INFLUENCE OF FREEZING ON THE SURVIVAL OF *MAGNAPORTHE ORYZAE* AND WEATHER CONDITIONS THAT FAVOR BLAST EPIDEMICS IN RICE

by

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Abstract

Wheat blast, caused by *Magnaporthe oryzae* pathotype *triticum*, has emerged as a serious problem for wheat production in South America and recently emerged as a threat to wheat production in Bangladesh. To prepare for the possible introduction of wheat blast in to the United States, it would be helpful to identify areas of the country most at risk for blast epidemics. Because wheat blast occurs primarily in tropical and subtropical regions of the world, cold winter temperatures may restrict the establishment of the blast pathogen in the United States. Therefore, the first objective of this research was to quantify the freeze-thaw tolerance of the wheat blast pathogen in naturally infected wheat rachises from Bolivia and to measure the viability of the conidia after exposure to various treatments. The results indicate that exposing the fungus in moist residue to multiple freeze-thaw cycles is more damaging than exposing the fungus in moist residue to longer, single freezes. When in dry residue, the fungus was not harmed by the freeze-thaw cycles. Freezing and thawing of the wheat blast fungus in moist residue significantly affected its ability to produce viable conidia.

The second objective of this research was to identify environmental conditions that could be conducive for wheat blast epidemics by examining historical epidemics of rice blast, caused by *Magnaporthe oryzae* pathotype *oryza*. The dataset used in this analysis consisted of 60 site-years of historical observations of rice blast levels from Arkansas, Louisiana, and Texas. These observations were coupled with monthly and weekly summaries of hourly weather variables based on temperature, relative humidity, precipitation, and regional moisture indices. Classification trees and logistic regression were used to identify variables associated with rice blast epidemics. The results indicate that rice blast epidemics are favored by cooler April

temperatures and higher levels of precipitation in June. Preliminary models for rice blast based on these variables were able to correctly classify epidemic years with >75% accuracy. In the future, the results of this project will be used as part of a risk assessment for a wheat blast introduction and establishment in the United States.

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Chapter 1 - Literature Review

The heterothallic ascomycetous fungus, *Magnaporthe oryzae*, is the causal agent of the disastrous disease blast (Valent *et al*., 2013). Blast disease is associated with grasses of agricultural importance such as wheat (*Triticum aestivum*), rice (*Oryza sativa*), barley (*Hordeum vulgare*), and oats (*Avena sativa*) (Khang and Valent, 2010). This fungal species is divided into host-specialized populations including the *M. oryzae* pathotype *oryza* (MoO) causing rice blast across the globe; the *M. oryzae* pathotype *triticum* (MoT) causing wheat blast in South America and Bangladesh; and the *M. oryzae* pathotype *lolium* (MoL) causing gray leaf spot on perennial and annual ryegrass and tall fescue in the United States and Japan (Valent *et al*., 2013; Malaker *et al*., 2016). Generally these pathotypes are unable to infect other host species, although MoL isolates in the U.S. are genetically similar to MoT isolates from South America (Tosa *et al*., 2004). Some of the native MoL isolates have been shown to have the capability to infect wheat (Valent *et al*., 2013). The risk of introduction of wheat blast into the U.S. is high, not only because of the similarities between MoL and MoT isolates, but also due to the fungi's seed-borne nature and increased trade and travel between the U.S. and South America (Valent *et al*., 2013).

Rice Blast (*Magnaporthe oryzae* pathotype *oryza*)

Rice blast is one of the most important diseases of rice worldwide (Ou, 1985). Between 10 and 30% of rice yields are lost to blast annually (Talbot, 2003). It is estimated that the yields lost to this disease would feed 60 million people a year (Khang and Valent, 2010). In the Southcentral United States, where most of the country's rice is produced, yield losses can reach 100% in fields during favorable years (uaex.edu). MoO infects all aboveground parts of the rice plant and can infect at all growth stages from seedling to grain formation (Ou, 1985). The pathogen is capable of causing leaf blast, collar rot, nodal blast, and panicle blast depending on when and where the pathogen infects (Groth, 2006). Among these infection stages, panicle blast is the most destructive in terms of yield loss because it can cause incomplete grain filling and poor milling quality (Bonman *et al*., 1989; Webster and Gunnel, 1992). The main overwintering inoculum sources are infected residue, seed, and related host species (Guerber and TeBeest, 2006). Sexual reproduction can occur in vitro through the mating types MAT1-1 and MAT1-2 (Talbot, 2003). Despite the ability of MoO to perform sexual reproduction, most isolates sampled from the field are female-sterile or totally infertile resulting in low levels of fertility. This means the blast fungus is largely asexual on rice in the field (Khang and Valent, 2010).

Crop management greatly affects rice blast severity. Rice blast can be controlled by altering the planting date, avoiding overfertilization, maintaining flood depth, planting resistant cultivars, and applying fungicides (Chen *et al.*, 2015). In the past decade, major work has been done to identify over 80 dominant resistance genes from rice or wild relatives in order to develop resistant cultivars (Khang and Valent, 2010; Ballini *et al.*, 2008). Unfortunately, in many areas resistant varieties are quickly overcome by the disease due to the rapid genome evolution of the fungus (Khang and Valent, 2010). Some rice cultivars convey adult plant resistance as they mature. This means that less disease develops on more mature plants than on seedlings, and as the plant matures the infection is reduced. Hwang *et al.* (1987) demonstrated that cultivars conveying adult plant resistance had less yield loss than cultivars with low or no adult plant resistance (Hwang *et al*., 1987). Increased use of fertilizer, particularly nitrogen, makes rice blast infections more severe (Bonman, 1991; Kurschner *et al.*, 1992), so it is important to use the recommended rates and not overfertilize. It has also been shown that loss of flood depth increases rice plant susceptibility to blast infection (Guerber and TeBeest, 2006; Khang and Valent, 2010). Maintaining water depths and preventing overfertilization will reduce the susceptibility of rice to MoO and make for a less conducive environment for disease development. Single applications of azoxystrobin and trifloxystrobin are used to control blast in the southern United States (Groth, 2006). In a study by Groth (2006) it was found that a single application of azoxystrobin at heading effectively controlled blast, but two applications at boot and heading were more effective (Groth, 2006). Currently, fungicides remain the most popular choice of control for rice blast (Kaundal *et al.*, 2006).

Forecasting and simulation models have been developed in several rice producing countries including Italy, South Korea, Philippines, India, and Japan (Calvero *et al*., 1996; Biloni *et al*., 2006; Ishiguro and Hashimoto, 1991; Kaundal *et al.,* 2006). For prediction models, weather factors such as temperature, relative humidity, precipitation, dew point, and wind speed are used to determine whether a blast epidemic is more or less likely to occur that year (Biloni *et al*., 2006; Calvero *et al*., 1996). These weather factors are based on the optimum developmental conditions for rice blast. Prediction models have the power to aid growers in making fungicide decisions more rationally by optimizing the timing and frequency of application of fungicides (Ou, 1985; Biloni *et al*., 2006; Kaundal *et al*., 2006). To our knowledge, no prediction model has been developed for the Southcentral United States where rice is predominantly produced in the country. Weather variables that prove important in disease systems in other areas of the world won't necessarily be important in a prediction model for the United States, but could give clues as to which weather factors may be useful.

