2010

Effects of environmental variables and crop growth on development of brown rust epidemics in sugarcane

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EFFECTS OF ENVIRONMENTAL VARIABLES AND CROP GROWTH ON DEVELOPMENT OF BROWN RUST EPIDEMICS IN SUGARCANE

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science
In

The Department of Plant Pathology and Crop Physiology

by
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B.S., EARTH University, Costa Rica, 2005
December, 2010

Dedication

To my father Jacinto Barrera, my mother Guillermina Ayala and my friends Jose Ricardo Ortiz and Miriam Gil for believing in my dreams when I was a teenager.

Acknowledgments

First of all, I would like to thank Dr. Jeffrey Hoy for providing me the guidance, encouragement and advice through the completion of this project, and for helping me develop scientific skills.

I also want to thank Dr. Michael Grisham, Dr. Clayton Hollier, Dr. Raymond Schneider and Dr. Mary Catherine Aime for serving in my research committee and for providing valuable advice in the design, execution and revision of the experiments results. Thanks to Dr. Michael Grisham for providing sugarcane plots for monitoring brown rust in Shriever LA, and Dr. Clayton Hollier for loaning his weather stations for collecting the environmental data in this study. Thanks to Dr. Bin Li from the Experimental Statistics Department at Louisiana State University for his support in the statistical analysis of the data.

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Abstract

Natural epidemics of brown rust, caused by *Puccinia melanocephala,* affecting sugarcane were studied to determine the crop and/or environmental factors that affect epidemic onset, severity and eventual decline. Environmental and crop growth variables were monitored along with disease severity in two susceptible cultivars, LCP 85-384 and Ho 95-988, each grown at a different location in Louisiana. During two seasons, correlation and multiple regression analyses identified leaf wetness and temperature as important determinants of disease severity for both cultivars. The results suggested that crop growth variables were not determinants for epidemics. Controlled conditions experiments assessing the interaction of leaf wetness and temperature demonstrated that changes in one variable will influence the effect of the other and identified minimum and maximum values required for infection. Increasing leaf wetness duration from 7 to 10 or 13 hours resulted in greater infection at an optimal temperature range of 17 to 27 C. Minimum requirements for leaf wetness and temperature were 7 hours and 17 C. Minimal infection occurred at 29 and 31 C. Severe epidemics in both cultivars began to decline once maximum ambient daily temperature was 32 C or higher. Lower disease severity during the 2010 epidemic in Ho 95-988 allowed an analysis of the effects of conducive and limiting conditions on brown rust severity. Lower severity resulted from a combination of unfavorable temperature and leaf wetness conditions that delayed onset then reduced the rate of disease increase. An accumulation of 23-25 leaf wetness conducive days after the daily minimum temperature exceeded 17 C preceded the onset of disease on young leaves in all three epidemics suggesting cumulative leaf wetness days might provide an epidemic predictor. The study results suggest that the

occurrence of limiting temperatures determines the initiation and decline of a brown rust epidemic under Louisiana climatic conditions. The availability of leaf wetness is then the most important determinant of disease severity during the epidemic period. The study results suggest that temperature and leaf wetness can provide the basis for a disease advisory or forecasting system that predicts the threat of a severe epidemic and improves recommendations for fungicide use.

Chapter 1. Literature Review

Today, over 75% of sucrose produced in the world comes from sugarcane (Huntrods *et al.,* 2010). It is a crop grown in over 110 countries, mainly in tropical and subtropical regions (Fig. 1.1). It occupies an area of 107.11 million ha and has a total production of 7431.5 million metric tons of cane worldwide (FAO, 2009). Out of these countries, Brazil has the highest area (8.598 million ha), while Peru has the highest productivity with 131.8 tons of cane/ha (FAO, 2009).

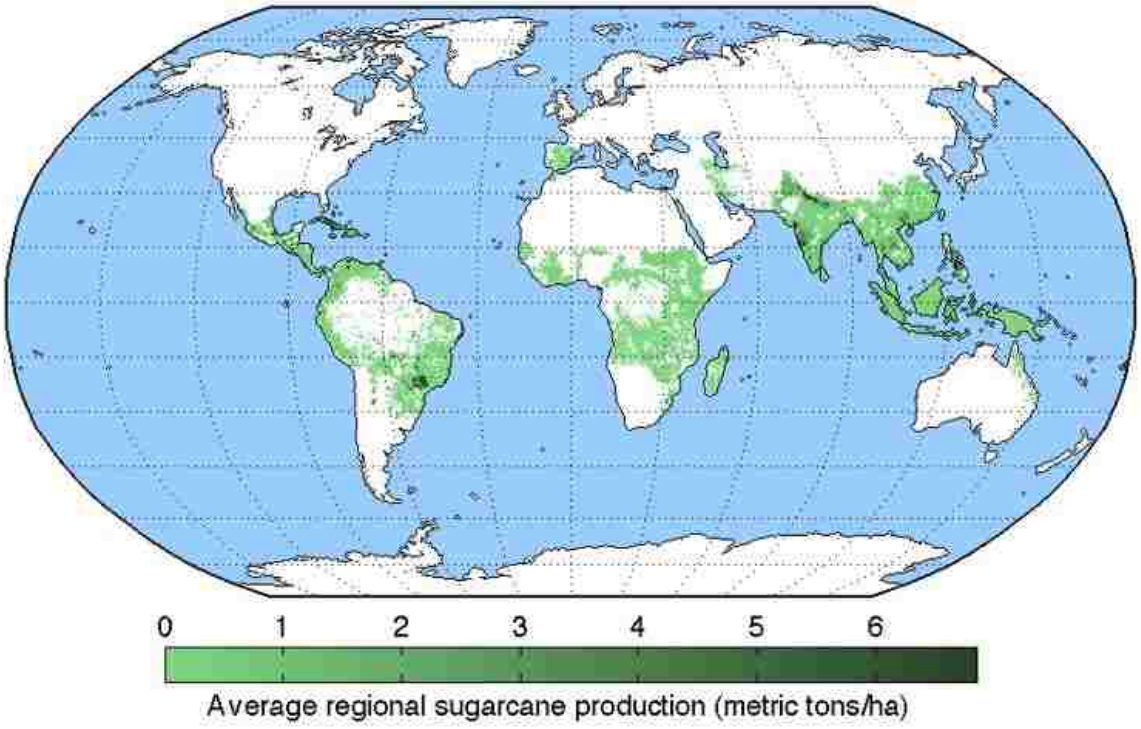

Figure 1.1 Distribution of sugarcane crop worldwide. Source: University of Minnesota Institute on the Environment with data from: Monfreda, C., N. Ramankutty, and J.A. Foley. 2008.

In the United States, sugarcane is grown for sucrose in Florida, Louisiana, Texas, and Hawaii. Florida produces 48% of the total cane sugar (Baucum and Rice, 2009), while Louisiana produces nearly 43%. The rest is produced in Texas and Hawaii

(NASS, 2010). Sugarcane production has an important role in the Louisiana economy. The crop is grown on nearly 182,000 ha, and its production can exceed 14 million tons of cane, with an economic impact of $1.7 billion to the cane growers and raw sugar factories of the state (American Sugar Cane League, 2009**)**. Additionally, the economic activity generated by this crop provides employment for approximately 27,000 workers in the production and processing of sugar.

Among the 120 diseases that were described by Rott *et al.* (2000) on sugarcane, brown rust is considered to be one of the diseases capable of causing severe losses and one that demands a continuous resistance selection effort in breeding programs. Yield reductions of 10-40% due to brown rust are common on susceptible varieties (Comstock *et al.,* 1992), and severe epidemics can reduce yield by up to 40-50% (James, 2004). Before 1978, the disease was only present in scattered locations in Africa and Asia, without causing important economic impacts (Purdy *et al.,* 1985). However, after its introduction into the Americas during that year, it became a major concern in many production areas because of its immediate severe effect on cultivar B4364, which accounted for most of commercial growing area in the Caribbean (Liu, 1980). As a result, many industries were forced to remove susceptible cultivars from production. It is likely that its movement to the Western Hemisphere occurred via transoceanic high altitude air current transportation of urediniospores from Western Africa to the Caribbean (Purdy *et al.,* 1983).

The amount of loss caused by brown rust was initially assessed following introduction by comparing the productivity in years before versus the years after its

development in susceptible cultivars. For example, reports of losses in four mill areas in Mexico indicated that 50% of the yield produced by the susceptible cultivar B4362 was lost due to rust infection during the season 1981-1982, compared to the season 1979-1980 (Purdy *et al.,* 1983). Losses in yield of up to 33% were reported in the Dominican Republic for 1978 (Comstock *et al.,* 1992). A replicated pot trial study (Comstock *et al.,* 1992) indicated reductions of up to 24% in sucrose yield in the susceptible cultivar CP 72-1210. In Louisiana, sucrose yield losses up to 22% were reported from a susceptible cultivar, LCP 85-384, during the 2004 to 2006 growing seasons (Hoy and Hollier, 2009).

Puccinia melanocephala Syd. & P. Syd, the causal organism of brown rust, belongs to the Phylum Basidiomycota, Class Pucciniomycetes, Order Pucciniales, Family Pucciniaceae, Genus *Puccinia* (Dixon *et al.,* 2010). Although sugarcane is the main host, *P. melanocephala* infection also has been observed on other species within the Poaceae family, including sugar grass (*Erianthus fulvus)*, and *Narenga porphyrocoma* (Raid and Comstock, 2000). Dixon (2008) reported infection by *P. melanocephala* on *Eulalia fastigiata,* Miscanthus *(Miscanthus* spp.), common bamboo *(Bambusa* vulgaris*), Phyllostachys aurea,* and sorghum (*Sorghum* spp). Both urediniospores and teliospores have been described. Urediniospores are very common during epidemics, while teliospores have been observed towards the end of the growing season. Basidiospores have been found, but they do not initiate infection in sugarcane (Purdy *et al.,* 1983). An alternate host is not known.

Typical symptoms of the disease include initial yellow spots or flecks on both leaf surfaces. Subsequent lesions become reddish-brown, and sporulating pustules develop in the abaxial leaf surface within 10-14 days (Asnaghi *et al.,* 2001). Severely infected

leaves have large numbers of pustules that can coalesce, causing large areas of leaves to become necrotic. Young leaves may then die prematurely (Comstock and Raid, 2000). Even though the disease rarely kills the plant, it can cause reductions in stalk diameter, stalk length, and in the number of stalks per plant, thus affecting both tonnage and sucrose yields (Raid and Comstock, 2006).

Traditionally, withdrawing susceptible cultivars of sugarcane from cultivation, then breeding and selecting resistant cultivars has been the only measure for brown rust management (Purdy *et al.,* 1983; Raid and Comstock, 2000). The requirement for brown rust resistance places an additional burden on the selection process resulting in the elimination of agronomically promising cultivars (Raid and Comstock, 2000); however, breeding has provided control for the disease and has reduced economic losses (Asnaghi *et al.,* 2001). Unfortunately, the development of resistant cultivars is not an everlasting cure for the problem. Newly developed cultivars are threatened by the possible mutation of *P. melanocephala* into new races. The occurrence of races of the pathogen has not been studied extensively (Shine *et al.,* 2005), but there have been numerous reports of cultivar shifts from resistance to susceptibility to brown rust. For example, the cultivar CP78-1247 showed resistance to brown rust in Florida before 1985. However, in 1988, it showed extremely high susceptibility, and yields were as low as 40% of expected (Raid *et al.,* 1989). Other cultivar shifts were observed in Florida, including CP 79-1580 (Dean and Purdy, 1984), CP 72-1210 (Raid *et al.,* 1981), CP 74-2005, and CL 73-239 in 1989 (Shine *et al.,* 2005). In Louisiana, a shift from resistance to susceptibility was observed in the cultivar LCP 85-384, and severe to moderate

symptoms have been observed in four previously resistant commercial cultivars that were released since 2003 (Hoy, 2008; Hoy unpublished).

Replacing newly susceptible cultivars in the field is not an easy process in sugarcane even if suitable replacement cultivars are available. This makes the economic impact of sudden shifts in cultivars to susceptibility more severe. Vegetative propagation and a fallow period prior to planting make the planting of sugarcane expensive. To recover these costs, it is necessary to obtain multiple annual harvests of stalks from a planting. Early plow-out of sugarcane results in large economic losses. In addition, the availability of healthy seedcane of replacement cultivars for planting may be limited. As a result, for a new resistant cultivar to be ready for establishment in the field, it can take several years. These circumstances can result in periods of time in which the yields and profitability of the industry can be seriously affected by shifts in susceptibility to brown rust (Hoy, 2008). The use of fungicides as an alternative control measure in these cases might be an option to minimize losses in the event of sudden disease outbreak (Hoy and Savario, 2007). Studies conducted in Louisiana have showed that application of pyraclostrobin fungicide can reduce rust development and minimize yield loss in rust susceptible cultivars (Hoy *et al.,* 2009). Brown rust can reduce tonnage yield by 4-8 tons depending on the length of time brown rust is affecting the cane, but well-timed applications of fungicides can prevent this loss (LSU AgCenter, 2010).

Timing of fungicide applications requires basic knowledge of the conditions leading to severe brown rust epidemics. This is particularly important given the fact that fungicides are expensive and can potentially exert negative impacts on the

environment. Thus, the use of fungicides must be optimized in order to achieve maximum returns. Epidemiological research can result in a better understanding of the factors that affect disease development and provide the basis to formulate disease advisory systems that aid in the decision making for appropriate timing for chemical control activities.

In the case of brown rust, different studies have provided important information on the factors affecting disease development. Comstock and Raid (2000) suggested that moisture and temperature are the most important environmental factors that affect rust infection. Urediniospore germination in water was reported from 4 to 40 C; however, the optimum is in the 21-26 C range (Ryan and Egan, 1989). Sotomayor (1983) reported the inhibition of germination at temperatures above 34 C, while Comstock and Raid (2000) reported a dramatic decline of spore germination and low levels of infection above 30 C under field conditions in Florida. In addition, aerial concentrations of urediniospores detected in spore traps decreased after the occurrence of maximum ambient daily temperatures of 30 C (Irey, 1987).

Leaf wetness or high relative humidity for a period longer than 8 hours is considered to be necessary for infection along with favorable temperatures (Comstock and Raid, 2000). Likewise, up to 97% of urediniospore germination and up 87% of appressoria were formed after 6 hours of inoculation at 20-25 C (Sotomayor *et al., 1983*). Other important factors that have been reported to affect the development of the disease are plant age and growth stage. Susceptible cultivars are more susceptible to infection between 3 and 6 months of age, while plants older than 6 months show reduced infection even under favorable conditions (Comstock and Ferreira, 1986).

However, under field conditions, the relationship among all these environmental factors, crop development, and pathogen inoculum with disease severity remains unclear.

A comprehensive study of the epidemiology of brown rust is needed in order to understand the combination of factors that result in severe epidemics and to gather information that can help in the formulation of a disease advisory system. With this tool, decision making in regard to fungicide applications could be improved and potential yield losses minimized. The objective of this study is to determine the relationship between crop growth and environmental variables resulting in severe epidemics that can serve as a base for the formulation of a disease advisory system for brown rust in sugarcane.

Chapter 2 contains a study of the interaction of leaf wetness and temperature: two variables that are considered to be the key for the development of brown rust. The results provided a base for the interpretation of data originating from field epidemics presented in Chapter 3. Chapter 3 presents, describes, and analyzes the patterns of environmental and crop growth variables for natural epidemics in susceptible cultivars during 2009 and 2010. Actual values for key variables are compared to determine conducive and limiting conditions that determine epidemic severity and that might be used for the development of a disease advisory/forecasting system.

Chapter 2. Effects of Leaf Wetness and Temperature on Brown Rust Infection in Sugarcane

2.1 Introduction

Brown rust, caused by *Puccinia melanocephala* Syd. & P. Syd, can cause severe yield losses in susceptible sugarcane (*Saccharum* inter-specific hybrids) cultivars in most sugar production regions (Raid and Comstock, 2000). Yield reductions of 10-40% due to brown rust are common for susceptible cultivars (Comstock *et al.,* 1992), and severe epidemics can cause 40-50% loss (James, 2004). Several variables are associated with disease severity, including host resistance and pathogen genetics (Asnaghi *et al.,* 2001; Raid and Comstock, 2000; Shine *et al.,* 2005), plant growth stage (Comstock and Ferreira, 1986), weather conditions (Comstock and Raid, 2006; Irey, 1987; Sandoval *et al.,* 1983), and plant nutrition and soil characteristics (Anderson and Dean, 1986; Anderson *et al.,* 1990; Johnson *et al.,* 2007). Leaf wetness and temperature have been suggested to be the most important environmental variables affecting brown rust development in susceptible cultivars (Raid and Comstock, 2006; Sandoval *et al.,* 1983; Purdy *et al.,* 1983).

Leaf wetness defined as free moisture on the leaf surface for at least 6 hours has been demonstrated to be a requirement for urediniospore germination in temperatures ranging from 5 to 30 C (Sotomayor *et al.,* 1983). Infection was observed to be highest when plants were incubated with leaf wetness for 14 hours in the dark following inoculation (Olds, 1982). Water originating from dew periods has been suggested to be the most important source of moisture during epidemics (Raid and Comstock, 2006). Rain did not correlate with rust infection grade in one study (Comstock and Ferreira, 1986), and although it creates leaf wetness, rain also can have

a detrimental effect by washing urediniospores from the leaf surface (Raid and Comstock, 2006).

The effect of temperature on brown rust development also has been studied. Sotomayor *et al.* (1983) reported urediniospore germination and appressorium formation occurs over a wide temperature range from 5-34 C, with an optimal range from 15 to 30 C. Other studies (Hsich *et al,* 1977; Sahni and Chona, 1965) found optimal germination between 21 and 26 C, and urediniospore germination was found to rapidly decrease with temperatures exceeding 30 C (Raid and Comstock, 2000). Urediniospores were reported to rapidly lose viability when temperatures increase above 35 C (Purdy *et al.*, 1983).

Information is lacking concerning the interaction of leaf wetness and temperature on leaf infection by *P. melanocephala.* The objective of this study was to investigate the interaction of leaf wetness and temperature to improve understanding of the brown rust infection process and how this interaction might affect epidemic development.

2.2 Materials and Methods

Urediniospores were collected with a vacuum device (Model DC515, DEWALT Industrial Tool Corp., Baltimore, MD) from naturally infected leaves of multiple susceptible cultivars during 2009 and 2010, tested for viability by plating on water agar at room temperature, and preserved at –80 C. These spores were later used to inoculate leaves on plants that were then exposed to different combinations of temperature and hours of leaf wetness. Urediniospore viability was determined by plating on water agar at room temperature at the time of each inoculation and ranged from 22 to 30% during the experiments.

Plants of brown rust susceptible cultivar Ho 95-988 (Tew *et al.,* 2005) produced from single-bud cuttings in the greenhouse were used in the experiments. Plants were between 60 and 85 days old at the time of inoculation. The substrate used for growing the plants was a 1:1 mixture of silt loam soil and sand. Two weeks prior to inoculation with the pathogen, plants were fertilized with 15-11-15 N-P-K.

Plants were inoculated with a urediniospore suspension containing approximately 1×10^6 spores/ml. To achieve this, 0.2 grams of spores were suspended in a solution containing 14 ml of distilled water and 0.1% Tween 20. Spore concentration was adjusted with a haemocytometer. Inoculum was applied to both sides of a leaf with a brush until a film of moisture was visible. To maintain defined periods of leaf wetness, the inoculated leaf was introduced in a horizontally positioned glass test tube (70mL, 25x200 mm) containing 3 ml of distilled water (Fig. 2.1). Tubes were sealed with Parafilm (Pechiney Packaging Company, Chicago, IL) then held in a stable horizontal position with tube racks inside incubators at a given temperature in the dark. During the infection period, temperature was monitored with a thermocouple temperature sensor (Model 3667s, Spectrum Technologies, Plainfield, IL), and the range was $\pm$ 1 C. After the leaf wetness period for a given treatment was completed, the glass tube was removed from the leaf, and the leaf was allowed to air dry. Plants were then placed on shelves at 23 C $\pm$ 1 C with 12 h/day artificial lighting for 14 days. Disease severity was assessed after 14 days by counting the total number of lesions with necrotic tissue for each inoculated leaf. Lesion density per area was calculated by dividing the total number of lesions by the leaf area in cm^2. The leaf area was determined by image

analysis with Assess software (APS Press, American Phytopathological Society, St. Paul, MN).

