS development. NBLS severity was less affected by the N rate in a resistant hybrid. Hence, selection of cultivars and N applications are two important factors that should be included as part of integrated management of NBLS.

The study results demonstrated that tillage and seeding densities affected plant populations, NBLS incidence and severity. Plant population was reduced under stale seed bed systems at higher seeding densities from 389-500 seeds/m^2. At lower seeding density, disease severity was not much different between the tillage systems. *Cercospora janseana,* a pathogen that survives in crop debris, can be affected by seeding density and tillage system. However, there is a complex interplay between the disease and cultural practices that probably affects pathogen life cycle factors and, in turn, the impact of disease on yield. The agronomic effects of seeding density and tillage can be confound in the presence of NBLS, and the effect of disease on yield can be confused by the interaction of cultural practices. These results suggest that, in the absence of other control measures, there is no benefit to increasing the seeding density above the recommended range (444 seeds/m^2) and that NBLS incidence will be lower under conventional tillage.

Recommendations for growers based on the results of the current study

- Management of narrow brown leaf spot of rice should be integrated approach that reduces NBLS development. Disease development starts in lower canopy and moves to the upper canopy as the season progress. Splashing rain and wind helps to spread the fungal spores to upper leaves hence disease develops within the canopy. Although NBLS development starts from the lower canopy, its development on flag leaves impacts yield loss the most.
- *Cercospora janseana* overwinters on the crop residues and soil debris, acts as the initial inoculum, and during the season can survive on seasonal weeds. Clean and well prepared seed beds are preferred over the stale seed bed in order to reduce the initial inoculum.
- NBLS development increases with an increase in seeding density regardless of tillage systems.
- Early planting of cultivars (mid-March through mid-April) is recommended to reduce NBLS severity. Disease development starts early (within 64 days after sowing depending upon the susceptibility level of the cultivars) in later plantings (mid-May).
- NBLS development is affected by the susceptibility of the cultivar. Early onset and quick NBLS development occur in very susceptible and susceptible cultivars. NBLS on early planted (mid-April), very susceptible to moderately susceptible cultivars, can be better managed by applying propiconazole fungicide at the early boot to late boot stage, but in

late planted rice, fungicide application at the panicle initiation is a more efficient application time. Resistant cultivars are not immune to NBLS. Disease does develop in resistant cultivars but at lower rate, therefore, resistant cultivars do not no need a fungicide application.

- Nitrogen application has an effect on NBLS development. Nitrogen deficient plants are more prone to NBLS. Higher nitrogen also increases the NBLS severity on very susceptible and moderately susceptible cultivars but no effect of nitrogen application on NBLS has been found with use of moderately resistant to resistant cultivars.

The current studies answered many questions about the NBLS development and recommend that time of planting, selection of tillage systems, seeding density, cultivars susceptibility levels and nitrogen management have an integral influence on NBLS management. Nevertheless, there are questions that were raised while developing an integrated disease management program for NBLS:

- Results of the current study revealed that NBLS severity increased in the late planting but investigation as to the reasons for this increase should be explored. It is theorized that either conducive environmental conditions for NBLS development or an increase in *C. janseana* inoculum could be the possible causes of higher NBLS severity in the late season. Future studies of inoculum quantification and role of environment in NBLS development might reveal the reasons for more NBLS in late planted rice.
- Moderately susceptible and resistant cultivars have expressed quantitative resistance to NBLS but exact reasons of this resistance are not known. Understanding the quantitative aspects of disease rate reducing resistance (incubation period, latent period, inoculum

quantification and quality, and lesion development on different cultivars) would further improve the NBLS epidemiology and management.

- NBLS severe outbreak of 2006 resulted in economically significant yield losses but since then disease has developed at low to moderate rates and the current studies were performed under these conditions. Single fungicide application at either early boot or panicle initiation, depending upon cultivars and time of planting, was found to be sufficient and effective. Under high disease pressure, effectiveness of single fungicide application may not have the same results, therefore, multiple fungicide applications need to be investigated under higher NBLS development.
- The results of nitrogen management studies have shown that nitrogen rate plays an important role in NBLS development and severity is affected by the susceptibility of the cultivars. Further studies related to source of nitrogen, nitrogen uptake, nitrogen use efficiency and leaf nitrogen percentage based on the soil nutrient and tissue analysis would help to improve the understanding of the differential interaction of cultivars and NBLS. The role of nitrogen in the absence or presence of other factors affecting disease development such as fungicide applications and the role of macro and micro nutrients need to be studied.
- Effect of seeding density was studied along with the tillage methods. Evidence of interplay of these two factors are found in this study. Individual effect of these factors and their interaction with cultivars of different susceptible levels need to be quantified.

VITA

Kirandeep Kaur, daughter of the late S. Devinder Singh and Manjit Kaur, was born in 1982, in Kot-Kapura, Punjab, India. She received her primary education in Kot-Kapura and graduated from high school in March, 2000. She was awarded the Bachelor of Science in Agriculture (Hons.) with a major in Forestry from Punjab Agricultural University, Ludhiana, in 2005. She did her Masters in Forestry and Natural Resources with a major in tree breeding and minor in Biochemistry from Punjab Agricultural University, in 2007. After finishing her masters, she worked as a research associate for two years in a project for development of cultural and management practices of *Jatropha curcas* and development of cost effective methods of micro-propagation of *Jatropha curcas*. Two years later, she was accepted as a graduate student in the Department of Plant Pathology and Crop Physiology, Louisianan State University in 2009. She has been working on developing an integrated disease management system for narrow brown leaf spot of rice. She currently lives in Baton Rouge, married to Dhananjay Mani. Kiran is currently a doctoral candidate in the Department of Plant Pathology and Crop Physiology at Louisiana State University.