The literature largely agrees on the favorable conditions of rice blast development. Leaf wetness is one of the most important factors in the blast disease system and is required for conidia to germinate (Teng *et al.,* 1991; Hamer *et al*., 1988). The optimum temperature for conidia to germinate is between 25-28°C (Sueda, 1928; Ou, 1985; Suzuki, 1969), and the appressorium forms at an optimum temperature between 16 and 25°C (Suzuki, 1969). Conidia are produced at a relative humidity of 93%, and production increases as relative humidity increases (Ou, 1985; Suzuki, 1975). Sporulation does not occur below 9°C or above 35°C or below 89% relative humidity (Suzuki, 1975). Mycelium growth is most efficient at 93% relative humidity (Abe, 1941). Calvero *et al.* (1996) found in South Korea, consecutive days with relative humidity greater than 80%, number of days with relative humidity greater than 80%, consecutive days with precipitation, and number of days with precipitation were useful in predicting maximum lesion number, final lesion number, and panicle blast incidence. In the Philippines, the same researchers found mean maximum temperature, consecutive days with precipitation, number of days with wind speed greater than 3.5 ms^{-1}, mean relative humidity, and precipitation frequency were important in predicting blast (Calvero *et al*., 1996). For predictive models in India, developed by Kaundal *et al*. (2006), rainfall was demonstrated to be the most important predictor followed by rainy days per week, minimum and maximum relative humidity, and minimum and maximum temperatures (Kaundal *et al.*, 2006). Due to the water requirement for conidial germination appressorium function, and specific optimum temperatures for disease development, it is not unexpected that precipitation, relative humidity, and temperature are reoccurring variables in these models (Talbot, 2003; Khang and Valent, 2010; Bourett and Howard, 1990).

Magnaporthe oryzae Disease Cycle

The rice blast disease cycle has been thoroughly studied and is described here. Wheat blast appears to follow a similar disease cycle (Tufan *et al.*, 2009). Severe epidemics are sporadic and are more likely to occur when warm weather with high humidity occurs (Urashima *et al*., 1993). The blast pathogen survives between growing seasons as mycelium in plant residue and seeds (Agrios, 1997; Suzuki, 1975). A study done by Faivre-Rampant *et al.* (2013) on rice indicated that blast is primarily located in the seed coat and that conidia are produced shortly after the infected seed germinates. These conidia infect coleoptiles, primary roots, and produce mycelium that infect primary leaves and secondary roots (Faivre-Rampant *et al*., 2013). Once the temperature and humidity begin to rise in early spring, conidia are formed on the residue (Suzuki, 1975) and transported from plant to plant by water drops and wind. When a conidium lands on a host leaf surface, it produces an adhesive substance called spore tip mucilage which attaches it to the leaf (Hamer *et al*., 1989). At optimum conditions, the conidia germinate within two hours when free water is present (Hamer *et al*., 1988) and the temperature is between 25 and 28°C for rice blast (Suzuki, 1969), and between 25 to 30°C for wheat blast (Cardoso *et al*., 2008). The germ tube eventually swells and forms an appressorium (Bourett and Howard, 1990) at an optimum temperature of 16 and 25°C (Suzuki, 1969). At this stage a specialized hypha, the penetration peg, punctures the plant cuticle with the highest turgor pressure known in any organism (80 times atmospheric pressure) (Howard *et al*., 1991). Penetration can occur between 10 and 32°C, but the optimum temperature is 24°C (Hemmi and Abe, 1932). After penetration, the hypha differentiates into a branched bulbous, invasive hypha that grows for 8-12 hours within the first infected plant cell (Kankanala *et al*., 2007). The invasive hyphae then move to adjacent plant cells presumably through plasmodesmata (Kankanala *et al.*, 2007). The fungus uses a biotrophic invasion strategy and 7 to 8 days after infection, diamond shaped lesions are formed that reduces photosynthesis (Burrell, 1974; Kankanala *et al*., 2007). Lesions develop at an optimum temperature of 26 to 28°C, but can develop at varying temperatures after longer latent periods (Hemmi *et al*., 1939). Aerial conidiophores are formed within the lesions creating a gray appearance (Khang and Valent, 2010). The conidiophore usually develops 5 or more conidia arranged sympodially at the tips (Talbot, 2003), and are formed at optimum temperatures ranging from 25-28°C (Suzuki, 1975). Sporulation does not occur below 9°C or above 35°C or below 89% relative humidity, but sporulation does increase with relative humidity at 93% and above (Suzuki, 1975). The spread of blast occurs mainly via airborne conidia (Urashima *et al*., 2007).

Wheat Blast (*Magnaporthe oryzae* pathotype *triticum*)

Wheat blast was first detected by Igarashi in the Paraná State of Brazil in 1985 (Igarashi, 1986, Urashima *et al*., 1993). Since then, the disease has spread to wheat producing areas in Brazil, Bolivia, Paraguay, Northern Argentina, and most recently, Bangladesh (Kohli *et al.*, 2011; Malaker *et al*., 2016). Similar to MoO, MoT is capable of infecting all aboveground parts of the wheat plant, but infection of the head is the most devastating phase of the disease (Kohli *et al.*, 2011). Symptoms can occur on all parts of the head including awns, glumes, and the rachis (Igarashi, 1990). Infected rachises become bleached at and above the point of infection and results in shriveled seeds or completely prevents grain filling (Cruz, 2013; Igarashi, 1990). At the infection point of the rachis, the tissue begins to turn brown to black and will later become gray due to sporulation (Urashima *et al*., 2009). This can result in 100% yield losses in fields (Kohli *et al*., 2011). Predominantly, head blast has been observed in the field without leaf lesions and the contribution of inoculum from the leaves has been debated (Cruz *et al.*, 2015). A recent study by Cruz *et al*. (2015) suggests older leaves of wheat may be more susceptible to blast than younger leaves (Cruz *et al.*, 2015). In the same study, the researchers observed senescence of the three lowest leaves on the wheat plant, but no other obvious blast symptoms. At flowering the plants were sampled and substantial sporulation was found on the senesced, basal leaves of susceptible cultivars. This study suggests that the inoculum produced on infected lower leaves within wheat fields may play a role in wheat blast outbreaks (Cruz *et al*., 2015). Blast of wheat, particularly head blast, is usually widespread throughout large production fields, which suggests there may be other sources of inoculum (Valent *et al*., 2013). It is often assumed in South America that the conidia causing head infections originate from blast infected weeds surrounding the fields. These weed sources include *Digitaria sanguinalis, Echinocloa crusgalli, Cenchrus echinatus*, among other species (Kohli *et al*., 2011).

Leaf lesions of wheat blast are very similar to rice blast in that the lesions are diamond shaped with tan centers and dark margins. When the lesions are sporulating they appear to be gray in the center (Valent *et al*., 2013). The sexual cycle of the blast pathogen has never been observed in nature on any host, but has been documented in the laboratory (Yaegashi and Udagawa, 1978). Wheat blast isolates taken from the field show high levels of sexual fertility. These isolates cross to form viable ascospores and microconidia (Urashima *et al*., 1993). Ascospores are produced in asci within perithecia (Yaegashi and Udagawa, 1978) and microconidia are produced within phialides, but their function is unknown (Chuma *et al*., 2009). The high level of sexual fertility in vitro suggests that the wheat blast pathogen may perform sexual recombination in the field (Valent *et al*., 2013).