Temperatures tested were 15, 17, 19, 21, 23, 25, 27, 29 and 31 C, while leaf wetness hours tested were 4, 7, 10, and 13. All temperatures were evaluated in combination with each of the leaf wetness periods. Every combination consisted of four replicates. The experiment was performed twice.

The effect of temperature, leaf wetness and their combined effects on infection were analyzed with the Randomization test (R statistical program, Free Software Foundation, Boston MA, USA). Due to lack of normality in the experimental data, statistical analyses of the different levels of temperature and leaf wetness were made with Friedman's non-parametric test (Infostats, University of Cordoba, Argentina).

Figure 2.1. Brown rust inoculated leaves inside glass tubes.

2.3 Results

Due to significant differences between the two experiments, results are presented separately for each. Leaf wetness duration and temperature affected

infection by *P. melanocephala* in both experiments (P<0.001), and differences were detected among wetness periods and temperatures (Figs. 2.2 and 2.3, Table 2.1). A leaf wetness period of at least 7 h was required for successful infection. In both experiments, leaves exposed to 4 h of leaf wetness did not develop symptoms regardless of the temperature at which they were incubated, and no infection occurred with a 7 h wetness period at 15 C. A few lesions developed with a 7 h wetness period at 17 and 19 C. Increasing the length of the leaf wetness period from 7 to 10 or 13 h resulted in a higher number of lesions at some temperatures, but the amount of increase varied between the two experiments (Figs. 2.1 and 2.2). Increasing wetness from 7 to 13 h resulted in an increase in lesion number at temperatures ranging from 17 to 27 C in both experiments. Infection levels were higher in Experiment 2 for leaf wetness periods of 7 and 10 h when temperatures were favorable for infection. Inoculation resulted in successful leaf infection for the entire temperature range of 15 to 31 C, but differences were detected among temperature treatments (Figs. 2.2 and 2.3, Table 2.1). Lesion development was negligible and significantly lower at 15 and 31 C in both experiments regardless of leaf wetness period. Temperature had a differential effect on lesion number depending on the length of the leaf wetness period. An optimal temperature range of 21 to 27 C was evident with a 7 h wetness period, but a 17 to 27 C optimal temperature range was detected with longer wetness periods. Appreciable infection occurred at 29 C only in Experiment 2. The interaction between temperature and leaf wetness with infection was significant (P<0.001) in both experiments.

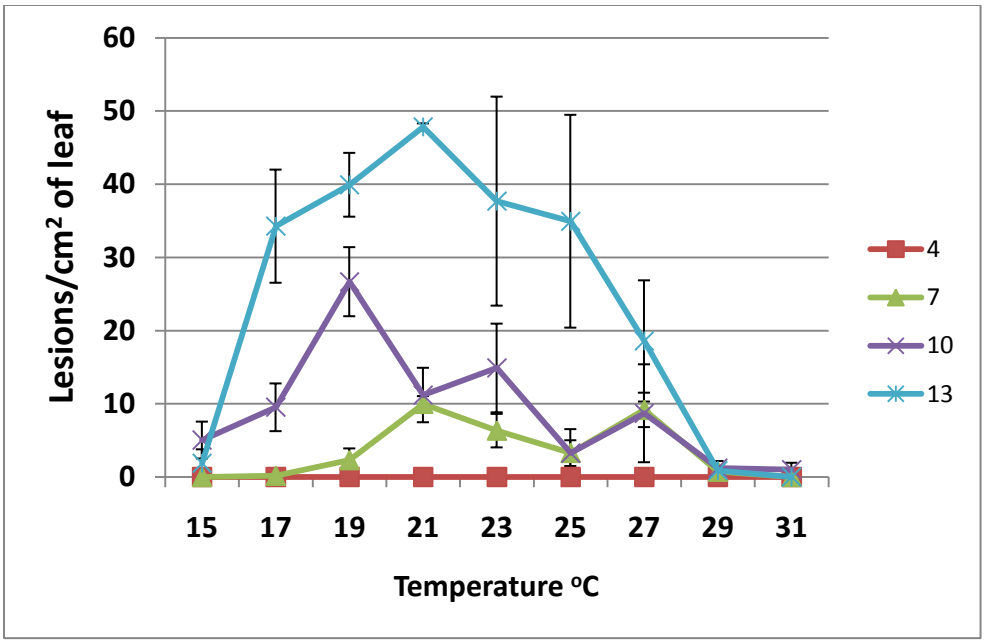

Figure 2.2. Effect of temperature and leaf wetness duration on brown rust infection assessed as lesion development in Experiment 1.

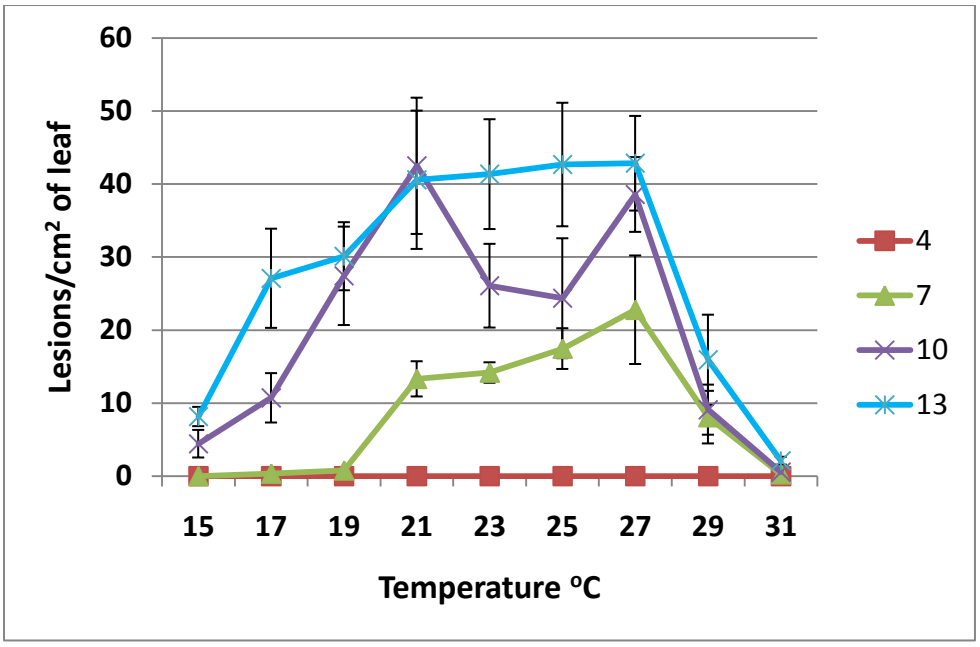

Figure 2.3. Effect of temperature and leaf wetness duration on brown rust infection assessed as lesion development in Experiment 2.

Table 2.1 Comparison of the effects of leaf wetness periods and temperatures on the development of brown rust lesions in sugarcane leaves for two experiments.

Variable levels	Sum of ranks and statistical classification in Exp. 1 [a]	Sum of ranks and statistical classification in Exp. 2 [a]
Leaf wetness		
4 hours	15 c	14 d
7 hours	28 b	26 c
10 hours	37 a	37 b
13 hours	40 a	44 a
Temperature ^{o}C		
15	20 c	15 de
17	26 abc	21 bcde
19	35 ab	26 abc
21	39 a	31 abc
23	37 ab	30 abc
25	31 abc	31 abc
27	34 abc	36 a
29	26 abc	21 cde
31	22 bc	14 e

[a]Sum of ranks for leaf wetness and temperature effects on number of lesions/cm^2 followed by different letters within a column and variable were significantly different ($P \leq 0.05$) as determined by Friedman test.

2.4 Discussion

Results from this study indicate that infection by *P. melanocephala* (assessed as number of lesions developed) is affected by leaf wetness and temperature, and changes in one variable will influence the effect of the other on infection. At least 7 h of leaf wetness were necessary for successful infection. Only low levels of infection occurred at the temperature extremes of 15 and 29 or 31 C. An optimal temperature range of 17 to 27 C was evident at leaf wetness periods of 10 and 13 h. Increasing the

length of the leaf wetness period from 7 to 10 or 13 h can result in the development of higher numbers of lesions within the optimum temperature range. Infection was always higher in this temperature range when leaf wetness increased from 7 to 13 h.

The reasons for variability between experiments in infection success at temperatures within the optimum range, particularly with 10 h of leaf wetness, are uncertain. The urediniospore source was different for the two experiments, and it is possible that the inoculation method may provide variable results under conditions favorable for infection. However, the results were consistent for the minimum and maximum leaf wetness and temperature conditions needed for infection. These are the values that would be of interest in field epidemiology studies.

The absence of symptoms observed with a leaf wetness duration of 4 h agrees with Comstock and Raid (2000), who reported a requirement for at least 8 h of exposure to liquid water for infection. The formation of lesions at all temperatures tested with an optimum range of 17 to 27 C agrees with uredioniospore germination results obtained by other authors. Optimal temperatures for urediniospore germination were found to be from 21 to 26 C (Sandoval, 1981; Liu, 1980; Sahni and Chona, 1965; Olds, 1982) and 15 to 30 C (Sotomayor *et al.,* 1983). Additionally, optimal appressorium formation was found to require at least 6 h of wetness (Sotomayor *et al.,* 1983).

Visible lesion formation was observed after the 8th day in this experiment, but symptom development continued until the 14th day after inoculation. Sotomayor *et al.,* (1983) reported the rupture of the epidermis and formation of urediniospores beginning 7 days after inoculation. A time period of approximately 8-11 days was assumed to be necessary between spore germination and the production of a new generation of spores

by Irey (1987). However, the latent period for brown rust and the factors that affect it have not been fully established.

The meaning of results from controlled conditions studies in relation to field interactions of leaf wetness and temperature affecting brown rust infection and severity is uncertain. The 7 h minimum requirement and higher infection with increasing leaf wetness duration may be directly applicable to field conditions. Applying minimum and maximum temperatures determined under constant controlled conditions to explain disease initiation and severity in the field where temperature is constantly in flux is more challenging; however, the results of this study confirm the results of previous related field studies. In Florida, ambient temperatures above 30 C were found to result in reduced disease severity (Comstock and Raid, 2000) and lower aerial concentrations of urediniospores (Irey, 1987). In Hawaii, maximum brown rust development was observed when mean minimum monthly temperatures were below 20 C (Comstock and Ferreira, 1986).

The minimum and maximum values determined in this study for leaf wetness and temperature and the results on their combined effects could be useful for comparisons with data from natural epidemics to improve our understanding of conditions required for epidemic onset, rate of increase over time and eventual decline of the epidemic. This could allow the development of a disease advisory or forecasting system to predict the initiation and potential severity of brown rust epidemics in susceptible sugarcane cultivars during each growing season.

Chapter 3. Study of the Effects of Environmental Variables and Crop Growth on Development of Brown Rust Epidemics in Sugarcane

3.1 Introduction

Brown rust, caused by *Puccinia melanocephala* Syd. & P. Syd., is an important disease of sugarcane (inter-specific hybrids of *Saccharum* L.) worldwide (Raid and Comstock, 2000). Following its discovery in the Americas in 1978, brown rust caused severe losses in susceptible sugarcane cultivars, especially the high yielding and extensively grown B4362 (Purdy *et al.,* 1985). In Florida, the disease incursion effectively eliminated the cultivation of several susceptible cultivars and forced the breeding program to begin screening for resistance (Comstock *et al*, 1992). Brown rust was first observed in Louisiana in 1979 (Koike, 1980).

The most effective way to manage brown rust has been with resistant cultivars (Asnaghi *et al*, 2003; Raid and Comstock, 2000). However, due to genetic variability and adaptability within the pathogen population, resistance to the disease has not been durable. Shifts from resistance to susceptibility have been reported in several cultivars in Florida, including CP 78-1247 (Raid *et al*., 1989), CP 79-1580 (Dean and Purdy, 1984), CP 72-1210 (Glaz and Coale, 1992), CP 74-2005 and CL 73-239 (Shine *et al.,* 2005), and LCP 85-384 in Louisiana (Hoy, 2008). Disease outbreaks on these newly susceptible cultivars have caused yield losses as high as 39% (Raid *et al.*, 1991). In Louisiana, 22% yield reduction was reported for cultivar LCP 85-384 (Hoy and Hollier, 2009).

The use of fungicides as an alternative management practice during periodic brown rust outbreaks is being evaluated (Hoy and Savario, 2007). However, fungicides

are expensive and their rational use requires a good understanding of the conditions leading to severe epidemics.

Considerable year-to year variation in brown rust epidemics occurs in susceptible cultivars, and disease severity can be strongly affected by the interplay between the pathogen, environmental variables, and crop growth. It has been suggested that plant age plays an important role in the natural decrease of epidemics under field conditions (Comstock and Ferreira, 1986; Bailey, 1979; Liu, 1980), with the crop being more susceptible when it is 3-6 months old and less susceptible when it is older than 6 months. However, high temperatures also have been linked to lower severity and the decline of epidemics. Thus, temperatures exceeding 35 C were reported to be limiting for the germination of urediniospores and infection under field conditions (Liu, 1980; Liu and Bernard, 1979). Additionally, disease severity decreased with ambient maximum daily temperatures above 30 C (Raid and Comstock, 2000), and a significant reduction in the number of trapped urediniospores in spore samplers followed the occurrence of maximum daily temperatures above 30 C (Irey, 1987). Long periods of leaf wetness and cool-to-warm temperatures have been reported as favorable for the development of brown rust (Comstock and Raid, 2000). Long periods of warm and humid weather also were reported to be favorable for the disease (Sandoval *et al.* 1983). Mild winters have been linked to early outbreaks of brown rust during the growing season, most likely by allowing overwintering of inoculum within the sugarcane fields (Irey, 1987).

Other factors that have been associated with brown rust severity include plant growth stage (Comstock and Ferreira, 1986), plant nutrition and soil characteristics (Anderson and Dean, 1986; Anderson *et al.,* 1990; Johnson *et al.,* 2007).

A thorough understanding of the conditions leading to the onset and development of severe brown rust epidemics is still lacking. An understanding of the factors affecting disease severity would be important for the successful establishment of an alternative management practice, such as fungicide application. The objective of this study was to investigate the roles of crop growth and environmental variables to determine disease conducive and limiting conditions that affect the onset, severity, and decline of brown rust epidemics in sugarcane. Achieving this objective could allow the formulation of a disease advisory or forecasting system.

3.2 Materials and Methods

3.2.1 Monitoring of Variables under Field Conditions

Natural epidemics of brown rust were monitored in susceptible cultivars at two locations in 2009 and 2010. During 2009, a single field of cultivar Ho 95-988 (Tew *et al.,* 2005) was monitored at the research farm of the USDA-ARS Sugarcane Research Unit in Schriever, LA (latitude 29.7126N, longitude -90.8273W), and a single field of cultivar LCP 85-384 (Milligan *et al.,* 1994) was monitored at the Sugar Research Station of the Louisiana State University Agricultural Center in St. Gabriel, LA (latitude 30.257N, longitude -91.099W). Both cultivars were resistant to brown rust at the time of release but have since become highly susceptible. During 2010, a single field of Ho 95-988 was monitored at the USDA-ARS farm, and single fields of Ho 95-988 and LCP 85-384 were monitored at the Sugar Research Station. Measurements of leaf wetness, ambient temperature, leaf surface temperature, rainfall, relative humidity, plant height, canopy cover, shoot population, number of green leaves per shoot, and disease severity were

taken for the epidemic period during each season (April 4-July 16, 2009; April 4-July 17, 2010).

Rain, ambient temperature, leaf temperature, leaf wetness, and relative humidity data were recorded every 15 minutes with a single weather station (Watchdog, Model 700, Spectrum Technologies, Plainfield, IL) arbitrarily located inside each sugarcane field. Temperature at the leaf surface was measured for leaves at different positions on the plant. Data were taken for single leaves in -2 and +4 positions based on the Kuijper leaf numbering system (Clements and Ghotb, 1968) in which leaves are assigned negative numbers acropetally and positive numbers basepitally relative to the youngest fully emerged leaf (with the collar joining the leaf blade and sheath visible). Leaf +4 is lower and partially shaded, while -2 is an upper leaf still emerging from the leaf whorl and fully exposed to the sun at the top of the developing canopy. Temperature was recorded with thermocouple temperature sensors (Model 3667s, Spectrum Technologies). The lead for the sensor was attached to the adaxial leaf surface with adhesive tape. Leaf wetness sensors (grid-type electrical resistance, Model 3666, Spectrum Technologies) were placed at the level of -2 and +4 leaves. In preliminary monitoring with sensors on three similar leaves, temperature showed variation within 1.5 C and leaf wetness periods varied within 30 min, so only single leaves of each type were monitored in an individual field during the experiments. Leaves produced by the apical meristem emerge continuously through a leaf whorl at the shoot apex. Therefore, the temperature sensors on upper and lower leaves and wetness sensors were adjusted weekly.

To evaluate crop development, 40 plants were arbitrarily selected every week from each field, and the number of green leaves per plant and plant height were recorded from the soil line up to the base of the youngest fully emerged leaf. Additionally, shoot population was monitored every week by counting the number of shoots in four replicates of 3-m-long row sections within the field. Finally, crop canopy development was monitored weekly with three digital pictures taken at fixed points within the field, from the soil surface in the middle of the inter-row facing the sky. Pictures were analyzed with Assess software (APS Press, American Phytopathological Society, St. Paul, MN) to determine percent canopy cover at each date.

Brown rust was assessed on a weekly basis by arbitrarily collecting 15 leaves at the same position on the plant from each field. In severe brown rust epidemics, the young, recently emerged leaves with maximum photosynthetic potential become infected. Therefore, epidemic severity over time was monitored by collecting the youngest and second youngest (+1) fully emerged leaves at each weekly sampling within the fields. The total number of lesions and number of sporulating pustules were determined for a 10-cm-long section in the middle of each leaf the same day they were collected. Finally, the leaves were scanned in their entirety using a CanoScan 8600F scanner (Canon Inc., Lake Success NY) to produce digital images that were analyzed using Assess software for diseased area quantification.

3.2.3 Statistical Analyses

Correlation analyses taking into consideration a symptom expression latent (lag) period of 8-14 days were conducted for each of the variables with disease severity from April 4 up to the peak of the epidemic in each field for the two seasons (R-Statistical

Program, Free Software Foundation, Boston, MA). Thus, data for each variable from the week preceding a disease severity measurement were tested for correlation. Daily mean values were generated for rain, upper leaf surface temperature (maximum, minimum, and mean), lower leaf surface temperature (maximum, minimum, and mean), lower leaf wetness (sensor inside developing canopy), upper leaf wetness (sensor at the top of the developing canopy), ambient temperature (maximum, minimum, and mean), and relative humidity (maximum, minimum, and mean). Measurements of plant height, number of green leaves per plant, shoot population, and canopy cover were obtained on a weekly basis.

The correlation analysis allowed the identification of variables most correlated with disease severity. To refine the analysis of the environmental variables identified and to determine if specific periods during the day were more conducive for the disease, correlation analyses with disease were performed with hourly observations for environmental variables. All correlations were tested for significance with the Randomization test (R-Statistical Program).

The occurrence of brown rust sporulating pustules on the lower leaves served as an indicator of the presence of inoculum. Sporulating pustules were present on plants in the Ho 95-988 and LCP 85-384 fields during 2009 and the Ho 95-988 field at the USDA-ARS farm during 2010. Freezes capable of killing all above-ground sugarcane plant tissue occurred during the 2009-2010 winter. As a result, sporulating pustules did not develop on plants in the Ho 95-988 and LCP 85-384 fields at the Sugar Research Station during the epidemic period of 2010. Therefore, data for the selected variables were not analyzed for these two fields.