Controlling wheat blast is challenging because of the lack of resistant varieties and ineffective fungicides when conditions are favorable (Valent *et al*., 2013). Wheat blast is not controlled by the same fungicides that are able to control rice blast (Khang and Valent, 2010). Mixtures of triazoles and strobilurins are used in South America and applied during heading to control blast in moderately resistant varieties (Kohli *et al.*, 2011). Although, fungicides have been shown to be ineffective when sprayed on susceptible varieties during severe epidemic years (Urashima *et al*., 2009), a study by Cruz *et al.* (2015) suggests that applying fungicides to the leaves, before heading, may also be effective in controlling blast (Cruz *et al.*, 2015). U.S. winter wheat varieties and spring wheat varieties have been screened using blast isolates from Brazil and Bolivia. Kohli *et al.* (2011) reported that wheat varieties showing resistance to a select amount of blast isolates may not show resistance to natural field populations (Kohli *et al*., 2011), therefore it is important to test resistant varieties in field plots in South America as well. Very few resistance genes have been identified for wheat blast. Two have been identified in the variety Thatcher (Zhan *et al*., 2008), and resistant varieties that have been derived from the CIMMYT line, Milan, have been deployed throughout South America (Kohli *et al.,* 2011). Adjusting the planting date of wheat is an important control strategy for South America, because the early planted wheat is more likely to be heading when the conditions are most conducive for blast (Mehta *et al*., 1992). Deep plowing of wheat residue and destroying alternate hosts are also control strategies used in South America (Valent *et al*., 2013; Mehta *et al*., 1992).

Climate is an important factor in the distribution of blast. Kohli *et al.* (2011) reported that blast is favored by frequent rain for several days at an average temperature between 18 and 25°C during flowering. If these conditions are followed by hot, humid, and sunny days, a very conducive environment is created for blast to develop (Kohli et al., 2011). The maximum blast intensity observed by Cardoso *et al*. (2008) was at 30°C and increased with increasing wetting periods (Cardoso *et al*., 2008). According to these conditions, blast requires tropical and subtropical environments to survive (USDA: New Pest Response Guidelines). Similar to MoO, MoL overwinters in seeds and residue (Ou, 1985; Harmon *et al*., 2005). Gray leaf spot was reported by Harmon *et al*. (2005) to be significantly reduced by alternations of freezing and thawing (Harmon *et al*., 2005). In this study the conidial production was measured from samples of perennial ryegrass in the field and laboratory. Findings suggest that the fungus can survive

Indiana winters in infected residue, but the population is reduced significantly and may not be sufficient enough to serve as the primary inoculum source for summer outbreaks. Freeze-thaw cycles (24 hour periods of 4°C and -20°C) on fresh, infected residue reduced the population to undetectable levels. The results of this study suggest that there is a transition zone for the survival of MoL, and that the threat of this disease decreases with increasing latitude (Harmon *et al*., 2005). MoO has been reported to have sensitivity to cold temperatures (Ou, 1985). Findings reveal that only about one-fifth of hyphae survive for 50-60 days at -4 to -6°C (Abe, 1935; Ou, 1985).

Cruz (2013) predicted that MoT would not be able to survive overwintering in the low-temperature regions of the U.S., but would not be limited in the lower half of the U.S. (Cruz, 2013). According to this study, approximately 75% of the areas that produce winter wheat are not at risk for wheat blast outbreaks, but the remaining areas may experience conditions for wheat blast outbreaks. The states at greatest risk for wheat blast establishment are Alabama, Arkansas, Florida, Georgia, Illinois, Indiana, Iowa, Kansas, Kentucky, Louisiana, Michigan, Mississippi, Missouri, Nebraska, North Carolina, Ohio, Oklahoma, Pennsylvania, South Carolina, Tennessee, Texas, Virginia, and West Virginia (Cruz, 2013). Haley (2011) calculated the average number of freeze thaw days for each state. A freeze thaw day event is defined as any time the temperature crosses the freezing point (Haley, 2011). In December alone, eastern Kansas experiences 15-20 freeze thaw days and western Kansas experiences 20-25 freeze thaw days (Haley, 2011). Since wheat blast developed in tropical and subtropical regions, it is unclear as to whether the fungus would be able to survive the numerous freezing and thawing events it would be exposed to in parts of the United States.

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Chapter 2 - Effects of freezing on the survival and reproduction of *Magnaporthe oryzae* pathotype *triticum*, the causal agent of wheat blast

Abstract

Wheat blast, caused by *Magnaporthe oryzae* pathotype *triticum* (MoT), has emerged as a serious problem for wheat production in South America. To prepare for the possible introduction of wheat blast in the United States, it would be helpful to identify areas of the country most at risk for blast epidemics. Because wheat blast occurs primarily in tropical and subtropical regions of the world, cold winter temperatures may restrict the establishment of the blast fungus in the United States. Therefore, the objective of the research was to quantify the freeze-thaw tolerance of MoT in naturally infected wheat rachises from Bolivia and to measure the viability of the conidia after exposure to various treatments. Treatments included blast-infested rachises that were 1) moist and exposed to five freeze-thaw cycles, 2) moist and exposed to a single, five-day freeze with no thaw periods, 3) dry and exposed to five freeze-thaw cycles, 4) control with no freeze cycles, and 5) moist and exposed to a single, 24-hour freeze. Exposing the fungus in dry residue to five freeze-thaw cycles had no effect on its ability to produce viable conidia relative to the control. When the fungus was in moist residue however, five freeze-thaw cycles reduced the number of viable conidia produced by 66% relative to the control and 83% relative to the fungus in dry residue exposed to five freeze-thaw cycles. The fungus in moist residue exposed to a single 5-day freeze was not significantly affected compared to the control, but produced 65% less viable conidia than the fungus in dry residue exposed to five freeze-thaw cycles. The fungus in moist residue exposed to a single 24-hour freeze also was not significantly different from the

control, but produced 72% less viable conidia than the fungus in dry residue exposed to five freeze-thaw cycles. In conclusion, freezing and thawing of the wheat blast fungus in moist residue significantly affected its ability to produce viable conidia.

Introduction

Magnaporthe oryzae pathotype *triticum* (MoT) is an ascomycetous fungus that causes wheat blast in South America. First identified by Igarashi in Brazil in 1985, the wheat blast pathogen has since spread to wheat producing areas in Brazil, Bolivia, Paraguay, Northern Argentina, and most recently, Bangladesh (Igarashi, 1986; Urashima *et al*., 1993; Kohli *et al.,* 2011; Malaker *et al*., 2016). Wheat blast is capable of infecting all aboveground parts of the wheat plant and can result in 100% yield loss in individual fields during conducive years. The most yield reducing phase is the infection of the spike (Kohli *et al.*, 2011). Spike infections result in shriveled seeds and seed abortion causing severe losses in yield and grain quality (Igarashi, 1990).

Magnaporthe oryzae is divided into host specific populations called pathotypes. Two major pathotypes of *Magnaporthe oryzae* are already present in the United States: *Magnaporthe oryzae* pathotype *oryza* (MoO) which causes rice blast, and *Magnaporthe oryzae* pathotype *Lolium* (MoL) which causes a major turf grass disease, gray leaf spot, on perennial and annual ryegrass and tall fescue (Valent *et al*., 2013). The blast diseases are quite similar in that they have comparable disease cycles and overseason as mycelia and conidia in seeds and residue (Tufan *et al.*, 2009; Ou, 1985; Harmon *et al*., 2005). MoL isolates from the United States and MoT isolates from South America are genetically similar, and some MoL isolates appear to have the ability to infect wheat (Tosa *et al*., 2004; Valent *et al*., 2013). A host shift of the MoL pathotype to MoT is a potential pathway of wheat blast introduction into the United States.

Climate has a large influence on the distribution and disease incidence of *Magnaporthe oryzae*. Harmon *et al*. (2005) reported that the gray leaf spot pathogen is reduced to undetectable levels by alternations of freezing and thawing in the laboratory. In fields located in Indiana, Harmon found MoL can survive the winters in infected residue, but the pathogen population is reduced significantly (Harmon *et al*., 2005). These results suggest that the threat of gray leaf spot decreases with increasing latitude and there is a transition zone for the pathogen's survival. Since wheat blast and gray leaf spot are genetically similar, MoT could behave similarly to MoL and be restricted by winters in the United States. Although it has not been tested; the wheat blast pathogen is considered by some to need tropical and subtropical environments to survive and spread (USDA: New Pest Response Guidelines). If this is correct, MoT may not be able to survive the harsh winters of the United States' Midwest (Cruz, 2013).

The United States is currently the largest exporter of wheat in the world (USDA 2016). Despite being the largest exporter, the United States still imports wheat from Brazil (Cruz, 2013). Due to the seed-borne nature of MoT, this pathogen may be introduced to the United States from Brazil, which could result in disease establishment (Valent *et al*., 2013). Cruz (2013) performed a quantitative pathway analysis to estimate the probability of MoT entry into the United States (Cruz, 2013). The analysis suggested that conditions in North Carolina were favorable for wheat blast in seven out of every ten years.

To prepare for the possible introduction of wheat blast in to the United States by either a host shift of MoL or introduction of infested seed or residue from South America, it would be helpful to identify areas of the country most at risk for epidemics. Because of the results with MoL highlighted previously, a better understanding of the influence of freeze-thaw cycles on the blast fungus may be an important component of the risk assessment. In this study, our objective was to evaluate the influence of freeze-thaw cycles on the reproduction potential of the blast fungus and germination rate of conidia.

Materials and Methods

These experiments were performed in a biosafety level 3 laboratory (BSL-3) at the Biosecurity Research Institute on the Kansas State University campus. Naturally infested wheat spikes were used in the experiments to test native isolates of MoT from the field. The wheat blast susceptible variety "Atlax" was grown during the winter season in the Warnes province of Santa Cruz, Bolivia. It was planted April 15, 2015 and harvested August 10, 2015. A severe wheat blast epidemic developed at the site. The dry residue was harvested by hand and placed into plastic bags for storage. Before beginning the experiments, all parts of the plant (seeds, glumes, leaves, and rachises) were tested for sporulation. The spores produced were counted and it was concluded that the rachises had the best sporulation. To prepare the rachises for testing, the glumes and seeds were removed so only the rachis was remaining. The rachises were then mixed together so that they were chosen randomly for the treatments. One replicate of each treatment consisted of five rachises, not surface sterilized, placed in a glass Petri plate (10 cm) holding two filter paper disks (9 cm). For each treatment there were five replicates. The replicates of the first treatment were moistened with 3 mL of unsterilized, distilled water and randomly placed under continuous fluorescent illumination (25 $\mu m/m^2/s$) at an average of 22°C for eight hours (Cruz *et al.*, 2012). These conditions were considered incubation hours. After eight hours, the replicates were randomly placed into an Isotemp refrigerator freezer (Fisher Scientific) and experienced five freeze-thaw cycles. A freeze-thaw cycle is defined as 23 hours of freezing at approximately -15°C and one hour of thawing under the continuous fluorescent illumination at approximately 22°C. Each hour of thawing was considered an incubation hour. After the five freeze-thaw cycles, the replicates experienced 72 additional incubation hours for a total of 84 incubation hours. The replicates of the second treatment were moistened with 3 mL of unsterilized, distilled water and had eight hours of incubation before placement in the freezer for a continuous five days with no thaw periods. After the 5-day freeze, the replicates experienced 76 additional incubation hours for a total of 84 incubation hours. The replicates of the third treatment were left un-moistened and exposed to five freeze-thaw cycles. After the freeze-thaw cycles, 3 mL of unsterilized, distilled water were added to the replicates and they were incubated for 84 hours. The fourth treatment was considered the control. The replicates were moistened with 3 mL of unsterilized, distilled water and then experienced 84 incubation hours with no freeze periods. The replicates of the fifth treatment were moistened with 3 mL of unsterilized, distilled water and had eight incubation hours before placement in the freezer and exposed to a single 24-hour freeze. After the 24-hour freeze, the replicates experienced 76 additional incubation hours for a total of 84 incubation hours. The treatments were started within five days of each other at specific times so that they would all be quantified on the same day. The day before quantification, each treatment replicate received 1 mL of additional unsterilized, distilled water.

Once a treatment experienced 84 hours of incubation total, the five rachises from one replicate were placed in a 10 mL screw-cap tube with 3 mL of unsterilized, distilled water. The tube was rapidly swirled with a 150-watt digital vortex mixer for 40 seconds to create a conidial suspension. Next 10 µl were pipetted from the conidia solution into a C-Chip DHC-N01-5 Neubauer improved hemocytometer (In Cyto). Conidia were counted at ×20 magnification with a compound light microscope (Olympus BHC). Conidia concentrations were calculated based on the total amount of conidia in four of the hemocytometer fields. After the conidia were counted, 150 mL of the conidia solution was spread onto 1.5% water agar in Petri plates (6 cm) using a sterile glass rod. These steps were carried out simultaneously for each replicate of the treatment. At least 12 hours after plating the conidial solution on water agar, the germination of conidia for each replicate was determined. The first 30 conidia found on the agar plate were recorded as either germinated or not germinated. If a germ tube was visible the conidium was considered germinated. The experiment was arranged in a complete randomized design and was repeated four times.

The total conidia production and germination rates were multiplied together to calculate the amount of viable conidia produced per replicate. The viable conidia were averaged across replicates for each treatment, and analyzed using SAS PROC GLIMMIX (SAS Studio 3.4; SAS Institute Inc.) with a log normal distribution. The germination rates were analyzed separately using the GLIMMIX procedure in SAS with a Gaussian distribution. Run (repeat of experiment) and run-by-treatment were considered random effects and treatment a fixed effect. The treatment means were compared using Tukey's honestly significant difference procedure ($\alpha=0.05$). Covariance parameter estimates (run-by-treatment interaction) were checked on both analyses to confirm the experiments were reproducible across runs. There were no run-by-treatment interactions; therefore, results from all four repeats of the experiment were combined for analysis.

Results

The reproduction of MoT from infested rachis pieces was influenced by exposure to freezing temperatures. However, the moisture status of the crop residue greatly influenced the response. In this analysis, exposing the fungus in dry residue to freeze-thaw cycles had no effect ($p=0.2947$) on its ability to produce viable conidia relative to the control (Figure 2.1, Table 2.1). However, when the fungus in moist residue was exposed to five freeze-thaw cycles the number of viable conidia produced was decreased by 66% relative to the control. These treatments were found to be significantly different (p=0.0122) (Table 2.1). The fungus in the moist residue exposed to five freeze-thaw cycles produced 83% less viable conidia than the fungus in the dry residue exposed to five freeze-thaw cycles. Significant differences were also found between these two treatments (p=0.0004). The fungus in the moist residue exposed to a single 5-day freeze did not produce significantly fewer conidia compared to the control (p=0.1931), but produced 65% less viable conidia than the fungus in the dry residue exposed to five freeze-thaw cycles. This resulted in significant differences between the treatments (p=0.0050). The fungus in moist residue exposed to a single 24-hour freeze was not significantly different from the control, but produced 72% less viable conidia than the fungus in the dry residue exposed to five freeze-thaw cycles: this was significant (p=0.0061).

The germination rates followed a similar pattern as the conidial production. Fungus in the moist residue exposed to five freeze-thaw cycles, a single 5-day freeze, and a single 24-hour freeze had mean germination rates of 38%, 40%, and 42% respectively. However, fungus in dry residue exposed to five freeze-thaw cycles and the fungus exposed to no freezing had germination rates of 77% and 61%, respectively.