Multiple regression analyses (R Statistical program) were performed with the 2009 and 2010 data to determine the relative importance of the different variables in the expression of disease. The models produced were compared for both cultivars at each location during the severe 2009 epidemic. The results determined the variables most involved in the development of brown rust epidemics. The epidemic data sets for these variables were then compared to determine if conducive or limiting values of these variables could be identified and whether their occurrence had any potential predictive value for future epidemics.

3.3 Results

Disease severity data were collected for both the youngest and second youngest fully emerged leaves. A review of the data was made to choose one for further analysis of the epidemics. In 2009, the second youngest leaf reached a maximum of 35% disease severity, while the youngest reached 25% in LCP 85-384. In Ho 95-988, the maximum values were similar with 21% and 20% for the youngest and second youngest leaves, respectively. In 2010, maximum disease severity for the second youngest leaf was 3.9%, while the highest disease severity for the youngest leaf was 2.4%. Considering the higher levels of disease severity measured for the second youngest leaf in two of three epidemics, the disease severity values obtained for the second youngest fully emerged leaf were chosen for further analyses of the epidemics.

3.3.1 Brown Rust Epidemics in 2009

The 2009 brown rust epidemic was characterized by early inoculum presence in the fields. By April 4, there were plants with sporulating pustules of brown rust in the plots of LCP 85-384 at St Gabriel and Ho 95-988 at Schriever. However, disease

severity did not begin to increase on the young, upper leaves until the week of May 22 in LCP 85-384 (Fig. 3.1) and the week of May 29 in Ho 95-988 (Fig. 3.2). Maximum disease severity was recorded on June 12 in LCP 85-384 with 35% of the leaf area occupied with lesions and on June 19 in Ho 95-988 with 19%. After these dates, disease severity on new leaves in the same position on the plant showed progressive reductions. By July 16, LCP 85-384 had only 1.8% of leaf area affected by the disease, while cultivar Ho 95-988 had 2.5%.

A seasonal shift from spring into summer occurred during the brown rust epidemic period. Crop growth shifted from tillering with an increase in shoot height to the stalk internode elongation phase during the epidemic period. Results for plant growth and environmental variables are presented and compared to disease severity in the two cultivars.

3.3.2 Plant Height

Plant height showed a steady increase in both cultivars over the entire epidemic and continued during the epidemic decline (Figs. 3.1 and 3.2). LCP 85-384 height reached 72.4 cm by the peak of the epidemic on June 12. Plant height continued to increase after the epidemic declined and increased on average 9.3 cm/wk over the recorded period. Ho 95-988 reached 87.6 cm by the peak of the epidemic on June 19 and increased 10.8 cm/wk during the recording period. Plant height increased in LCP 85-384 from 15 to 33 cm during the two weeks prior to epidemic initiation on the upper leaves on May 22, while in Ho 95-988, height increased from 30 to 44 cm in the two weeks prior to epidemic increase on May 29.

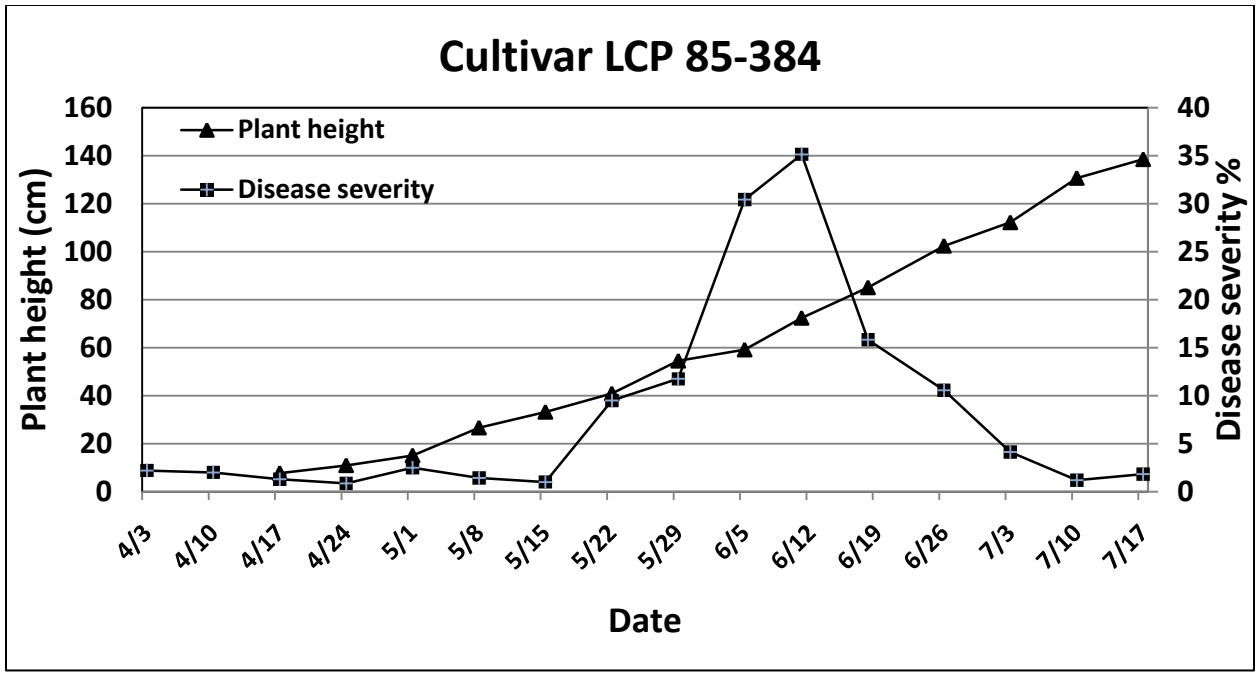

Figure 3.1. Plant height during 2009 brown rust epidemic in cultivar LCP 85-384 at St Gabriel, LA.

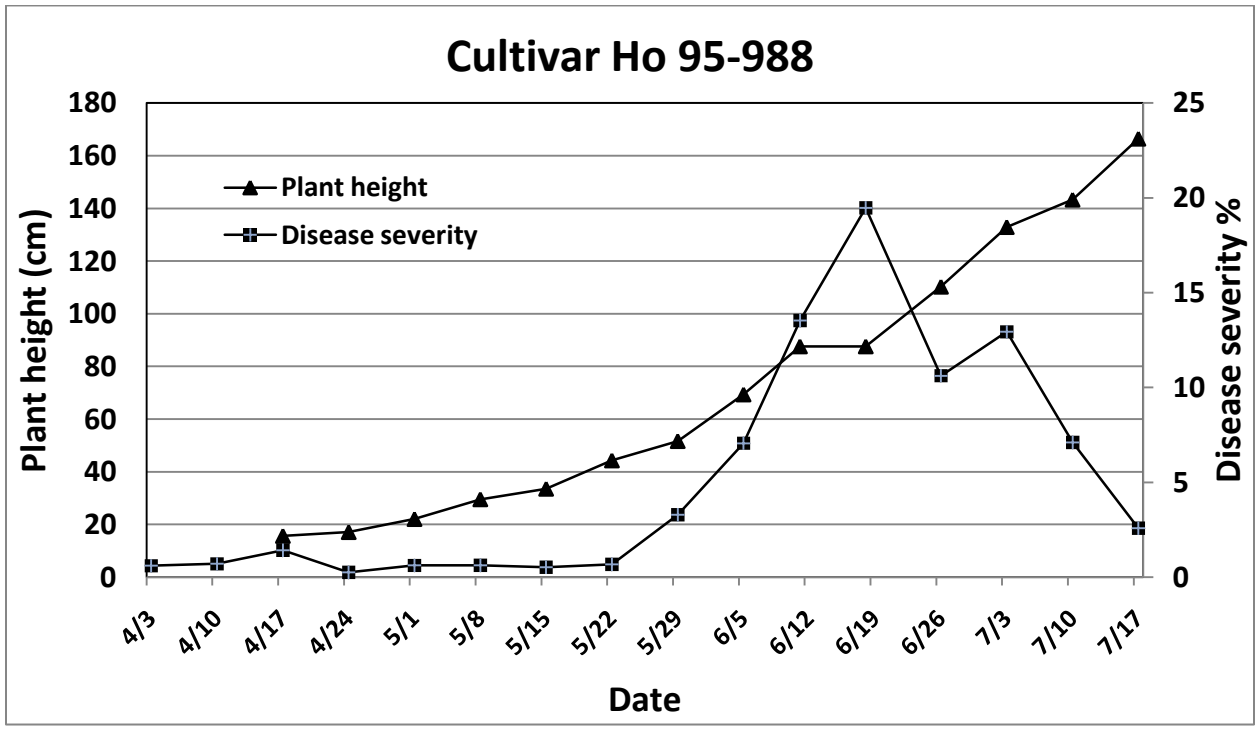

Figure 3.2. Plant height during 2009 brown rust epidemic in cultivar Ho 95-988 at Shriever, LA.

3.3.3 Shoot Population

Shoot population exhibited a rapid increase in the weeks prior to the onset of the epidemic in both cultivars and then stabilized or declined before the epidemic reached maximum severity (Figs. 3.3 and 3.4). The initial shoot number recorded in LCP 85-384 was 23 shoots/m of row on April 3 (Fig. 3.3). This increased to 63 shoots/m by May 15. The population then decreased to 24 shoots/m by July 16. In Ho 95-988, the initial shoot population on April 4 was 8 shoots/m (Fig. 3.4). By May 22, it increased to 40 shoots/m and remained at this level until June 12. The population then decreased to 20 shoots/m by July 16. Shoot population increased from 55 to 63 shoots/m for the May 8 and May 15 sampling dates prior to the onset of the epidemic in LCP 85-384. Shoot population increased from 25 to 40 shoots/m for the May 15 and May 22 sampling dates prior to the onset of the epidemic in Ho 95-988.

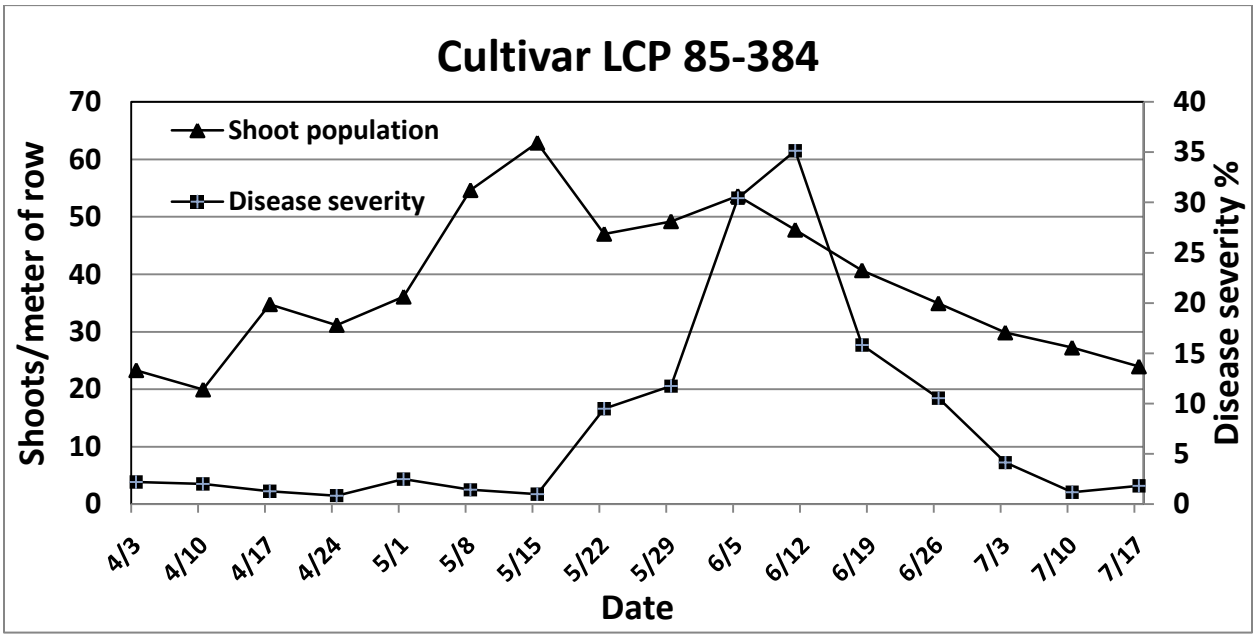

Figure 3.3. Shoot population during 2009 brown rust epidemic in cultivar LCP 85-384 at St Gabriel, LA.

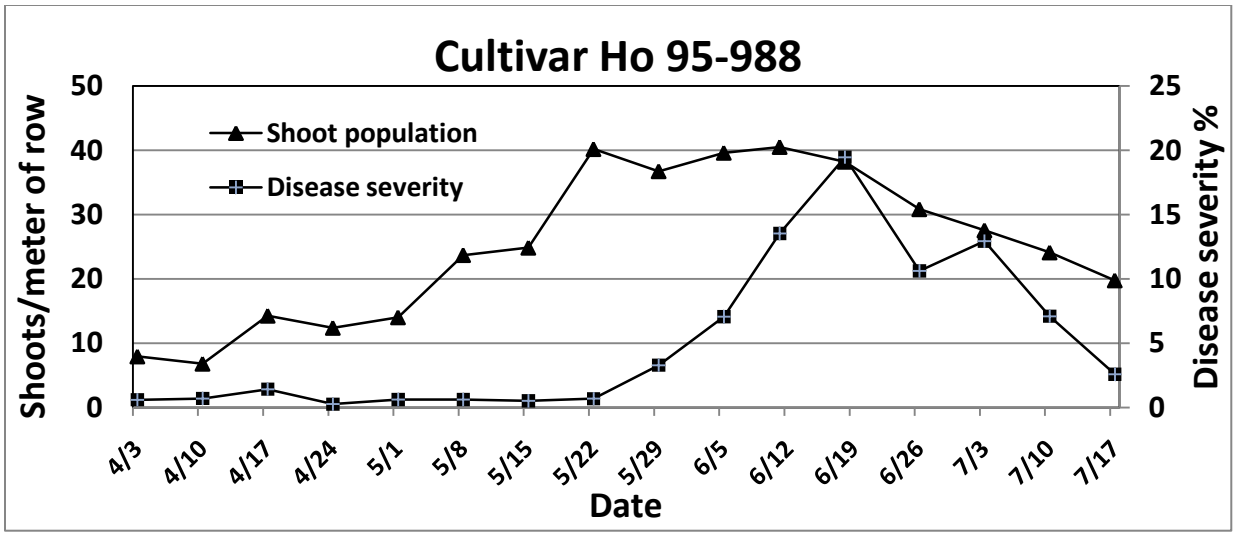

Figure 3.4. Shoot density during 2009 brown rust epidemic in cultivar Ho 95-988 at Shriever, LA.

3.3.4 Leaves per Shoot

The number of green leaves per shoot exhibited a limited range in variation over time for both cultivars in the weeks prior to epidemic development on the upper leaves (Figs. 3.5 and 3.6). Green leaf number per shoot ranged from four to seven for LCP 85-384 and five to seven for Ho 95-988. Leaf number did not increase until July when the epidemic had begun to decline.

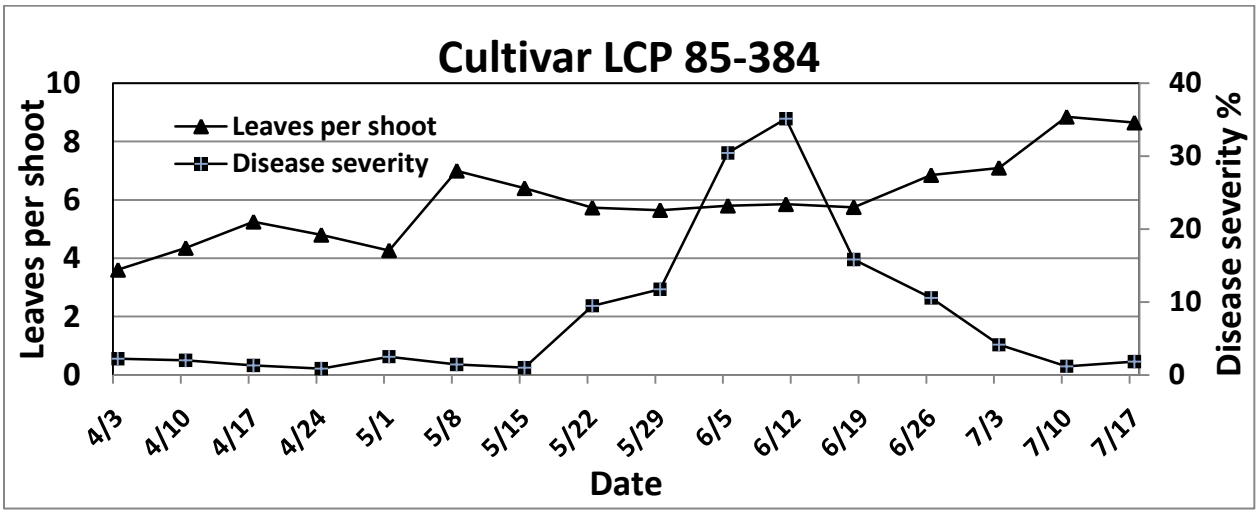

Figure 3.5. Number of green leaves per shoot during 2009 epidemic in cultivar LCP 85-384 at St Gabriel, LA.

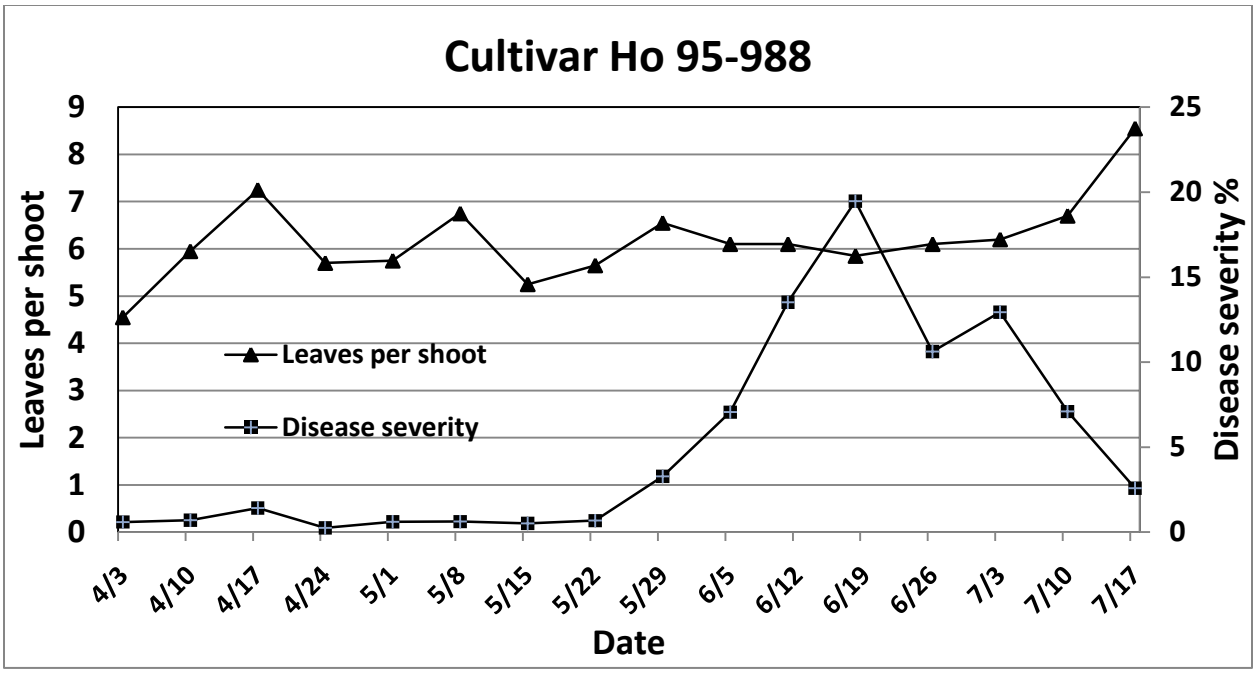

Figure 3.6. Number of green leaves per shoot during 2009 brown rust epidemic in cultivar Ho 95-988 at Shriever, LA.