Discussion

In this study, the wheat blast fungus in moistened wheat rachises was sensitive to freezing and thawing cycles. When the fungus experienced five freeze-thaw cycles in moistened residues, viable conidia production was reduced the most compared to single freeze events. This may be due to the variability in the spore counts and the reformation of ice crystals intracellularly during each freeze-thaw cycle. In a study by Morris *et al*. (1988), the effect of freezing and the viability after thawing of twenty different species of fungi was observed. One ascomycete species, *Sordaria fimicola*, was included in the study and shown to survive one freeze-thaw regime in the absence of glycerol. Lower rates of cooling resulted in shrinkage of the hyphae whereas faster rates of cooling caused intracellular ice formation and less shrinkage. The study showed that the shrinkage of the hyphae and the formation of intracellular ice did not affect the fungus in any way and the recovery rate was 100% (Morris *et al*., 1988). The formation of ice crystals for one freezing and thawing period did not affect the ascomycete in this study which is consistent with our results. Five freeze-thaw cycles has a more severe effect on MoT hyphae, perhaps because of the reformation of ice crystals after each thaw period.

MoT in dry residue exposed to five freeze-thaw cycles consistently gave the highest conidia counts and germination rates. This could be explained by reduced populations of competition fungi. Some of the competition fungi present may have been thermophilic, filamentous fungi (Griffin, 1994) that are unable to survive freezing temperatures, dry or moistened, but thrived in the control treatment, therefore suppressing the wheat blast fungus more.

This study also enables estimates of the reproduction potential of the blast fungus on naturally infected wheat residues. For example; in Bolivia, the planting density of wheat varies from 300-350 seeds per square meter (Manual de recomendaciones técnicas - Cultivo de trigo). Each of these seeds will produce 1.5 to 2.5 heads depending on the variety, environment, and cultural practices which translates to 450-875 spikes per square meter (personal communication, Javier Kiyuna, May 2, 2016). After harvest, the rachis of the wheat spike would be left behind in the field. In a heavily blasted field in Bolivia, this translates to 9-18 million blast conidia per square meter that could potentially provide inoculum for next year’s wheat crop. If the residue were to undergo one freeze-thaw cycle while moistened, this would reduce the inoculum load to

5-10 million conidia per square meter. To compare, if the residue was exposed to five freeze-thaw cycles while moistened, this would reduce the inoculum load even further to 3-6 million conidia per square meter.

In Kansas, the estimated results are similar. In the last five years the yield for winter wheat has ranged from 28-42 bushels per acre. Using the Estimating Wheat Yield guide from Kansas State University Research and Extension (MF-3044) (Martin *et al*., 2011), the estimated number of stems per foot for the yield range previously stated and 7.5-inch row spacing were used to create the range of 216-864 spikes per square meter. As with Bolivia, this range largely depends on cultural practices, variety, and the environment. After harvest, in a heavily infested wheat blast field, the fungus would be able to produce 4.5-18 million conidia per square meter. If the residue experienced one freeze-thaw cycle while moist, the population would be reduced to 2.5-10 million conidia per square meter. If the residue was exposed to five freeze-thaw cycles while moist, the population would be reduced even more to 1.5-6 million conidia per square meter. According to these results, five freeze-thaw cycles are unlikely to stop an epidemic from occurring, although it reduces the population significantly.

On average, northeast Kansas experiences 75-100 freeze-thaw events annually. In southern Texas, Louisiana, Florida, California, and Arizona, 0-25 freeze-thaw events occur annually (Haley, 2011). These areas coincide with acres planted to winter and durum wheat (NASS, 2015), and may be high risk areas for overwintering of wheat blast in the United States.

This report is the first to quantify the freeze-thaw effects on MoT-infested residue. The study indicates that freezing and thawing of moistened, infested tissue significantly reduces viable conidia production by 66% when compared to the control. This work provides insights into the possible introduction and establishment of MoT into the United States, suggesting that mild winters in areas of the United States where wheat is grown may contribute to the survival of the blast fungus and establishment in the United States. Areas in the United States where harsher winters occur would be less suitable for overwintering of the pathogen in residue and, consequently, less likely for wheat blast establishment and epidemics to occur; although more research is needed to support the findings reported here. Pathogen acclimation time, narrower temperature ranges, and longer cycles need to be tested in order to extend this research and more closely simulate naturally occurring environmental conditions.

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Figures and Tables

Figure 2.1 Average viable conidia production per replicate (5 rachises) by *Magnaporthe oryzae* pathotype *triticum* in naturally infected wheat residues exposed to one or more freeze-thaw (FT) cycles.

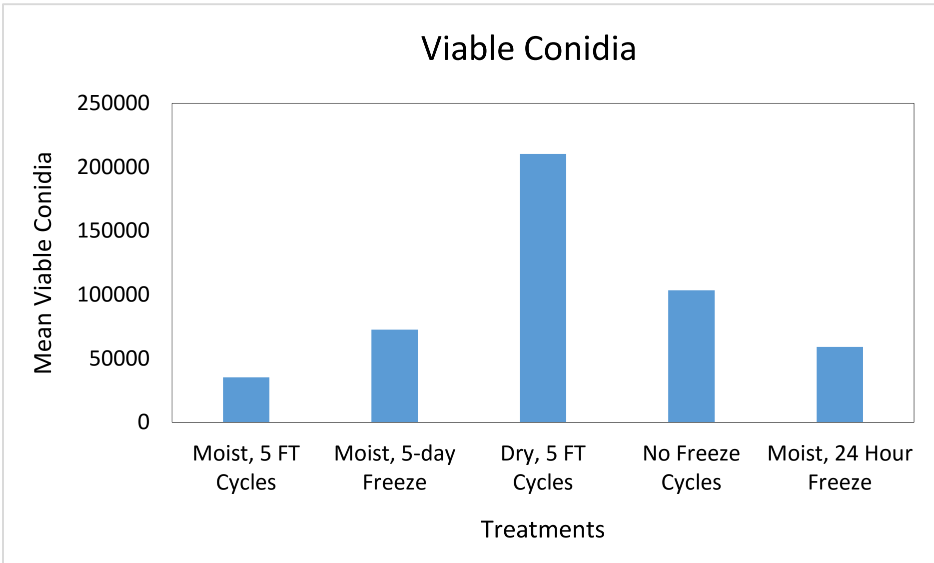

Table 2.1 Adjusted p-values for comparisons of mean viable conidia production per replicate (5 rachises) by *Magnaporthe oryzae* pathotype *triticum* following one or more freeze-thaw cycles.

Treatment (Mean Viable Conidia)	Moist, 5 FT Cycles	Moist, 5-day Freeze	Dry, 5 FT Cycles	No Freeze Cycles	Moist, 24 Hour Freeze
Moist, 5 FT Cycles (35,138)	--	0.5559	0.0004*	0.0122*	0.4930
Moist, 5-day Freeze (72,600)	0.5559	--	0.0050*	0.1626	1.000
Dry, 5 FT Cycles (210,338)	0.0004*	0.0050*	--	0.2947	0.0061*
No Freeze Cycles (103,438)	0.0122*	0.1626	0.2947	--	0.1931
Moist, 24 Hour Freeze (58,975)	0.4930	1.000	0.0061*	0.1931	--

Comparisons between treatments and the corresponding *p-values* (0.05).

The average viable conidia produced is under the treatment in parentheses.