3.3.5 Canopy cover

Overlapping of leaves from adjacent rows was not observed until May 15 in both cultivars (Figs. 3.7 and 3.8). After this date, canopy closure increased until the end of the experiment. Prior to the onset of the epidemic, canopy cover was 10% in LCP 85-384 and 22% in Ho 95-988. Canopy cover was 34% in LCP 85-384 and 76% in Ho 95-988 by the time the epidemic reached the maximum severity. Canopy closure continued after the epidemics began to decline.

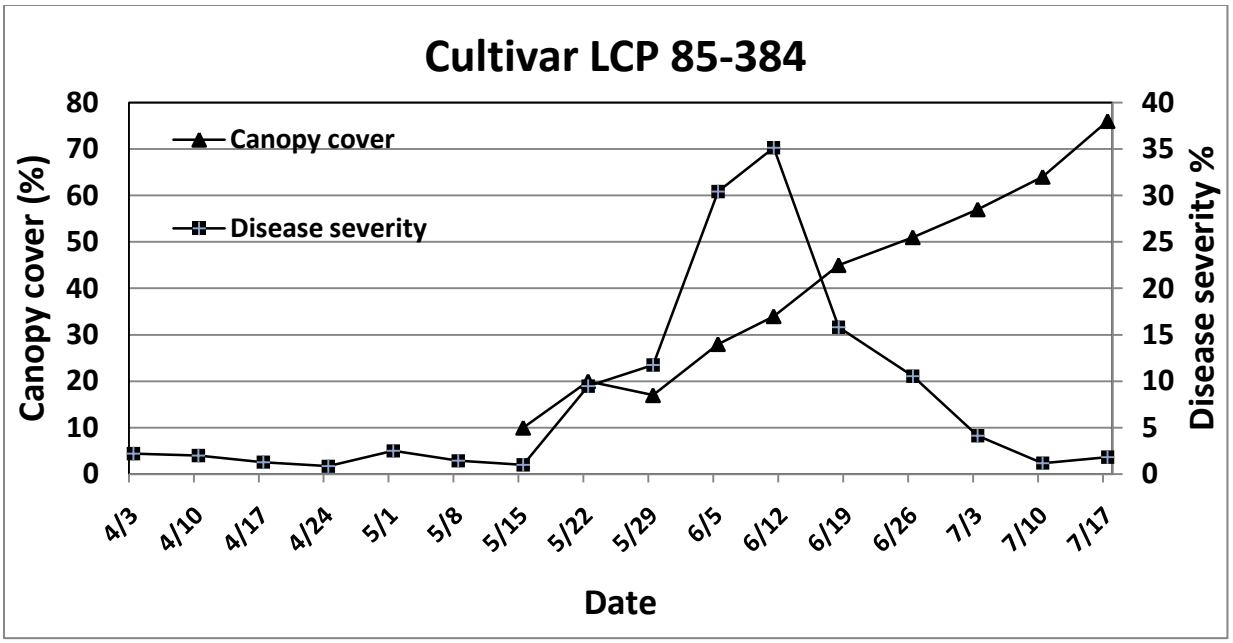

Figure 3.7. Canopy cover during 2009 brown rust epidemic in cultivar LCP 85-384 at St. Gabriel, LA.

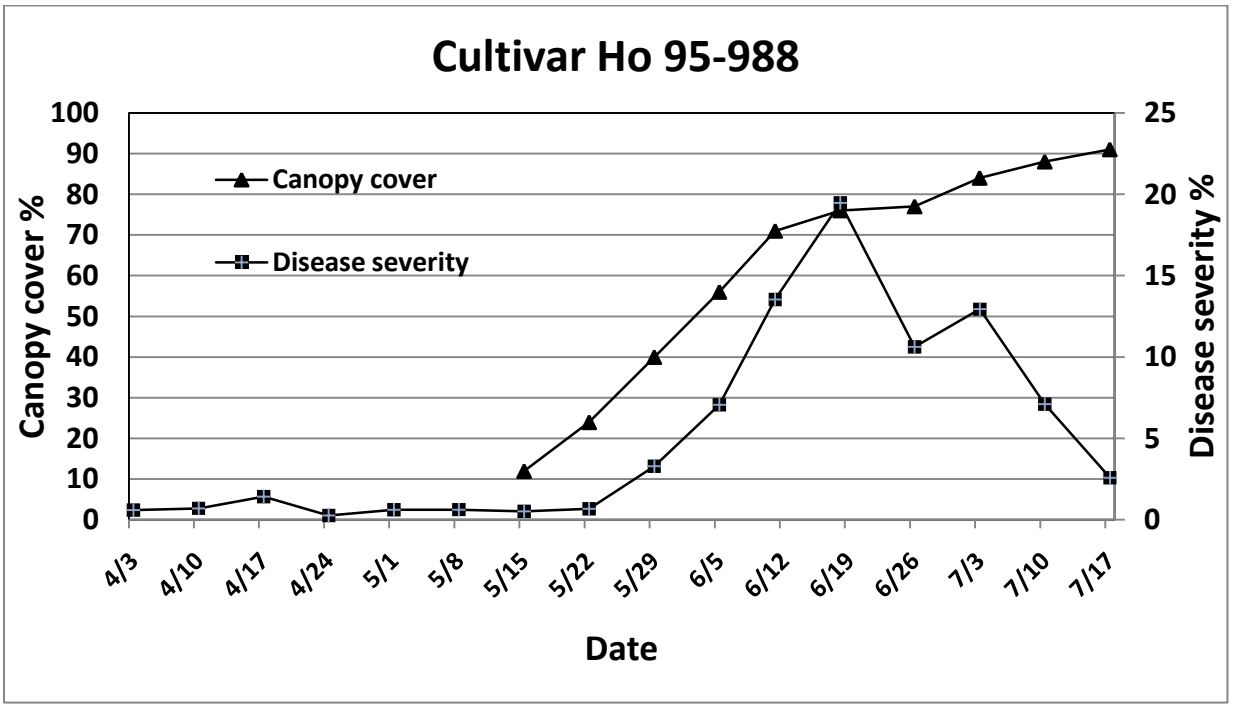

Figure 3.8. Canopy cover during 2009 brown rust epidemic in cultivar Ho 95-988 at Shriever, LA.

3.3.6 Leaf Wetness

The weekly mean for hours of leaf wetness per day measured on a lower leaf increased from 3 h/day on April 10 to 11 h/day on May 15 in LCP 85-384 (Fig. 3.9). Leaf wetness per day stayed above 8 h/day for the remainder of the epidemic. For Ho 95-988, the weekly mean for leaf wetness hours per day in lower leaves increased from 2 h/day on April 10 to 10 h/day on May 1 (Fig. 3.10). Leaf wetness period then fluctuated between 10 and 6 h/day for the rest of the epidemic.

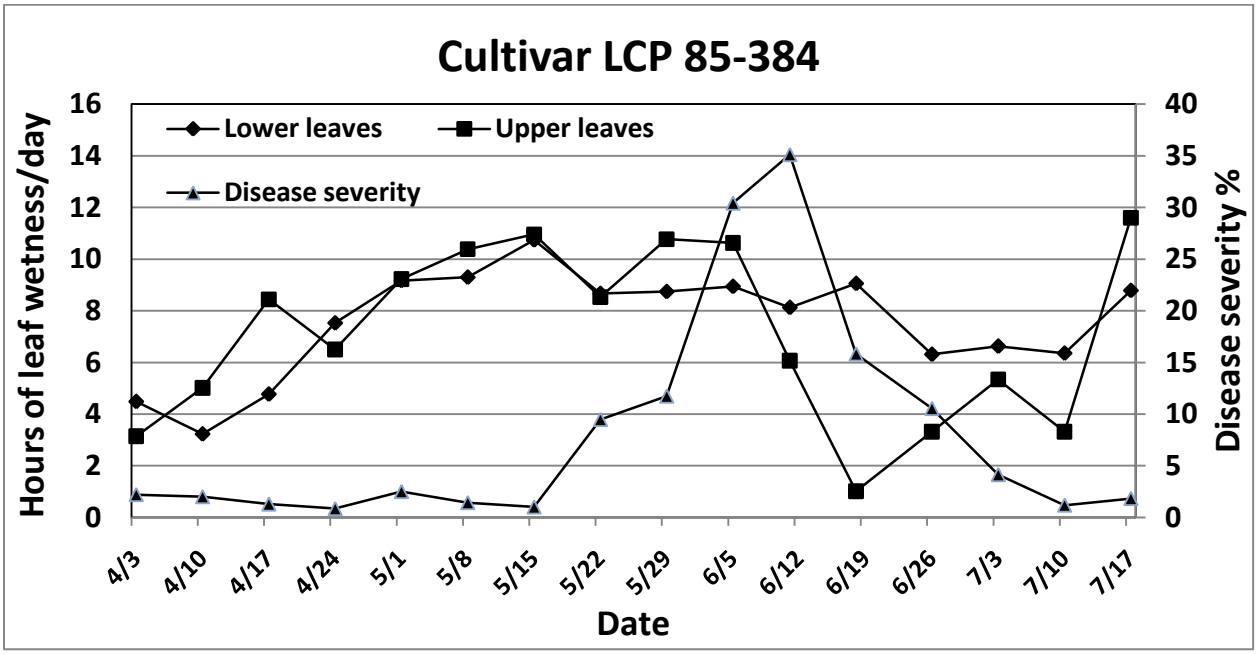

Figure 3.9. Weekly means for leaf wetness hours per day determined at the levels of upper and lower leaves during 2009 brown rust epidemic in cultivar LCP 85-384 at St. Gabriel, LA.

Daily hours of leaf wetness on an upper leaf exhibited similar increases to those measured on a lower leaf (Figs. 3.9 and 3.10). The weekly mean for hours of leaf wetness per day increased from 3 to 11 h/day between April 3 and May 15 then ranged from 8 to 11 h/day between May 22 and June 5 in LCP 85-384. The mean hours of leaf

wetness per day then decreased the week prior to and during the decline of the epidemic and ranged from 1 to 6 h/day between June 12 and July 10.

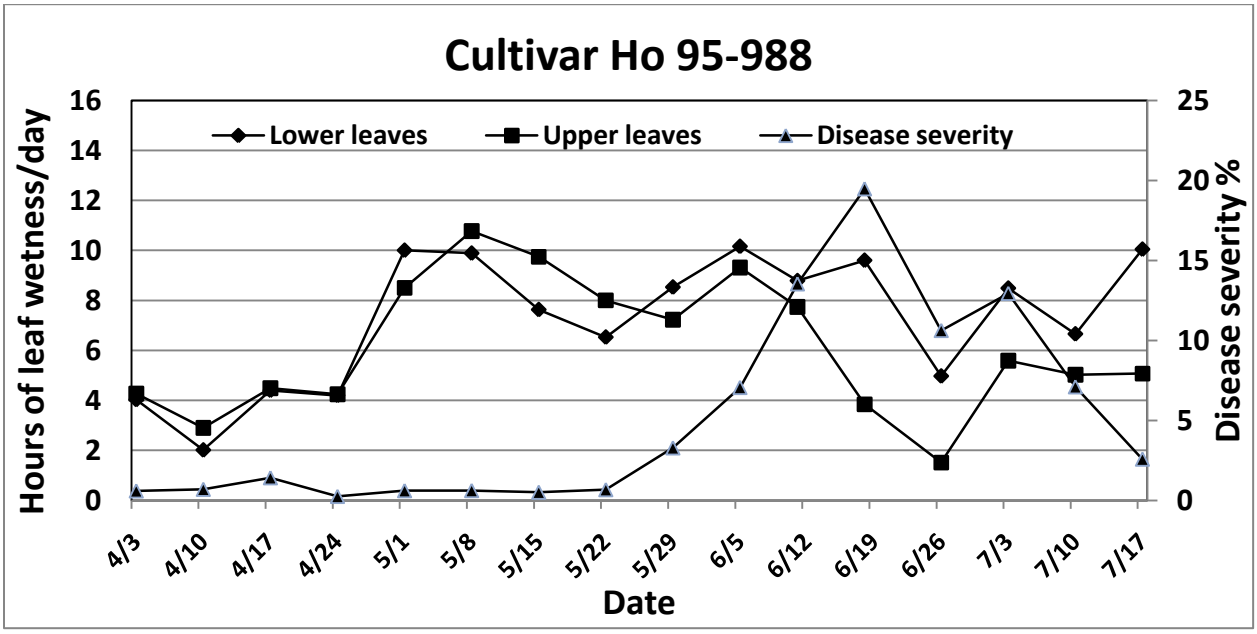

Figure 3.10. Weekly mean leaf wetness hours per day at the level of upper and lower leaves during 2009 brown rust epidemic in cultivar Ho 95-988 at Shriever, LA.

In Ho 95-988, the weekly mean for hours of leaf wetness per day on upper leaves increased from 3 to 11 hours between April 3 and May 8 and ranged from 7 to 10 h/day until June 5. The weekly mean then decreased to less than 6 h/day from June 19, the week prior to the decline of the epidemic, until the end of the experiment.

3.3.7 Temperature

Temperature at the surface of lower leaves increased during the first 6 wk of the epidemic in both cultivars (April 10-May 15) (Figs 3.11 and 3.12). In this period, the weekly mean temperature in LCP 85-384 changed from 18 to 26 C; however, in the week of May 22, mean temperature decreased to 22 C. After May 22, temperature

increased until the week of July 2 and reached 31 C. Mean temperature during the week of May 15, the week of epidemic onset was 26 C.

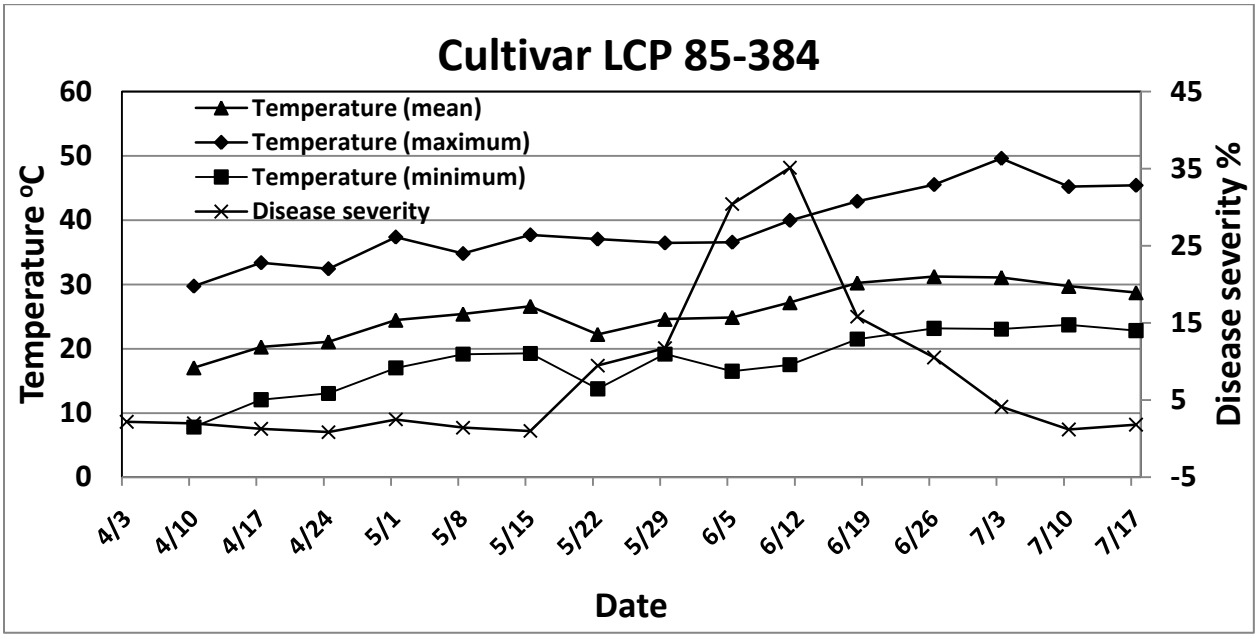

Figure 3.11. Weekly averages for mean, maximum, and minimum daily temperatures at the surface of a lower leaf during 2009 brown rust epidemic in cultivar LCP 85-384 at St Gabriel, LA.

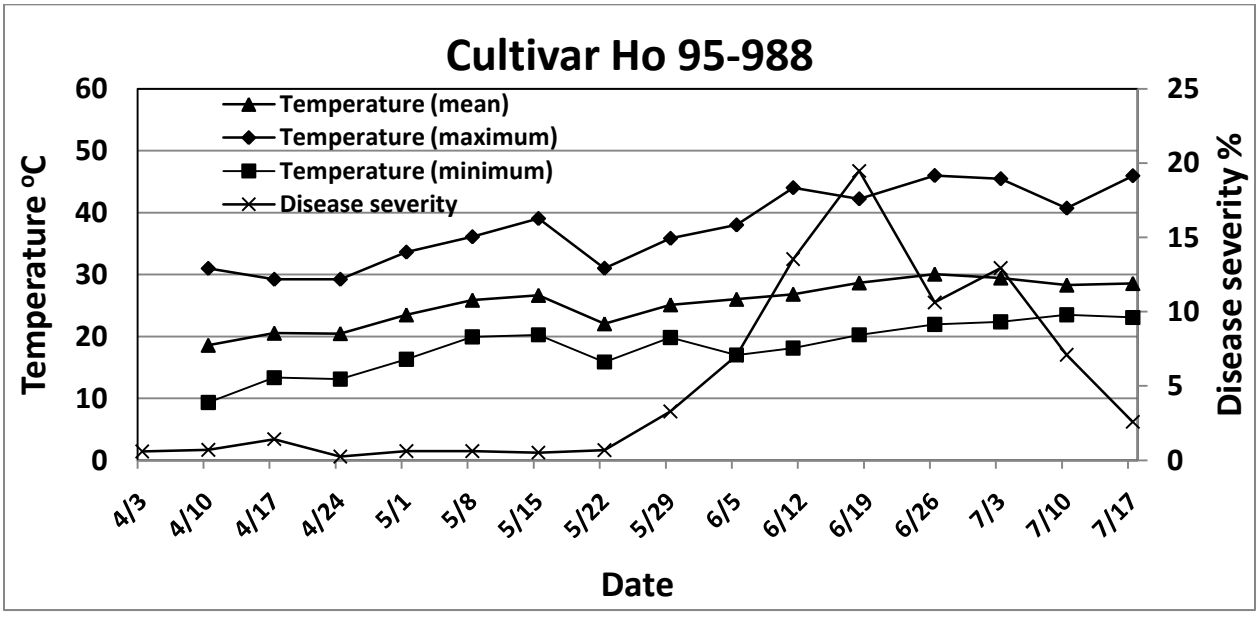

Figure 3.12. Weekly means for mean, maximum, and minimum daily temperatures at the surface of a lower leaf during 2009 brown rust epidemic in cultivar Ho 95-988 at Shriever, LA.

A similar pattern to mean temperature was observed for minimum temperatures at the surface of lower leaves in both cultivars (Figs. 3.11 and 3.12). The minimum temperatures increased in LCP 85-384 from 8 to 20 C between April 10 and May 15. Then, a decrease was observed by May 22, when the minimum temperature decreased to 14 C. The minimum temperature then increased to 19 C (May 29) and reduced slightly to 17 C by the peak of the epidemic on June 12. Minimum temperature during the week of May 15, the week that triggered disease growth, was 19 C. Minimum temperatures ranged from 22 to 24 C during the decline of the epidemics.

In the case of maximum temperatures, a slightly different pattern from mean and minimum temperatures was observed in both cultivars. In LCP 85-384, a gradual increase from 30 to 38 C occurred from April 10 - May 15 (Fig. 3.11). Only a slight reduction to 36 C was registered by the week of May 29. After that date, the mean maximum temperature increased to 40 C by the peak of the epidemic June 12 and then remained between 43 and 50 C during the rest of the epidemic. The mean maximum temperature during the week ending May 15 that triggered the epidemic was 38 C.

In the case of Ho 95-988, the mean and minimum temperature patterns were very similar to the ones observed for LCP 85-384 even though the location was different. Mean temperature on a lower leaf during the week of May 22 was 22 C, while the minimum temperature was 16 C. By the peak of the epidemic on June 18, mean temperature was 29 C, while the minimum was 20 C. After June 19, the mean temperature remained between 28 and 30 C during the decline of the epidemic, while the minimum remained between 22 and 23 C.

Maximum old leaf temperatures in Ho 95-988 showed some differences in relation to LCP 85-384. For example, a decrease in maximum temperature from 39 to 31 C was observed from May 15 to May 22. After May 29, maximum temperatures increased again to 42 C by the time the epidemic peaked the week of June 18. After that date, maximum leaf surface temperatures remained between 41 and 46 C.

The temperature patterns at the surface of an upper leaf during the epidemic were similar to the patterns for lower leaves in both cultivars (Figs. 3.13 and 3.14). Mean temperature during the week prior to epidemic onset (May 15) was 27 C in LCP 85-384 and 23 C in Ho 95-988 the week of May 22. Likewise, minimum temperature on upper leaves during the week of May 15 in LCP 85-384 was 19 C, while the maximum was 37 C. In Ho 95-988, the minimum recorded was 15 C and the maximum 37 C during the week of May 22. Mean, minimum and maximum temperatures during the peak of the epidemic in LCP 85-384 were 28, 17, and 42 C, respectively, while in Ho 95-988, they were 29, 20, and 44 C, respectively. After June 12, in LCP 85-384, mean temperature ranged from 29 to 30 C, the minimum between 21 and 23 C, and the maximum from 40 to 44 C. In Ho 95-988, the minimum ranged from 21 to 23 C, the mean from 29 to 30 C and the maximum from 44 to 46 C.

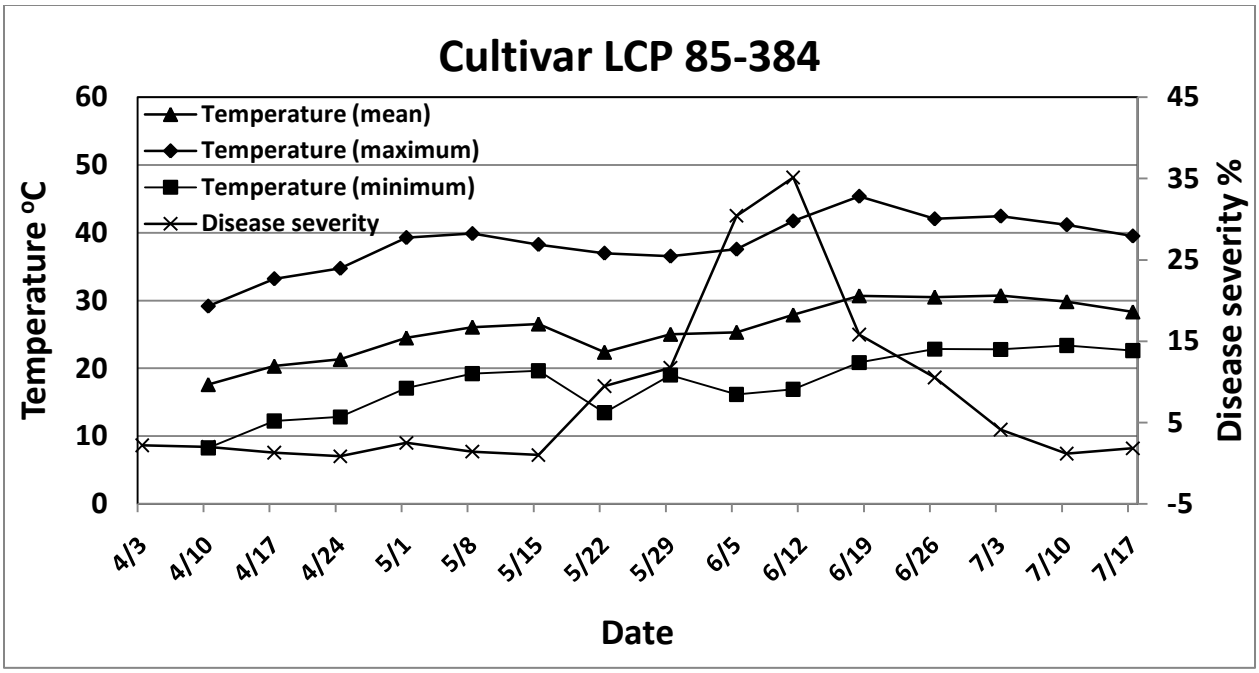

Figure 3.13. Weekly means for mean, maximum, and minimum daily temperatures at the surface of an upper leaf during 2009 brown rust epidemic in cultivar LCP 85-384 at St Gabriel, LA.

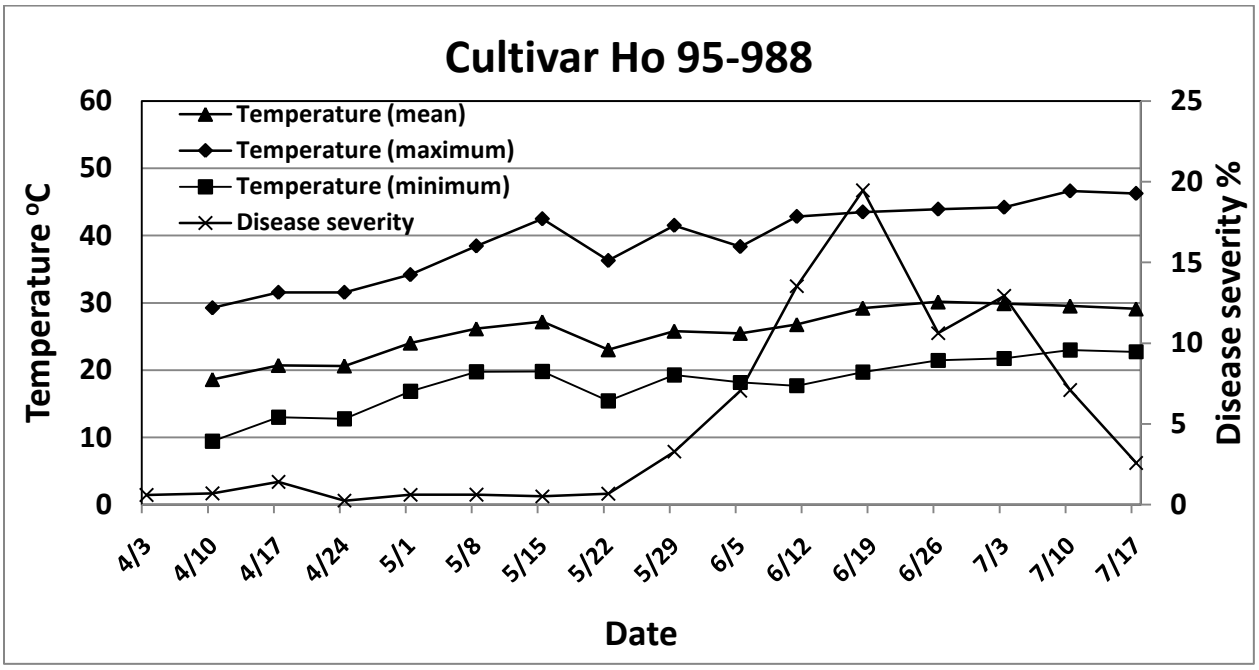

Figure 3.14. Weekly means for mean, maximum, and minimum daily temperatures at the surface of an upper leaf during 2009 brown rust epidemic in cultivar Ho 95-988 at Shriever, LA.

Maximum ambient temperatures were lower than the maximums registered at the surface of lower and upper leaves in both cultivars, while ambient temperature minimums were slightly higher for the ones recorded on upper and lower leaves (Figs. 3.15 and 3.16). As a result, the daily temperature variation ranges were narrower for ambient temperature. During the week of May 15, the week in which disease was initiated on young leaves in LCP 85-384, mean, minimum and maximum temperatures were 26, 20, and 32 C, respectively. In Ho 95-988, these were 22, 17, and 28 C, respectively. Mean, minimum and maximum temperatures in LCP 85-384 by June 12 were 25, 18, and 32 C, respectively. In Ho 95-988, for the week ending June 18, these values were 27, 22, and 33 C, respectively.

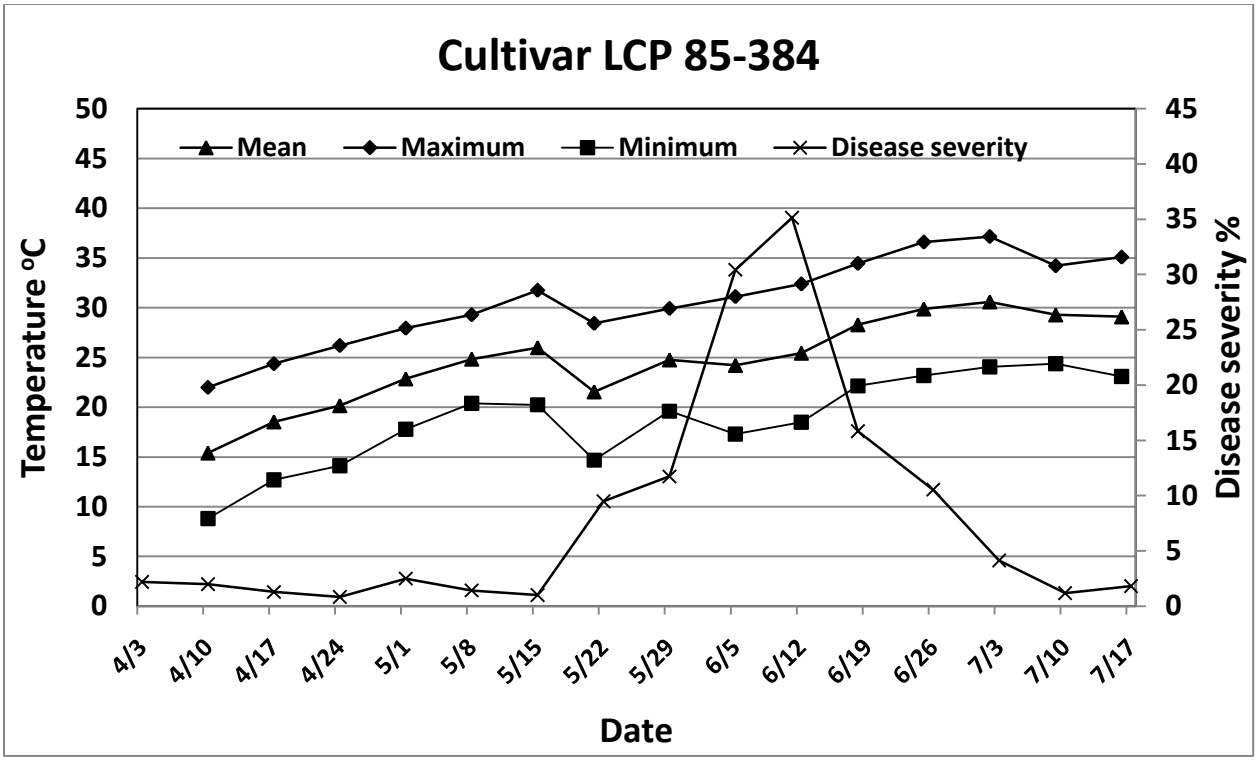

Figure 3.15. Weekly means for mean, maximum, and minimum daily ambient temperatures during 2009 brown rust epidemic in cultivar LCP 85-384 at St Gabriel, LA.

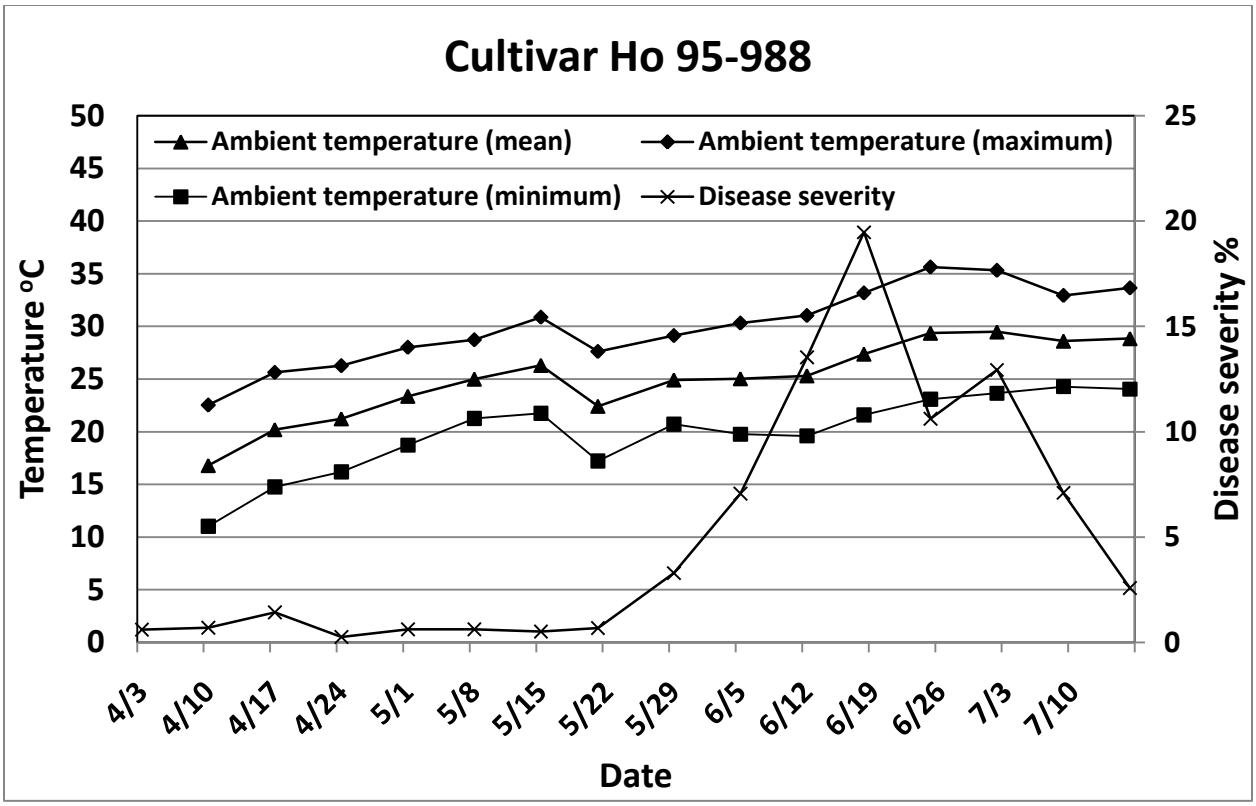

Figure 3.16. Weekly means for mean, maximum, and minimum daily ambient temperatures during 2009 brown rust epidemic in cultivar Ho 95-988 at Shriever, LA.

3.3.8 Relative Humidity

Relative humidity exhibited low variation before, during, and after the epidemic period. Thus, weekly mean daily relative humidity ranged from 62 to 74% during the monitoring period in LCP 85-384 (Fig. 3.17). In Ho 95-988, weekly means for daily relative humidity only ranged from 83 to 81% during the experiment (Fig 3.18). Maximum relative humidity ranged from 90-100% in both fields for the entire experiment. Minimum relative humidity exhibited the highest variation. The minimum weekly average in LCP 85-384 ranged from 29 to 53%, while in Ho 95-988, it ranged from 38 to 56%.

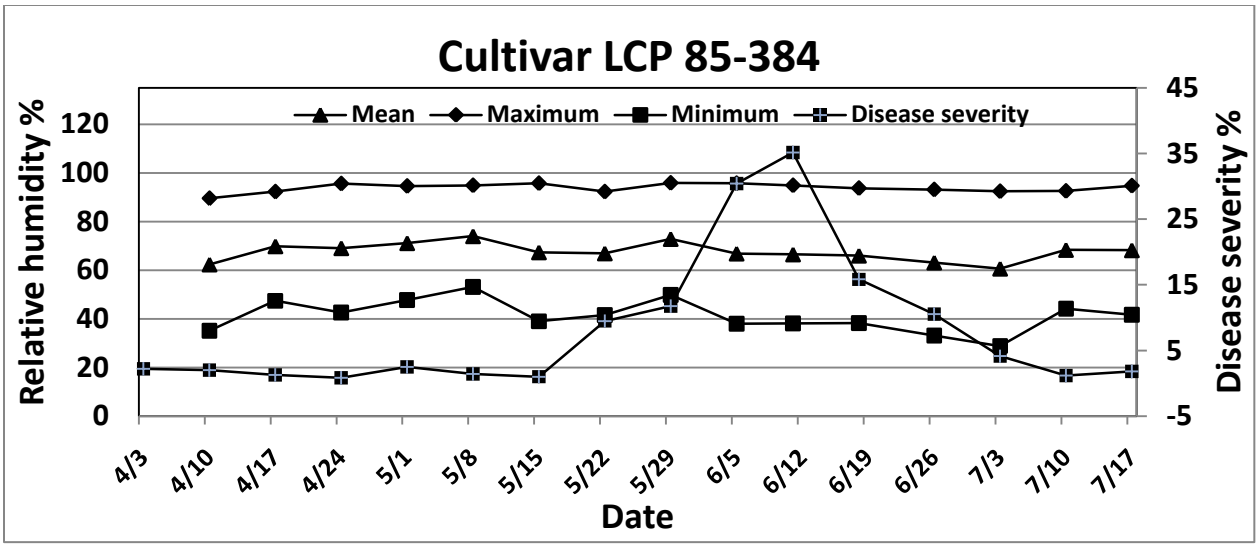

Figure 3.17. Weekly means for mean, maximum, and minimum daily relative humidity during 2009 brown rust epidemic in cultivar LCP 85-384 at St Gabriel, LA.

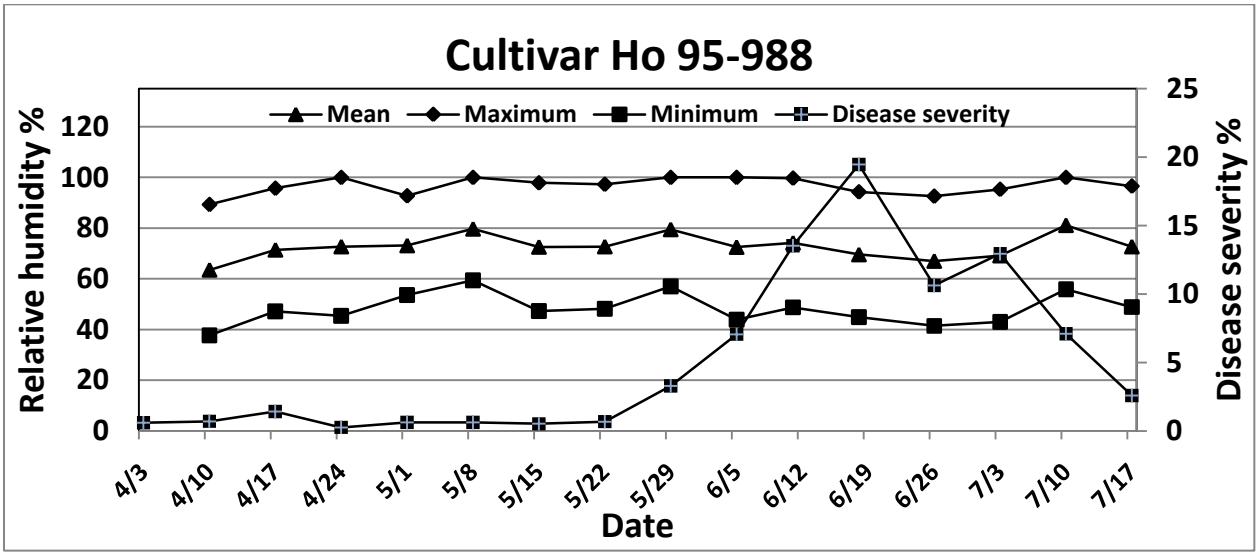

Figure 3.18. Weekly means for mean, maximum, and minimum daily relative humidity during 2009 brown rust epidemic in cultivar Ho 95-988 at Shriever, LA.