* Indicates significant differences

Chapter 3 - Weather conditions favoring epidemics of rice blast in the Southern United States

Abstract

Wheat blast, caused by *Magnaporthe oryzae* pathotype *triticum* (MoT), has emerged as a serious problem for wheat production in South America and recently emerged as a threat in Bangladesh. To prepare for the possible introduction of wheat blast in to the United States, it would be helpful to identify areas of the country most at risk for blast epidemics. Therefore, the objective of this research was to identify environmental conditions that could be conducive for wheat blast epidemics by examining historical epidemics of rice blast, caused by *Magnaporthe oryzae* pathotype *oryza* (MoO). The dataset used in this analysis consisted of 60 site-years of historical observations of rice blast levels from Arkansas, Louisiana, and Texas. These observations were coupled with monthly and weekly summaries of hourly weather variables based on temperature, relative humidity, precipitation, and regional moisture indices. Classification trees and logistic regression were used to identify variables likely associated with rice blast epidemics. The results indicate that rice blast epidemics are favored by cooler April temperatures and high levels of precipitation in June. Preliminary models for rice blast, based on these variables, were able to correctly classify epidemic years with >75% accuracy. The results of this project will be incorporated into a risk assessment for the possible introduction of wheat blast in to the United States.

Introduction

Wheat blast, caused by *Magnaporthe oryzae* pathotype *triticum* (MoT), is a fungal disease that first emerged in the Paraná State of Brazil in 1985 (Igarashi, 1986; Urashima *et al*., 1993). The pathogen has since spread to other wheat producing areas in South America, and most recently was detected in Bangladesh in February 2016 (Kohli *et al*., 2011; Malaker *et al*., 2016). Wheat blast is capable of infecting all aboveground parts of the plant and can result in 100% yield loss in fields during severe epidemics (Kohli *et al*., 2011). Currently, there is a lack of resistant wheat varieties and effective fungicides capable of controlling this disease (Valent *et al*., 2013).

Because wheat blast is a relatively new disease, the disease cycle, epidemiology, and biology of the pathogen are not fully understood. *Magnaporthe oryzae* is grouped into pathotypes based on the host range of the specific isolates. Rice blast, caused by *Magnaporthe oryzae* pathotype *oryza* (MoO), and gray leaf spot, caused by *Magnaporthe oryzae* pathotype *Lolium* (MoL), which infects annual ryegrass, perennial ryegrass, and tall fescue, are both prevalent in the United States (Valent *et al*., 2013). Since rice blast and gray leaf spot are caused by the same fungal species as wheat blast, similar characteristics among their epidemiology may be found. A team of collaborators from the United States, Brazil, Bolivia, and Paraguay have joined together in an effort to fully characterize wheat blast, define control methods, and perform a risk assessment of disease establishment, among other objectives. Therefore, the objective of this research was to identify environmental conditions that are conducive for wheat blast epidemics by examining historical epidemics of rice blast.

Rice blast predictive and simulation models have been created for rice producing areas of the world including Italy, South Korea, Philippines, India, and Japan (Calvero *et al*., 1996; Biloni *et al*., 2006; Ishiguro and Hashimoto, 1991; Kaundal *et al*., 2006). In general, these models use weather variables such as relative humidity, temperature, precipitation, dew point, and wind speed to determine the risk of rice blast epidemics (Biloni *et al*., 2006; Calvero *et al.,* 1996). To the best of our knowledge, no predictive models have been created for the rice producing regions of the Southern United States. Such a model could be very useful in aiding growers to make rational and timely fungicide decisions.

A review of rice blast epidemiology literature indicates leaf wetness is a very important factor for conidia germination and rice blast development (Teng *et al*., 1991; Hamer *et al*., 1988). Moistened conidia are able to survive relative humidity as low as 80% for a short amount of time, but are not produced below 89% relative humidity (Suzuki, 1975; Calvero *et al*., 1996). The optimum temperature range for germination is 25-28°C and 16-25°C for appressorium development (Suzuki, 1969; Sueda, 1928; Ou, 1985). According to Suzuki (1975) sporulation does not occur below 9°C or above 35°C (Suzuki, 1975). Kohli *et al*. (2011) reported that wheat blast is favored by continuous rain for several days at an optimum temperature between 18 and 25°C during flowering.

Materials and Methods

Historical observations of rice blast levels from the Southcentral United States, where a majority of the country's rice is produced, were obtained from collaborators in Arkansas, Louisiana, and Texas. For Arkansas, major epidemic years were acquired from the historical Arkansas Rice Disease Survey records and from Rice Disease Monitoring Plot Reports (provided by Dr. Rick Cartwright and Dr. Yeshi Wamishe). The years considered for Arkansas were 1986, 1987, and 1992-2013. Out of these years, seven were classified as epidemics. For Louisiana, the rice blast disease levels were obtained from the Crowley Rice Research Station (provided by Dr. Don Groth). These observations were based on fungicide testing results and records of regional epidemics in Southern Louisiana. The years used for Louisiana were 1984-2013. Within these years, four were classified as epidemics. Dr. Anna McClung and Dr. Shane Zhou provided historical rice blast epidemic information for Texas. The years used for Texas were 1993 and 2009-2013. Within these years, two were classified as epidemics. For all three locations, the years that did not have rice blast information were omitted from the analysis. The total data set included 13 epidemic years and 47 non-epidemic years.

Weather data was obtained from hourly importing weather stations maintained by the national weather service in key rice producing regions of Arkansas, Louisiana, and Texas. These locations included Stuttgart, Arkansas; Lafayette, Louisiana; and Port Arthur, Texas. The hourly weather data provided relative humidity (RH, %), precipitation (mm), and temperature (°C) for each year, and was summarized into monthly variables. Calendar years were edited to be specific to the rice season for these regions; therefore the biological year began in September and continued through August of the following year, as to represent temperature and moisture conditions likely to favor blast epidemics. In addition to hourly weather data, the standard precipitation index (SPI) was incorporated as a regional moisture index. The SPI is a probability index that provides a negative index for drought conditions and a positive index for wet conditions. The more negative the index is, the drier the soil conditions, and the more positive the index, the wetter the soil conditions (McKee *et al*., 1993; Guttman, 1999). The index typically ranges from -3 to 3; -3 representing very dry conditions and 3 representing very wet conditions. The monthly SPI was obtained from the National Climatic Data Center (NCDC). The climate districts were chosen based on where rice production occurs within each state and corresponded to the locations represented by hourly weather data (Figure 3.1).

Weather variables were created based on previous modeling efforts and experiments conducted in controlled environments. Moisture variables considered were SPI, sum of hours greater than 80% RH, sum of hours greater than 89% RH, and sum of monthly precipitation.

Temperature variables used were: sum of hours within favorable ranges (9-35, 16-28, 16-25), average monthly temperatures, and combinations of these temperature and moisture variables. Classification trees (CT) were used to select variables most likely to be associated with epidemics and non-epidemics (JMP Pro 11.0, SAS Institute Inc., Cary, NC). The variables were selected based on their likelihood-ratio chi-square statistic (G^2). In general terms, the higher the G^2 the more likely that variable is adequate at classifying epidemics and non-epidemics correctly.

To create variables more specific to the rice blast pathosystem, heading date variables were created. Specific heading dates provided by Don Groth were used for Lafayette. For Stuttgart and Port Arthur dates closest to 50% heading provided by the National Agricultural Statistics Service (NASS) were used for each year. The three weeks before the heading date were categorized as before heading and the three weeks (including the heading date) after heading were categorized as after heading. The heading date variables were combined with the original variables and analyzed in the same manner described previously using CT analyses and logistic regression.

The top fifteen variables with the highest G^2 were then used as independent variables in univariate and multivariate logistic regression models. The fit of the resulting models was evaluated based on the following criteria. The area under the receiver-operating characteristic (AUC) is scaled from 0.5 to 1. The closer the AUC is to one, the more epidemics and non-epidemics there are being classified correctly. The Akaike Information Criterion (AIC) was also used to measure how well the model fit the data. The lower the AIC, the better quality of the model. Accuracy was evaluated in terms of true positives (epidemics classified correctly), true negatives (non-epidemics classified correctly), false positives (non-epidemics classified as epidemics), false negatives (epidemics classified as non-epidemics), specificity (percentage of non-epidemics classified correctly), and sensitivity (percentage of epidemics classified correctly). The thresholds for determining model classification accuracy were based on Receiver Operating Characteristic (ROC) analysis.

Results

The classification trees identified multiple representations of temperature as strongly associated with rice blast epidemics. Four of these variables summarized temperatures in April. Specifically, fewer hours within the April temperature ranges of 9-35°C, 16-28°C, 16-25°C, and mean temperatures less than 19°C favored epidemics (9-35_4, 16-28_4, 16-25_4, and Temp_4, Table 3.1). This timeframe represents planting and early stages of seedling development for the rice producing regions of Arkansas, Louisiana, and Texas (Figure 3.2). Mean temperature less than 15.8°C in March (Temp_3), mean temperature less than 27.6°C in June (Temp_6), more hours in the 16-28°C July temperature range (16-28_7), and more hours in the 9-35°C August temperature range (9-35_8) were among other temperature variables that were identified as potentially important. July represents the heading development stage, August largely represents after heading and harvest, and March is before planting of the rice. When the time period was restricted to heading, the top temperature variables, according to the CT analysis, were the temperature range of 9-35°C after heading (9-35_AH) and mean temperature less than 28°C after heading (Temp_AH). These ranges represent optimum temperatures at which the pathogen would sporulate and develop.

Several moisture variables were identified by the CT analysis as well. The strongest association was with SPI greater than -0.55 in June (SPI_6, Table 3.1) which corresponds to when the rice crop is heading or just prior to heading (Figure 3.2). SPI greater than -1.0 in

August (SPI_8) and precipitation greater than 205.6mm before heading (Prec_BH) were among the top moisture variables. August is at the end of the heading development stage and when harvest begins in the rice plant. The variables listed previously coincide with planting and heading of the rice, which is when it is very vulnerable to disease.

The variables identified by the CT analysis were analyzed with logistic regression individually and combined with each other. The univariate model of SPI in June gave an AUC of .73, specificity of 77%, and sensitivity of 62% (Table 3.2). The univariate model of the 9-35°C temperature range in April gave an AUC of .72, specificity of 57%, and sensitivity of 85%. When SPI in June is paired with the 9-35°C temperature range in April, the AUC increases to .79, specificity is 77%, and the sensitivity is 85%. The mean temperature in April had an AUC of .71, specificity of 68%, and sensitivity of 85%. When paired with the SPI in June, the AUC is .77, specificity is 72%, and sensitivity is 85%. The remaining April temperature univariate models and bivariate models with SPI in June are similar to the results recorded here and are listed in Table 3.2. The heading date models did not fit the data as well, but could still be indicators of epidemic conditions. The mean temperature three weeks after heading had an AUC of .69, specificity of 49%, and sensitivity of 85%. When paired with the SPI in June the results were an AUC of .76, a specificity of 66%, and a sensitivity of 84%.

Discussion

This analysis documents the importance of moisture in the development of rice blast epidemics. The relationship with SPI was strongest in June. The average heading date of rice for the Lafayette data is July 1st, Stuttgart is August 1st, and Port Arthur is July 12th. Therefore the wetter conditions in June provide a more conducive environment for blast development right before heading. The heading date variable, precipitation three weeks before heading, had a strong correlation with epidemics as well. Moisture during this time period would help establish the disease on the leaves and favor reproduction just as the crop is entering the heading stages of growth that are vulnerable to the panicle blast phase of the disease.

Multiple temperature variables were also associated with blast epidemics. In general, cool temperatures in early spring appear to be most correlated with rice blast epidemics. April temperature had the strongest relationship and highest G^2 statistic when compared to the other temperature variables considered in this analysis. Rice is being planted in late March and April in the Southern United States, and cool temperatures during this time period may have indirect effects on the blast development. For example cool temperatures may delay planting. This delayed planting could have several downstream effects such as causing the crop to head later during a time frame that has increased leaf wetness and conducive temperatures that favor panicle blast epidemics. The delaying of planting due to cool spring temperatures may also delay flooding of rice fields, which can predispose the crop to blast as well (Don Groth, personal communication).

As mentioned previously, models from various rice producing regions around the world used RH, temperature, precipitation, among other variables to determine the risk of rice blast epidemics (Biloni *et al*., 2006; Calvero *et al*., 1996). Leaf wetness, high RH, and optimum temperature ranges are important factors for disease development according to the literature (Teng *et al*., 1991; Hamer *et al.*, 1988; Suzuki, 1975; Calvero *et al*., 1996). In this study, the monthly summaries of moisture during heading and cool April temperatures appear to be the driving variables in predicting rice blast epidemics. The variables specific to three weeks before and after the heading date indicate that precipitation three weeks before heading and optimum temperatures the three weeks after heading favor rice blast development. These results suggest that temperatures during heading are rarely limiting to the development of rice blast development. The system may be more driven by moisture that favors early disease establishment and inoculum at heading.

The threat of a wheat blast introduction in to the United States by a host shift or through trade is a prevalent risk that is currently being analyzed. This study characterizes environmental conditions which favor rice blast that could be useful in a risk assessment for modeling where wheat blast is a threat in the United States. The most favorable environmental conditions identified in this study will be combined with models for *Magnaporthe oryzae* in wheat and turf grass in order to create an overall risk assessment for the United States. Another outcome of this study is the logistic regression models can be used as part of a rice blast forecasting system for the Southern United States. Once the forecasting system is in place, it could be used to help alert growers of weather patterns associated with rice blast epidemics.

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Figures and Tables

Table 3.1 Important rice blast-associated weather variables defined by classification tree analysis.

Variable	G^2	Variable Description
Temp_4	12.1	Average monthly temperature in April
16-28_4	11.8	Monthly sum of hours within this temperature range in April
16-25_4	9.5	Monthly sum of hours within this temperature range in April
SPI_6	9.3	Standard precipitation index in June
Temp_3	9.3	Average monthly temperature in March
16-28_7	9.2	Monthly sum of hours within this temperature range in July
Prec_BH	8.7	Three week sum of precipitation before heading
9-35_4	7.8	Monthly sum of hours within this temperature range in April
9-35_8	7.3	Monthly sum of hours within this temperature range in August
SPI_8	7.3	Standard precipitation index in August
Temp_6	7.3	Average monthly temperature in June
9-35_AH	6.5	Three week sum of hours within this temperature range after heading
Temp_AH	5.2	Three week average of temperature after heading

The CT analysis identified 13 variables that are most strongly associated with rice blast epidemics. The variable column contains the abbreviation for each variable, the G^2 column shows the G^2 for each variable given by the CT analysis, and the variable description column defines each variable. The numbers and letters at the end of each variable represent the month or heading timeframe of that variable. For example: 4 represents April, 6 represents June, BH represents three weeks before heading, and AH represents three weeks after heading. **Bolded variables are most strongly associated with rice blast epidemics based on the logistic regression analysis in Table 3.2.**

Table 3.2 Logistic regression results between the top moisture and temperature variables that favor rice blast epidemics.