3.3.9 Rain

Rainfall varied at the two locations during the 2009 experiments (Figs. 3.19 and 3.20). In Ho 95-988, there was more cumulative precipitation from May 8 to June 5. Except for the week of May 22, all records within this period had precipitation above 12

mm/wk, while in cultivar LCP 85-384 all records during this period were below 10 mm/wk. This included the period prior to the onset of the epidemics in the different cultivars at each location.

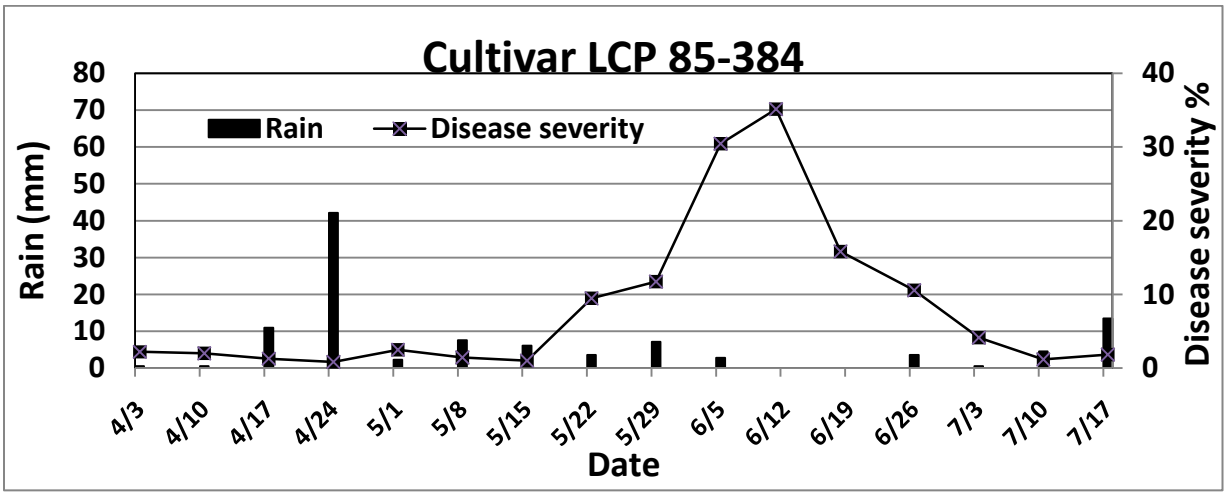

Figure 3.19. Weekly cumulative rainfall at St. Gabriel, LA during the 2009 brown rust epidemic in cultivar LCP 85-384.

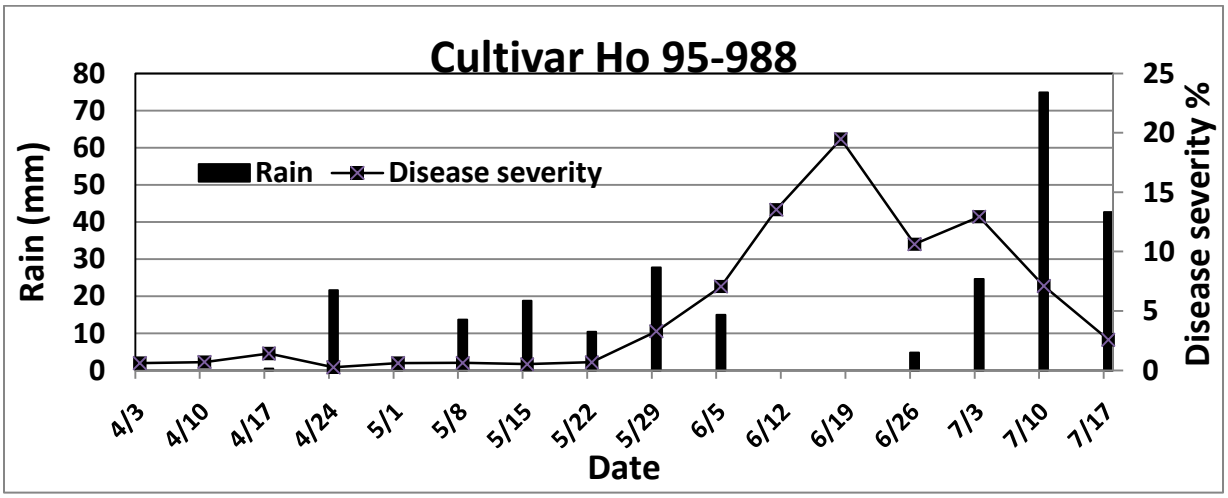

Figure 3.20. Weekly cumulative rainfall at Schriever, LA during the 2009 brown rust epidemic in cultivar Ho 95-988.

3.3.10 Comparison of Disease Assessment Methods

The assessment of disease included the enumeration of brown rust lesions on a 10 cm mid-leaf section and the determination of the percentage of sporulating pustules.

The number of lesions per 10 cm of leaf recorded each week exhibited a very similar pattern to the total percent leaf area with brown rust lesions determined by image analysis for both cultivars during the 2009 epidemics (Figs. 3.21 and 3.22). In Ho 95-988, the lesion count remained high for 2 wk after the peak of the epidemic as determined by image analysis (Fig. 3.22). This was due to the continued formation of small lesions on the second youngest fully emerged leaf that did not develop into pustules.

Sporulating pustules as a percentage of the total number of lesions per leaf reached a maximum value of 68% on May 1, exhibited a second peak of 45% on May 22, and then declined to less than 10% by mid-June in LCP 85-384 (Fig. 3.23). In Ho 95-988, the percentage of sporulating pustules exhibited a pattern of increase after May 1, reached a peak of 42% on June 5, 2 weeks before the peak of the epidemic, then declined to only 2% on June 26 (Fig. 3.24).

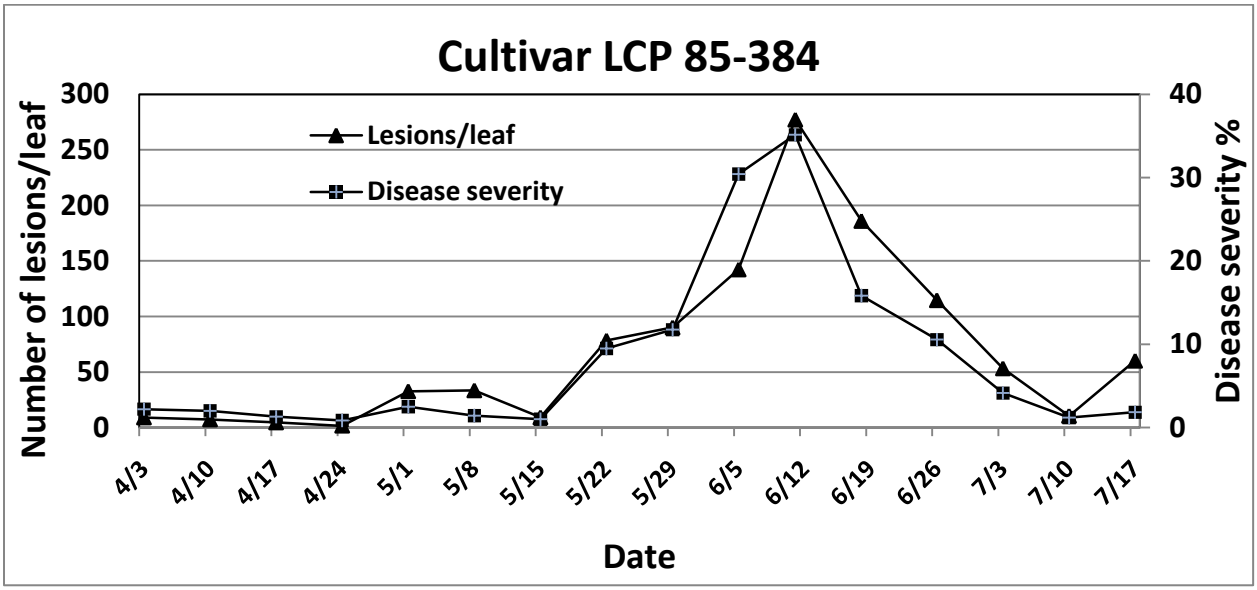

Figure 3.21. Comparison of number of lesions per 10 cm of leaf to disease severity determined by image analysis on entire leaf during the 2009 sugarcane brown rust epidemic in cultivar LCP 85-384 at St. Gabriel, LA.

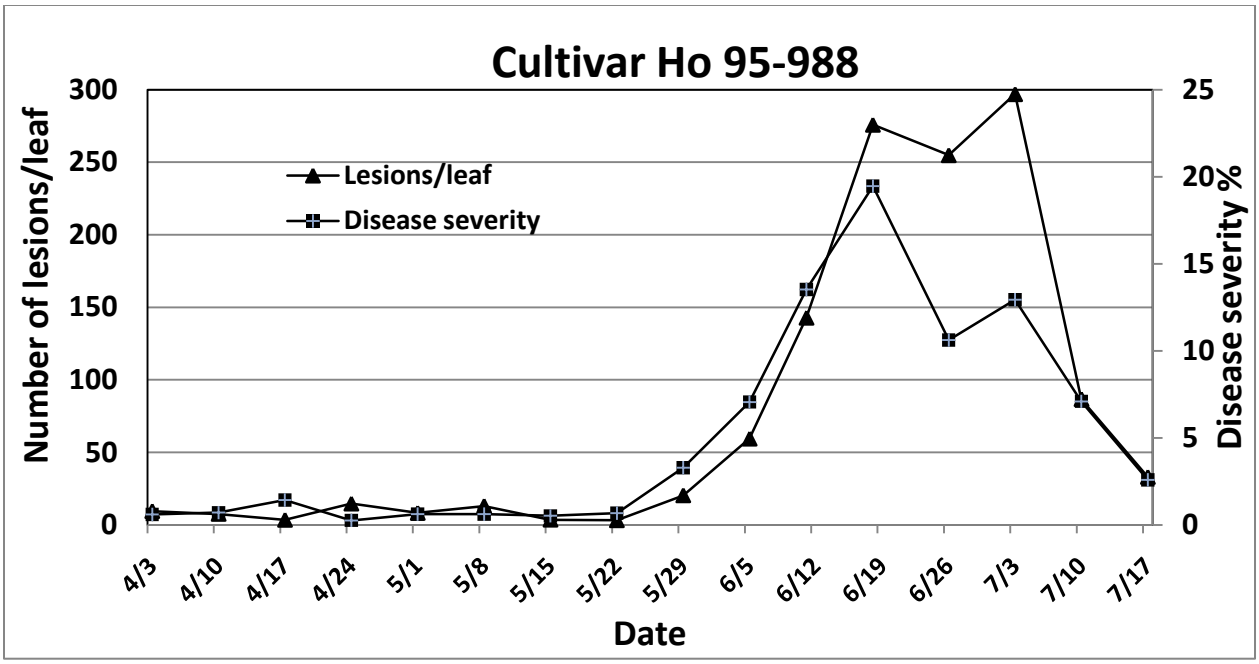

Figure 3.22. Comparison of number of lesions per 10 cm of leaf to disease severity determined by image analysis on entire leaf during the 2009 brown rust epidemic in cultivar Ho 95-988 at Shriever, LA.

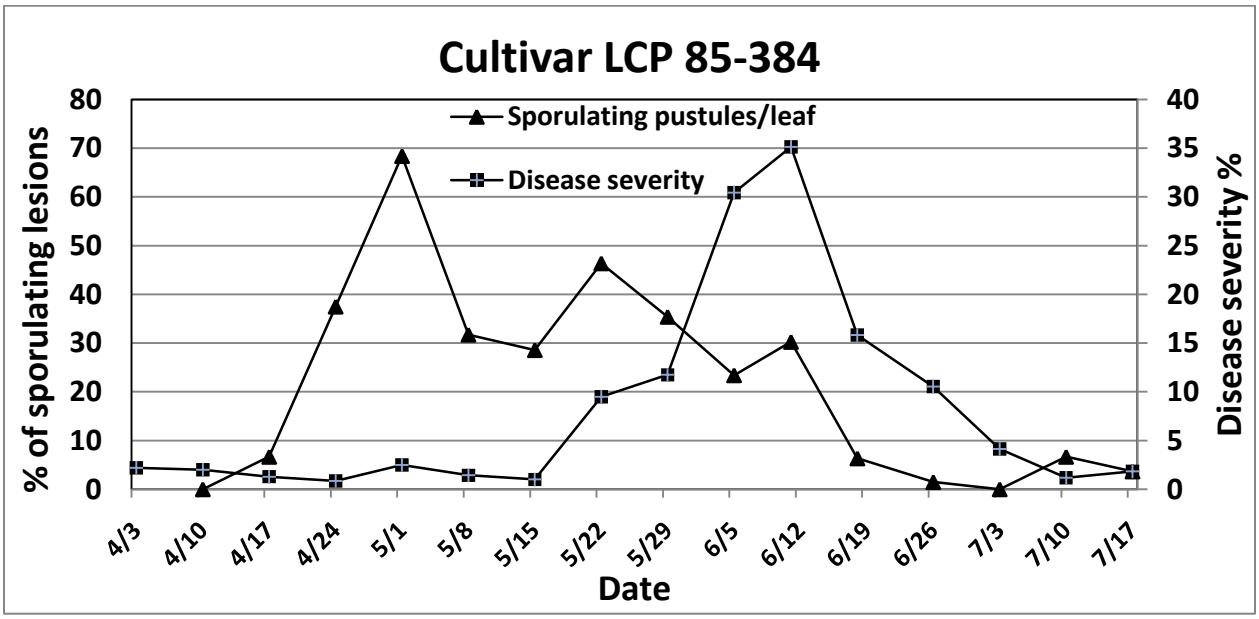

Figure 3.23. Comparison of percentage of sporulating pustules to disease severity during the 2009 brown rust epidemic in cultivar LCP 85-384 at St Gabriel, LA.

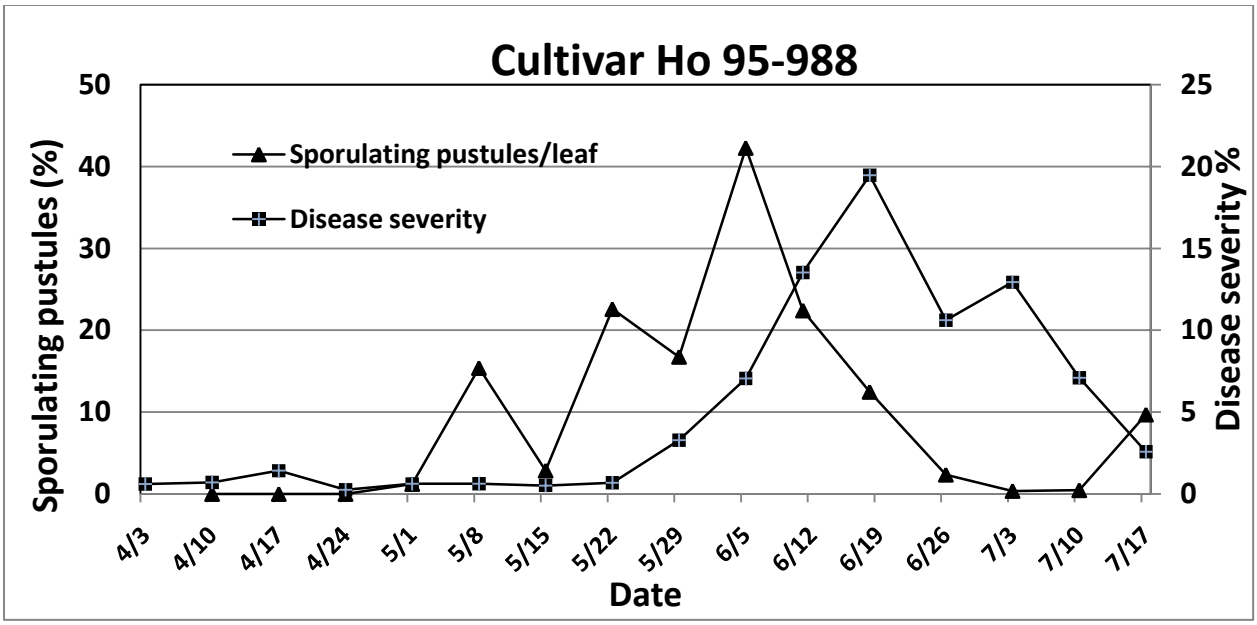

Figure 3.24. Comparison of percentage of sporulating pustules to disease severity during the 2009 brown rust epidemic in cultivar Ho 95-988 at Shriever, LA.

3.3.11 Correlation Analyses for the 2009 Epidemics

To identify variables affecting brown rust severity during 2009, a correlation analysis for all variables under study was performed for each of the cultivars. Data from the beginning of the epidemic up to the maximum levels of disease severity were used (April 4-June 12 in LCP 85-384, and April 4-June 19 in Ho 95-988). Data beyond the peak of the epidemics was not considered in order to study the potential effect of the variables on epidemic increase. The crop growth variables, plant height and canopy cover, had the highest correlation coefficients in both cultivars (Table 3.1). The correlation analysis also found that all environmental variables, except rain, minimum relative humidity, and mean relative humidity, had intermediate correlation coefficient values in the range of 0.2 to 0.8 in both cultivars.

Table 3.1. Correlation analysis comparing all variables with disease severity for two cultivars during the 2009 brown rust epidemics.

Variable	LCP 85-384		Ho 95-988	
	Correlation	p-value	Correlation	p-value
Canopy cover	0.7	0.41	0.99	0.00
Plant height	0.93	0.00	0.96	0.00
Shoot population	0.52	0.12	0.67	0.01
Number of leaves/plant	0.45	0.11	0.27	0.348
Leaf wetness on lower leaves	0.29	0.34	0.42	0.16
Leaf wetness on upper leaves	0.49	0.05	0.22	0.50
Temperature on lower leaves (mean)	0.43	0.20	0.48	0.17
Temperature on lower leaves (minimum)	0.36	0.36	0.28	0.50
Temperature on lower leaves (maximum)	0.23	0.61	0.73	0.07
Temperature on upper leaves (mean)	0.41	0.23	0.47	0.11
Temperature on upper leaves (minimum)	0.4	0.28	0.31	0.38
Temperature on upper leaves (maximum)	0.46	0.12	0.56	0.06
Rain	-0.18	0.74	-0.08	0.88
Ambient temperature (mean)	0.48	0.13	0.42	0.14
Ambient temperature (maximum)	0.6	0.03	0.56	0.03
Ambient temperature (minimum)	0.37	0.34	0.3	0.41
Relative humidity (mean)	0.02	0.94	0.15	0.60
Relative humidity (maximum)	0.46	0.08	0.45	0.11
Relative humidity (minimum)	-0.14	0.77	-0.07	0.88

3.3.12 Hourly Correlation Analysis

An hourly correlation analysis was conducted for all environmental variables to determine if there were periods of time during the day when environmental variables were more correlated with brown rust severity. Compared to the daily means,

temperature variables were more correlated with disease severity from 10:00 AM to 7:00 PM, while leaf wetness and relative humidity were more correlated with disease severity from 12:00 AM to 6:00 AM (Table 3.2).

Table 3.2. Comparison of environmental variable correlation coefficients for the mean of the more correlated time period during the day and the daily averages in two cultivars during the 2009 brown rust epidemic.

Variable	LCP 85-384		Ho 95-988	
	Hourly	Daily	Hourly	Daily
Leaf wetness on lower leaves	0.47	0.29	0.52	0.42
Leaf wetness on upper leaves	0.46	0.49	0.32	0.22
Relative humidity	0.32	0.02	0.44	0.15
Ambient temperature	0.57	0.48	0.53	0.42
Temperature on lower leaves	0.43	0.43	0.65	0.48
Temperature on upper leaves	0.53	0.41	0.52	0.47

3.3.13 Multiple Regression Analyses

Multiple regression analysis was used to evaluate combinations of selected variables to explain disease severity. In the 2009 epidemic, models combining four different variables provided high R^2 and significant p-values ($P<0.05$) (Table 3.3). When three variables were included in the analysis, the only significant models included leaf wetness (on upper, fully exposed leaves or lower, partially shaded leaves) or relative humidity; temperature on upper, fully exposed leaves or lower, partially shaded leaves or ambient; and plant height (Table 3.3). Two-variable models including only environmental variables were not significant for either of the cultivars in 2009 (Table 3.3).

Table 3.3. Multiple regression models combining different variables for the 2009 brown rust epidemics in cultivars LCP 85-384 and Ho 95-988.

Multiple regression models	LCP 85-384		Ho 95-988		Multiple regression models	LCP 85-384		Ho 95-988	
	R^2	p-value	R^2	p-value		R^2	p-value	R^2	p-value
4-variable models:									
LWL +TLL+SP+PH	0.98	0.01	1	<0.01	RH+TUL+SP+PH	0.95	0.03	0.99	<0.01
LWY+TLL+SP+PH	0.97	0.01	1	<0.01	LWL +AT+SP+PH	0.97	0.01	0.99	<0.01
RH+ TLL +SP+PH	0.94	0.03	1	<0.01	LWU+AT+SP+PH	0.96	0.02	1	<0.01
LWO +TUL+SP+PH	0.98	0.01	0.99	<0.01	RH+AT+SP+PH	0.94	0.04	0.99	<0.01
LWY+TUL+SP+PH	0.96	0.02	1	<0.01					
3-variable models:									
LWL+ TLL +SP	0.33	0.39	0.59	0.19	LWL+ TLL +PH	0.96	<0.01	0.92	0.01
LWU+ TLL +SP	0.3	0.45	0.9	0.01	LWU+ TLL +PH	0.88	0.02	0.98	<0.01
RH+ TLL +SP	0.3	0.45	0.7	0.09	RH+ TLL +PH	0.89	0.02	0.98	<0.01
LWL+TUL+SP	0.27	0.64	0.58	0.14	LWL+TUL+PH	0.96	<0.01	0.96	<0.01
LWU+TUL+SP	0.25	0.67	0.61	0.11	LWU+TUL+PH	0.88	0.02	0.96	<0.01
RH+TUL+SP	0.25	0.67	0.58	0.14	RH+TUL+PH	0.89	0.02	0.98	<0.01
LWL+AT+SP	0.44	0.23	0.45	0.17	LWL+AT+PH	0.95	0.01	0.95	<0.01
LWU+AT+SP	0.33	0.40	0.5	0.12	LWU+AT+PH	0.89	0.02	0.96	<0.01
RH+AT+SP	0.32	0.42	0.45	0.17	RH+AT+PH	0.89	0.81	0.98	<0.01
2- variable models:									
LWL+ TLL	0.28	0.23	0.44	0.17	RH+TUL	0.2	0.52	0.3	0.28
LWU+ TLL	0.29	0.22	0.73	0.02	LWL+AT	0.38	0.11	0.3	0.17
RH+ TLL	0.3	0.20	0.42	0.19	LWU+AT	0.34	0.15	0.38	0.09
LWL+TUL	0.19	0.53	0.31	0.27	RH+AT	0.34	0.15	0.3	0.17
LWU+TUL	0.19	0.53	0.46	0.12					

Abbreviations: LWU=leaf wetness on upper leaves, LWL=leaf wetness on lower leaves, TLL=temperature on lower leaves, TUL=temperature on upper leaves, SP=Shoot population, PH=plant height, AT=ambient temperature, RH=relative humidity.

3.4 Brown Rust Epidemics During 2010

Spring 2010 was preceded by winter freezes that killed all foliar tissues of sugarcane plants in the field as compared to the 2009 season in which live foliar tissues were observed throughout the preceding winter months. A comparison of the number of recorded hours below freezing and the minimum temperatures per freeze event during the 2008-2009 and 2009-2010 winters at the USDA research farm in Shriever, LA revealed 63 hours below freezing with a minimum temperature low of -2.8 C during 2008-2009, while 177 hours below freezing with two minimum temperatures of -7.2 C occurred during 2009-2010 (Table 3.4). The mean, minimum, and maximum monthly averages for temperatures during the winter 2009-2010 also were below 30-yr-average records, while the winter months for 2008-2009 exhibited averages slightly above the 30-yr-averages (Table 3.5). The severe winter adversely affected the overwintering of *P. melanocephala* and caused a probable delay and/or reduction in the availability of primary inoculum. Sporulating pustules were observed only on lower leaves of Ho 95-988 plants in Schriever, whereas none were observed on plants of Ho 95-988 or LCP 85-384 plants at St. Gabriel. Therefore, the only brown rust epidemic results available for 2010 were from Ho 95-988 at Schriever, LA.

Table 3.4. Date of occurrence, duration, and minimum temperature for freezes during the winter months in 2008-2009 and 2009-2010.[a]

2008-2009			2009-2010		
Date	Duration (hours)	Lowest temp (^{o}C)	Date	Duration (hours)	Lowest temp (^{o}C)
10/29/2008	1	0.0	11/27/2009	2	0.0
12/2/2008	6	-2.2	12/5/2009	5	-1.1
12/11/2008	3	0.0	12/6/2009	1	0.0
12/12/2008	5	-1.1	12/28/2009	1	-0.6
12/22/2008	6	-1.1	12/29/2009	8	-2.2
1/12/2009	1	0.0	1/3/2010	3	0.0
1/14/2009	4	-1.1	1/4/2010	5	-1.1
1/15/2009	2	0.0	1/5/2010	12	-3.9
1/16/2009	8	-2.2	1/6/2010	8	-3.3
1/17/2009	1	0.0	1/7/2010	2	-0.6
1/20/2009	4	-0.6	1/8/2010	24	-3.3
1/21/2009	8	-2.8	1/9/2010	20	-6.7
2/3/2009	3	-1.1	1/10/2010	16	-7.2
2/4/2009	2	-0.6	1/11/2010	9	-7.2
2/5/2009	4	-0.6	1/12/2010	6	-2.2
2/20/2009	3	0.0	1/13/2010	8	-3.9
3/1/2009	2	0.0	1/30/2010	1	0.0
			1/31/2010	7	0.0
			2/10/2010	2	-1.1
			2/12/2010	4	0.0
			2/13/2010	7	-2.2
			2/14/2010	4	-1.1
			2/16/2010	5	-2.8
			2/17/2010	3	-0.6
			2/18/2010	7	-1.1
			2/25/2010	5	-2.8
			3/4/2010	2	0.0
Totals hours	**63**	**-**	**Total hours**	**177**	**-**

[a] Temperature data originated from Louisiana State University AgCenter weather station in Schriever, LA.

Table 3.5. Temperature records for the winter and early spring months of 2008-2009 and 2009-2010. [a]

Month	2008-2009			2009-2010			Average (1971-2000)		
	Max	Min	Mean	Max	Min	Mean	Max	Min	Mean
November	21.6	7.5	14.6	20.8	8.1	14.4	21.6	8.8	15.2
December	18.8	7	12.9	14.8	5.3	10.1	17.4	5.4	11.4
January	17.9	5.4	11.7	13.7	2	7.8	15.8	4.3	10.1
February	20.2	7.3	13.8	12.6	2.3	7.4	17.8	5.8	11.8
March	23.2	11.6	17.4	19.6	6.8	13.2	21.8	9.6	15.7

[a] Means and average reference values are from weather station at the Louisiana State University AgCenter Ben Hur Research Farm, 12.5 km from St. Gabriel, LA.

The presence of brown rust lesions on Ho 95-988 plants within the plots was observed on April 17. However, even though the presence of inoculum was recorded early in the season, disease development was slow, and disease severity on the younger leaves did not exceed 1% until June 19 (Fig. 3.25). Low disease severity values continued to be recorded during late June and July with a severity maximum of 3.9% on July 10.

As for the 2009 epidemic, plant growth and environmental variables were monitored and compared to brown rust severity during 2010. Results are presented separately for each variable.

3.4.1 Plant Height

During the 2010 experimental period, plant height increased continuously. The overall weekly plant growth rate was 14.5 cm (Fig. 3.25). Plant height was 43.5 cm on May 29 the week before the increase of disease began then reached 2 m by the end of the experiment on July 17.

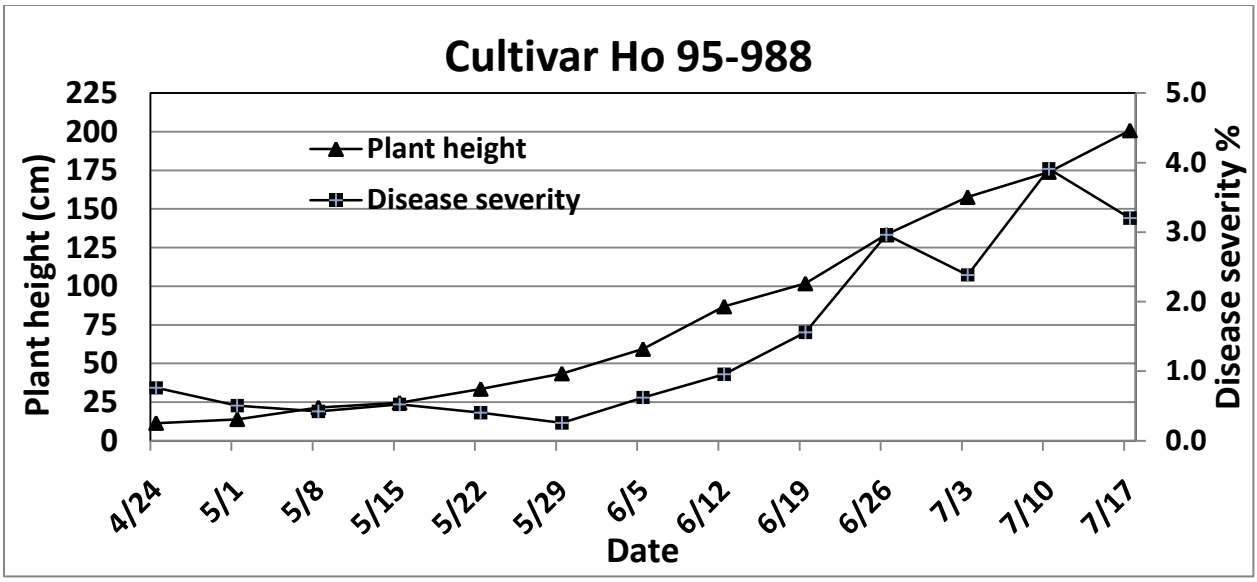

Figure 3.25. Plant height during the 2010 brown rust epidemic in cultivar Ho 95-988 at Shriever, LA.

3.4.2 Shoot Population

Shoot population increased until June 5 (Fig. 3.26). The maximum population was 34 shoots/m. After June 5, the population gradually decreased until the end of the experiment.

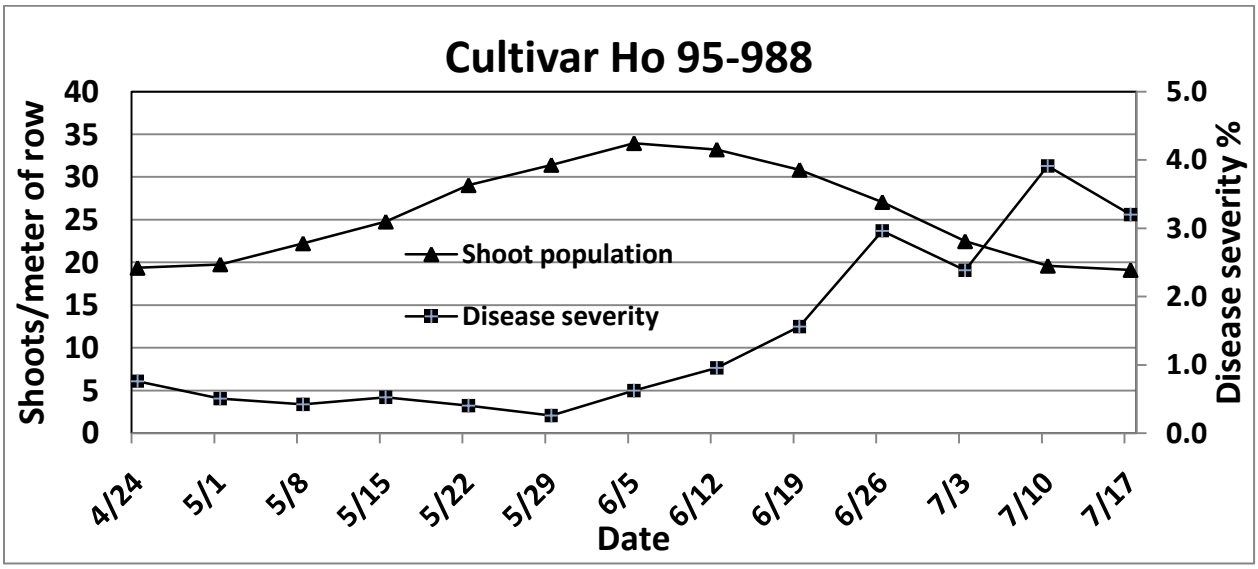

Figure 3.26. Shoot population during the 2010 brown rust epidemic in cultivar Ho 95-988 at Shriever, LA.

3.4.3 Leaves per Shoot

The number of green leaves per shoot increased during early May then showed little variation during the epidemic period (Fig. 3.27). From April 24 to May 8, the mean number of leaves per shoot changed from four to five. After the week of May 22, the mean number of leaves per shoot remained between six and seven as during 2009.

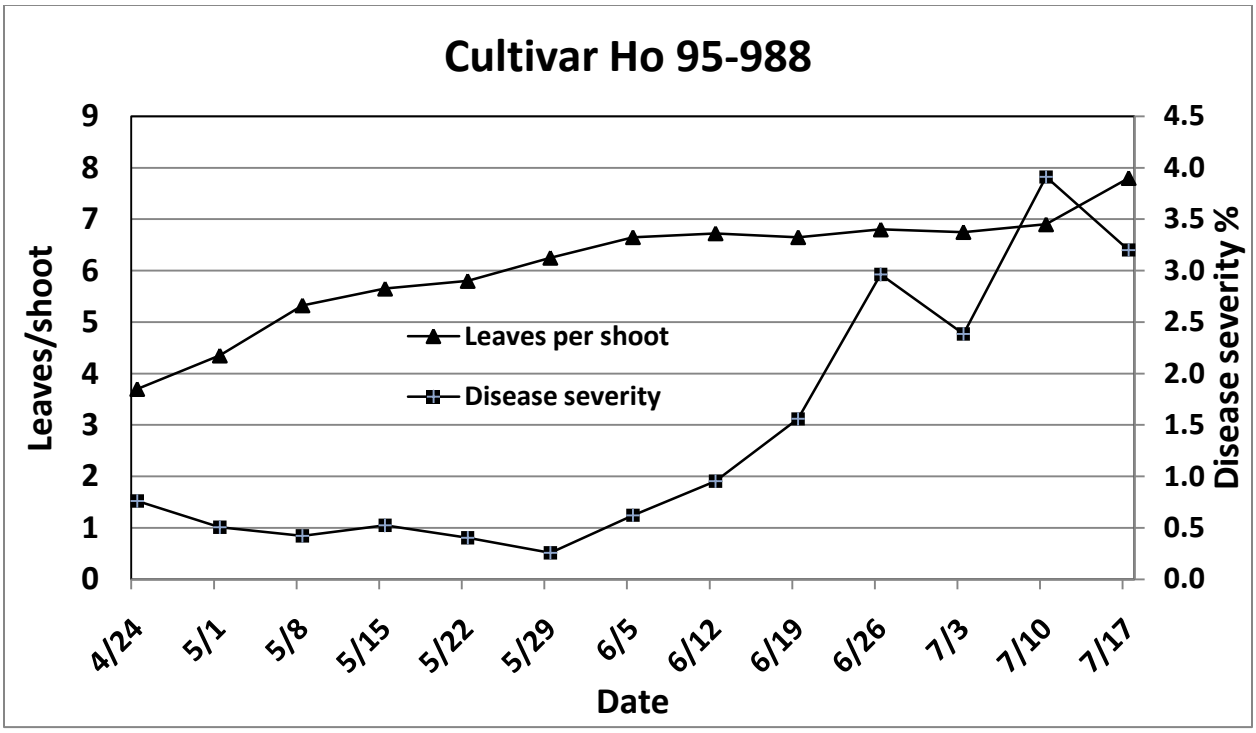

Figure 3.27. Number of green leaves per shoot during the 2010 brown rust epidemic in cultivar Ho 95-988 at Shriever, LA.

3.4.4 Canopy Cover

In 2010, the pattern of canopy development was similar to 2009. Canopy cover increased from approximately 20 to 80% from late May to late June (Fig. 3.28). The final canopy cover recorded was 86% on July 17. Canopy cover in the week before the beginning of the epidemic (May 29) was 24%.

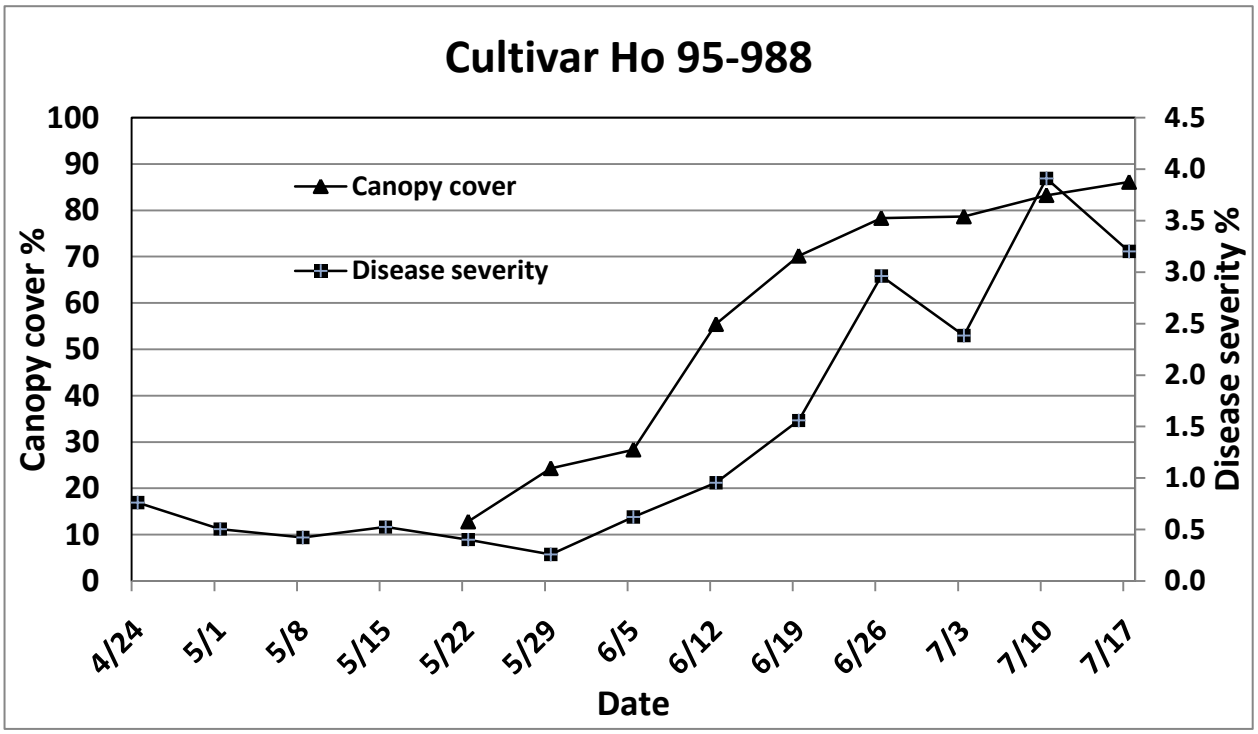

Figure 3.28. Canopy cover during the 2010 brown rust epidemic in cultivar Ho 95-988 at Shriever, LA.