Variable 1	Variable 2	AUC[a]	AIC[b]	Specificity[c]	Sensitivity	TP[d]	TN	FP	FN
SPI_6	-	.73159	60.8505	77%	62%	8	36	11	5
-	9-35_4	.71686	63.324	57%	85%	11	27	20	2
-	16-28_4	.72177	62.7563	57%	92%	12	27	20	1
-	Temp_4	.70867	63.7062	68%	85%	11	32	15	2
-	Temp_AH	.68903	60.8796	49%	85%	11	23	24	2
SPI_6	9-35_4	.79378	58.5545	77%	85%	11	36	11	2
SPI_6	16-28_4	.78642	58.9091	74%	85%	11	35	12	2
SPI_6	Temp_4	.76923	59.0236	72%	85%	11	34	13	2
SPI_6	Temp_AH	.76268	59.3733	66%	84%	11	31	16	2

Logistic regression results between the top moisture and temperature variables that favor rice blast epidemics.

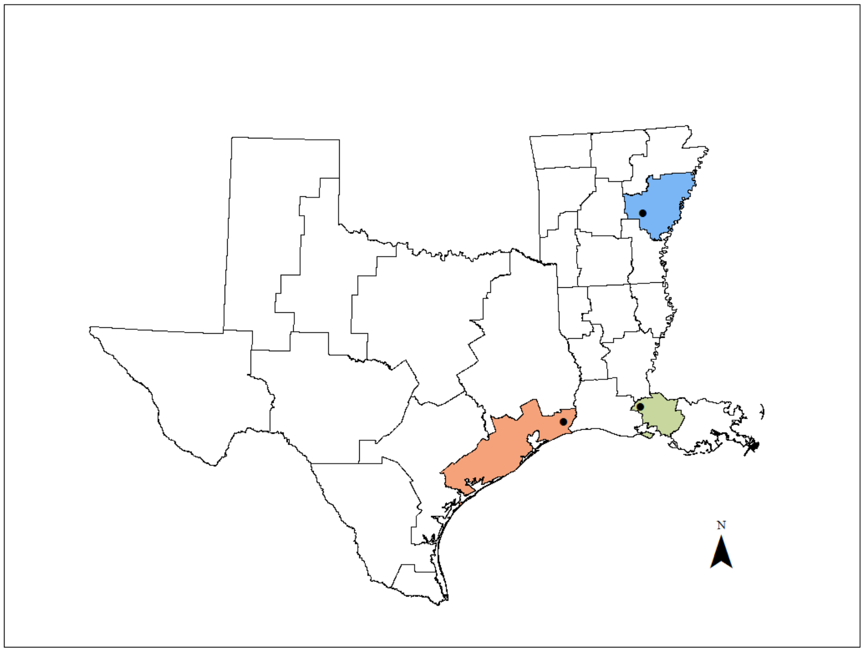
[a] The area under the receiver-operating curve (AUC) is scaled from 0.5 to 1. More epidemics are being classified correctly if the AUC is closer to one.

[b] The Akaike Information Criterion (AIC) measures the quality and fit of the model. Lower AICs are indicators of better models.

[c] Specificity is the percentage of non-epidemics being classified correctly and sensitivity is the percentage of epidemics being classified correctly.

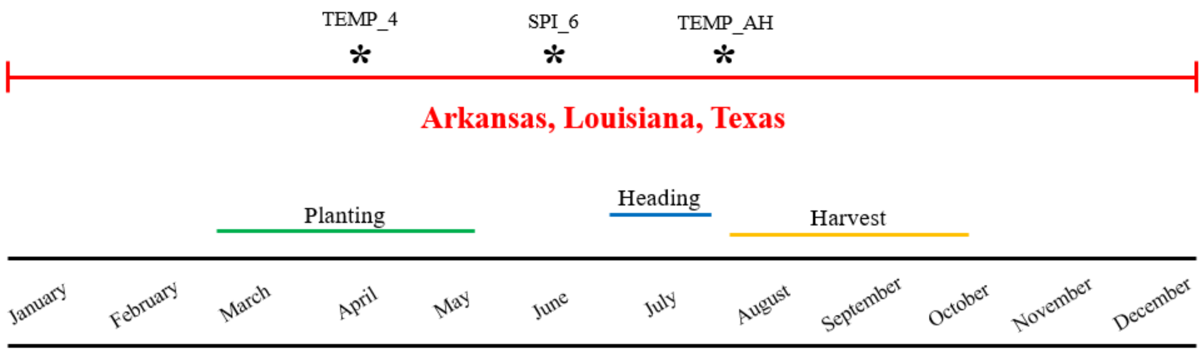
[d] TP (true positives) are epidemics classified correctly, TN (true negatives) are non-epidemics classified correctly, FP (false positives are non-epidemics) classified as epidemics, and FN (false negatives) are epidemics classified as non-epidemics.

Figure 3.1 Map of climate districts within Texas, Louisiana, and Arkansas that correspond to the Standard Precipitation Index and rice producing areas.



The solid circle within the colored climate districts corresponds to the location of the weather station that historical weather data was obtained from.

Figure 3.2 Rice production timelines for the Southcentral US and associated weather variables that influence rice blast epidemics.



TEMP_4 (average temperature in April) corresponds to the planting and seedling development stages of the rice crop. SPI_6 (Standard Precipitation Index in June) corresponds to the time period before the rice crop is heading and at the beginning of heading in some areas. The last variable (TEMP_AH) is the average temperature three weeks after heading.

Chapter 4 - Conclusions

This is the first report of the effect of freeze-thaw cycles on the reproduction of *Magnaporthe oryzae* pathotype *triticum*. The results document that multiple freeze-thaw cycles of the fungus on moist residue has a greater effect on conidia production than a single freeze event. Freeze-thaw cycles had little to no effect on the fungus when the residue was dry. The viable sporulation of the fungus was reduced by 66% relative to the control fungus. These results suggest that viable conidia are still being produced by the fungus. In the future it may be possible to expand or refine the results gained here by inoculating healthy wheat plants with the conidia solution made from each replicate. If disease establishment occurs, then the conidia are still able to cause disease. To take the study further, residue could be placed in an incubator that closely mimics the outside environment through more gradual transitions between temperature extremes. Therefore a better understanding of how the fungus would react to conditions in the United States would be measured. Because the freeze-thaw cycles that occur in Kansas are not as extreme as the ones used in this study, the fungus may be able to acclimate and survive better in the natural environment than indicated in this study. On the other hand, Kansas undergoes around 75-100 freeze-thaw cycles annually, even though not as extreme as the freeze-thaw cycles in this study, they could be more damaging because of the large number of cycles.

Analysis of rice blast epidemics in the Southern United States indicated that epidemics are favored by cool April temperatures and high moisture before heading. When these variables were paired together, an accuracy of >79% resulted. The main objective for this study was to identify weather variables associated with rice blast epidemics that may be useful in a wheat blast risk assessment for the United States. The most favorable environmental variables identified will be combined with models for *Magnaporthe oryzae* in wheat and turf grass in order to create an overall risk assessment for the United States. A risk assessment of wheat blast would provide insights into areas of the United States most at risk of a wheat blast introduction and establishment. The logistic regression models can be used to create a rice blast forecasting system for the Southern United States. The April temperature variables around planting and early seedling development could alert growers that early spring conditions are conducive for blast development. If there is high moisture in June, growers should monitor their fields closely for rice blast. The preliminary models from this study would need to be cross-validated and analyzed further to determine which ones are most accurate. Once the forecasting system is in place, it would alert growers of weather patterns associated with rice blast epidemics. When the risk of an epidemic is high, growers will be aware of the importance of scouting their fields so fungicide applications can occur in a timely manner.