3.4.5 Leaf Wetness

For the sensor placed at the level of lower leaves, weekly means for hours of leaf wetness per day ranged from 8 to 15 for the period from May 29 to July 3 when the epidemic was increasing (Fig. 3.29). In comparison, the weekly mean for leaf wetness hours per day ranged from 11 to 8 for the sensor located at the level of the young leaves during the same period. Means for leaf wetness hours per day remained high until mid-July.

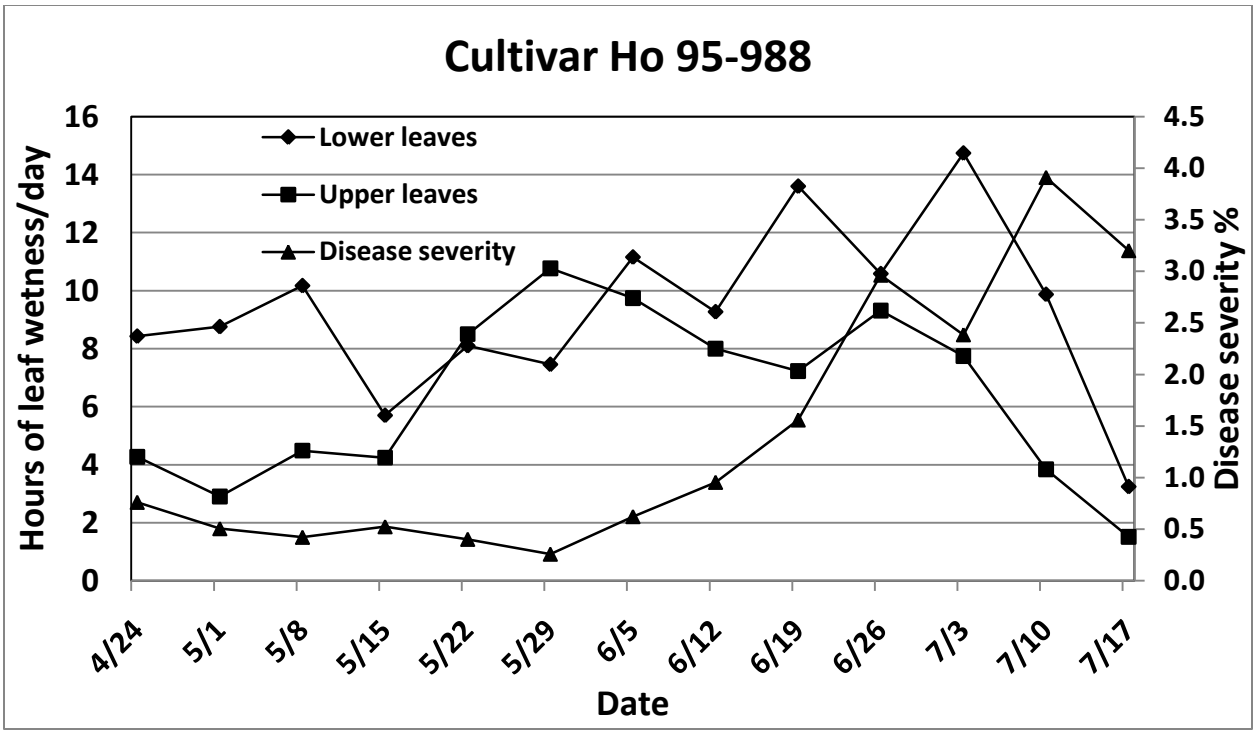

Figure 3.29. Weekly means for leaf wetness hours per day at the level of upper and lower leaves during the 2010 brown rust epidemic in cultivar Ho 95-988 at Shriever, LA.

3.4.6 Temperature

Mean daily temperature on lower leaves increased during the weeks of April 24 to May 8 from 22 to 26 C (Fig. 3.30). After May 1, the mean temperature ranged from 26 to 29 C for the entire epidemic. Mean daily temperatures on upper leaves were similar to mean temperatures on the lower leaf, except that mean temperatures for a 3-wk period from June 12 to June 26 reached 30 C, and maximum temperatures ranged from 45 to 50 C (Fig. 3.31). The slight disease severity increase to 3.9% by July 10 was preceded by 1 wk with maximum temperatures below 40 C during the week of July 3 for upper and lower leaves. Temperatures on upper leaves were not recorded until the week of May 22 due to low plant height and negligible differences with lower leaves (Fig. 3.31). The pattern on upper leaves over time was similar to lower leaves for the

mean and minimum temperatures, but as the plants grew, the maximum temperature at the surface of upper leaves increased relative to lower leaves.

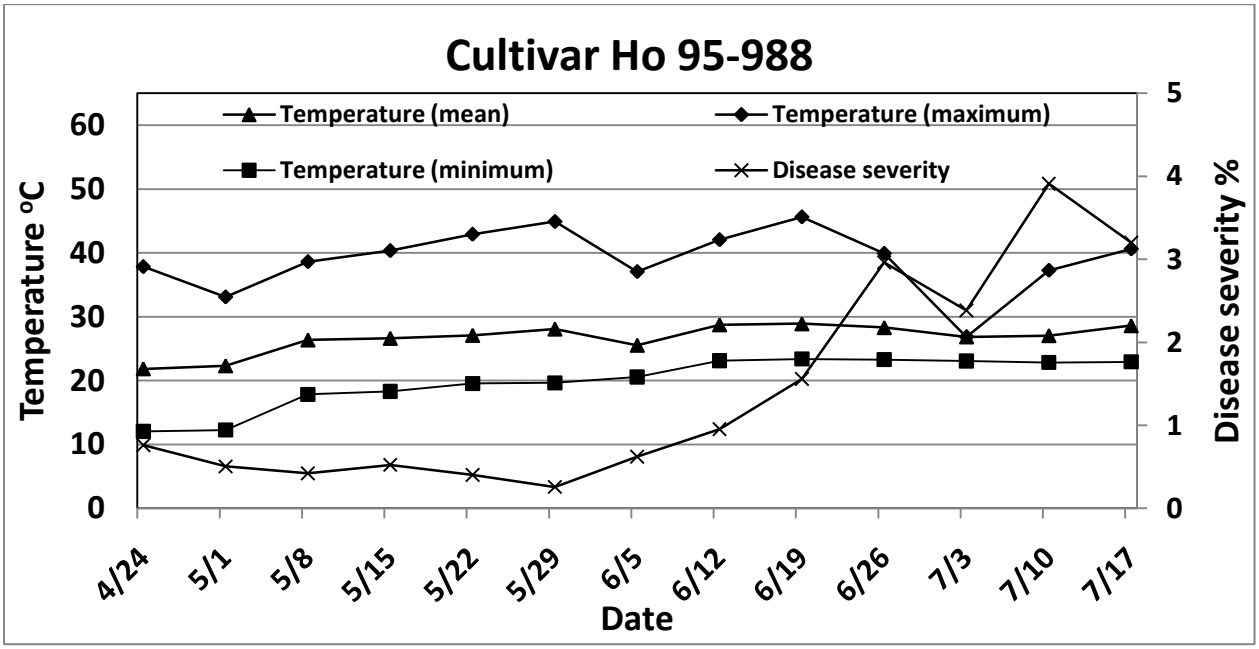

Figure 3.30. Weekly means for mean, maximum, and minimum daily temperatures on lower leaves during the 2010 brown rust epidemic in cultivar Ho 95-988 at Shriever, LA.

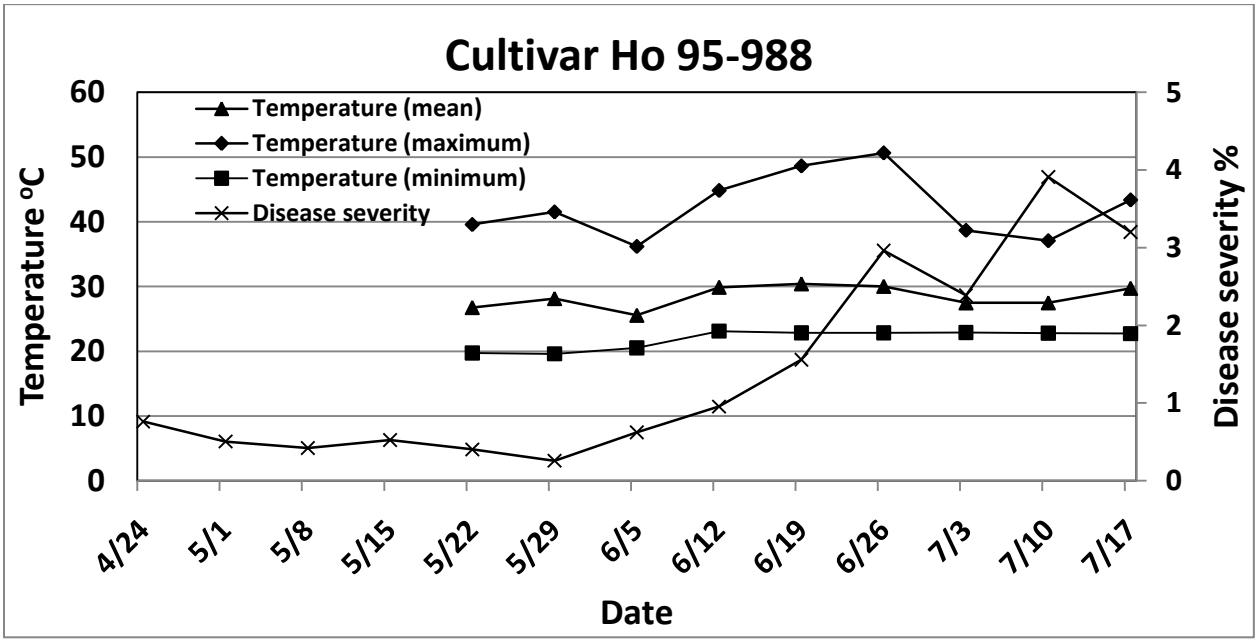

Figure 3.31. Weekly means for mean, maximum, and minimum daily temperatures on upper leaves during the 2010 brown rust epidemic in cultivar Ho 95-988 at Shriever, LA.

Ambient temperature was not as variable as the temperatures measured at the surfaces of upper and lower leaves. There was less difference between maximum and minimum temperatures during the day (Fig. 3.32). For example, a typical difference between maximum and minimum ambient temperature was 9 C, while on upper leaves, the lowest temperature difference recorded was 15 C. Weekly means for maximum daily temperature were approximately 30 C during May until the week of May 29 with a maximum of 34 C. The maximum daily temperature mean decreased to 30 C the week of June 5 then ranged from 33 to 34 C for the weeks of June 12 to June 26, decreased to 31 C the week of July 3 (the week prior to the epidemic peak), and increased thereafter. Mean and minimum temperatures exhibited little variation from June 12 to July 17. Mean temperature had the highest increase during the week of May 1, then the temperature remained between 25 and 29 C during the rest of the epidemic. Minimum temperature ranged between 20 and 24 C after May 8.

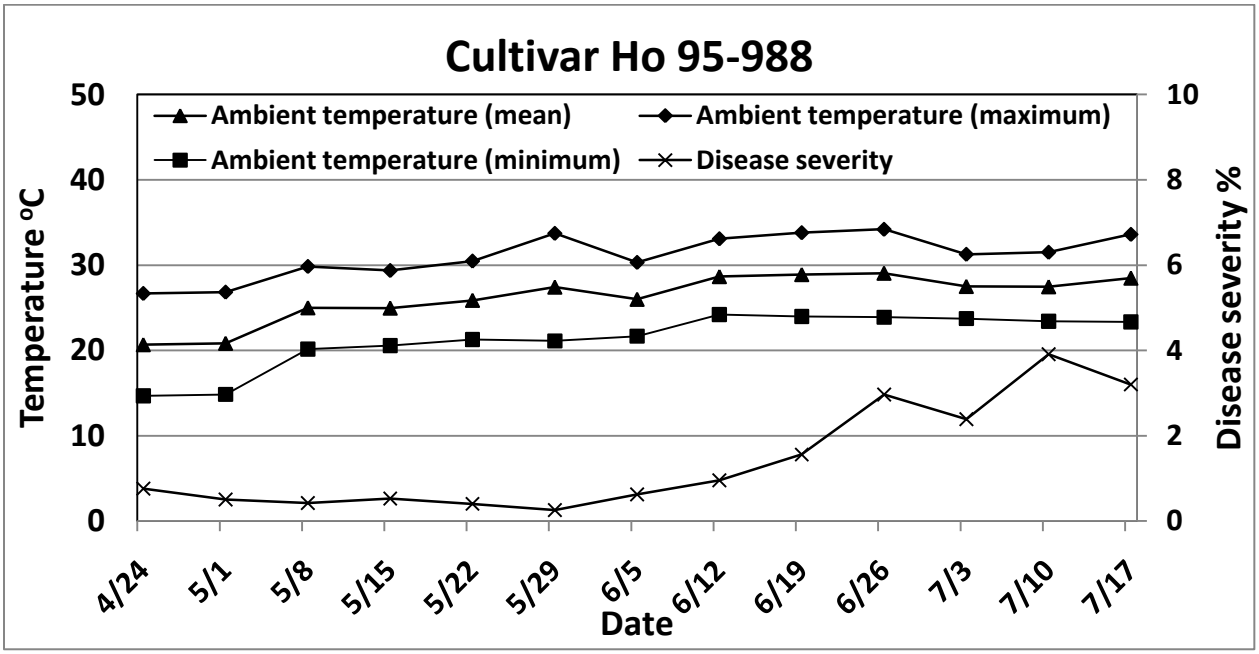

Figure 3.32. Weekly means for mean, maximum, and minimum daily ambient temperatures during the 2010 brown rust epidemic in cultivar Ho 95-988 at Shriever, LA.

3.4.7 Relative Humidity

Mean daily relative humidity exhibited only moderate variability during the experiment as in 2009 (Fig. 3.33) ranging from 74 to 91%. Minimum relative humidity exhibited the most variation ranging from 38.4 to 66%. The weekly mean for maximum relative humidity reached 100% every week, except the week of May 15.

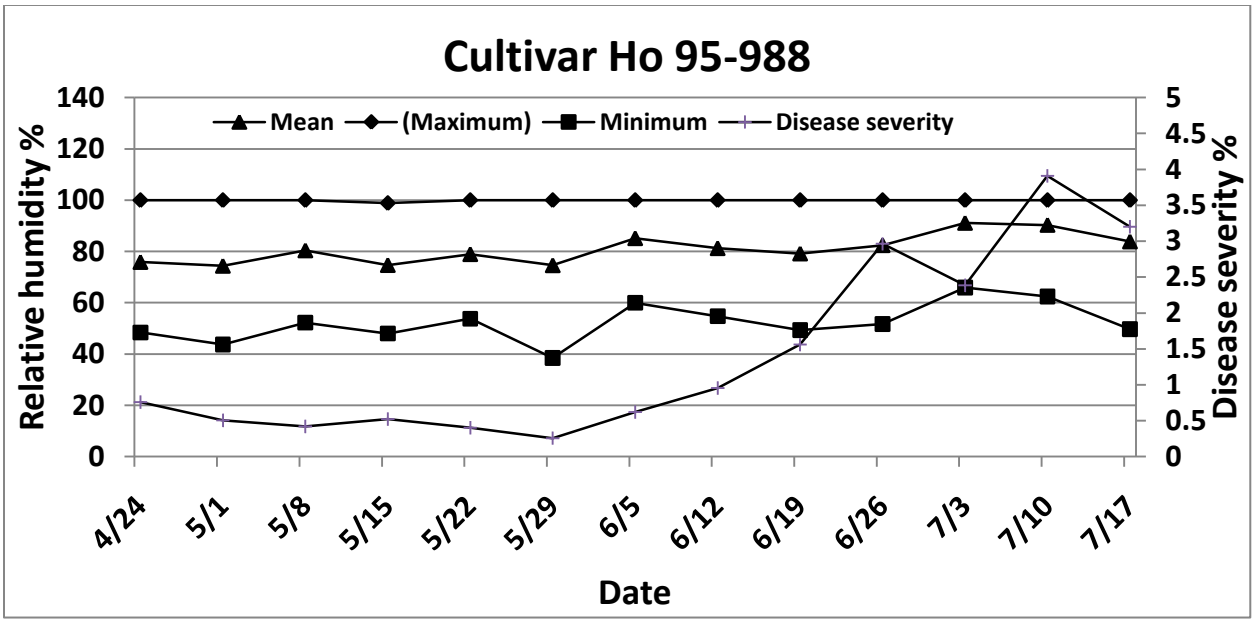

Figure 3.33. Weekly means for mean, maximum, and minimum daily relative humidity during the 2010 brown rust epidemic in cultivar Ho 95-988 at, Shriever, LA.

3.4.8 Rain

Rainfall was low during May in 2010 (Fig. 3.34). Rain was variable but occurred in greater amounts during the course of the epidemic. Higher cumulative rainfall was registered in the week preceding June 19 and the week preceding July 3. Cumulative rain was greater in 2010 than the 2009 experiment.

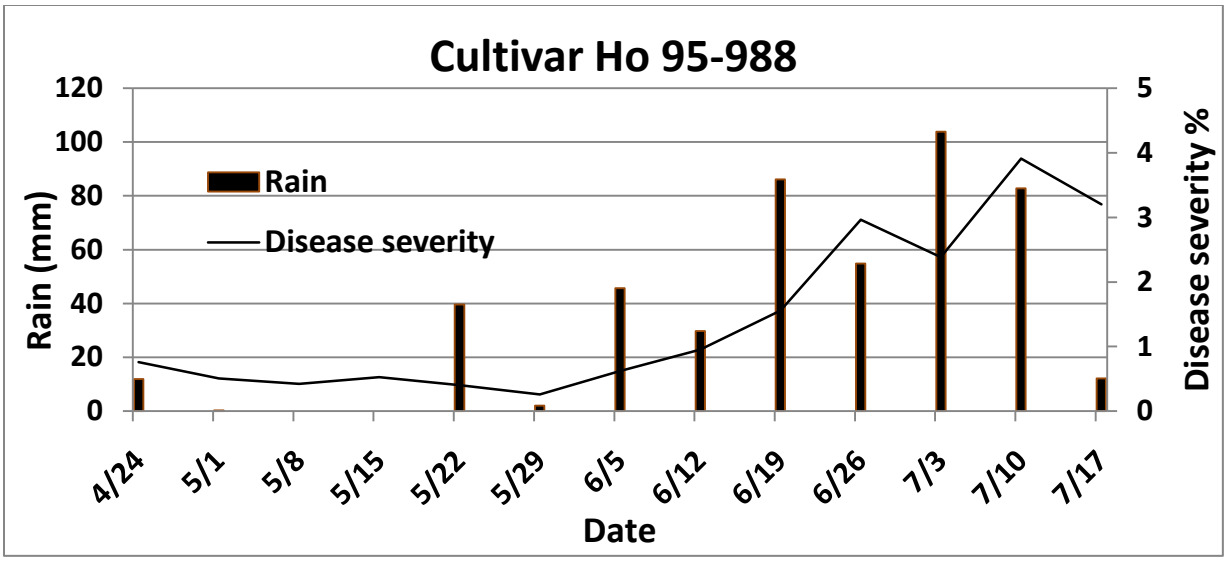

Figure 3.34. Cumulative weekly rain recorded at Schriever, LA during the 2010 brown rust epidemic in cultivar Ho 95-988.

3.4.9 Comparison of Disease Assessment Methods

As in the 2009 epidemics, lesions per 10-cm-section of leaf exhibited a similar pattern to disease severity determined by image analysis during 2010 (Fig. 3.35). The percentage of sporulating pustules varied but was generally low prior to the initiation of the epidemic then ranged from 15-25% during the epidemic (Fig. 3.36).

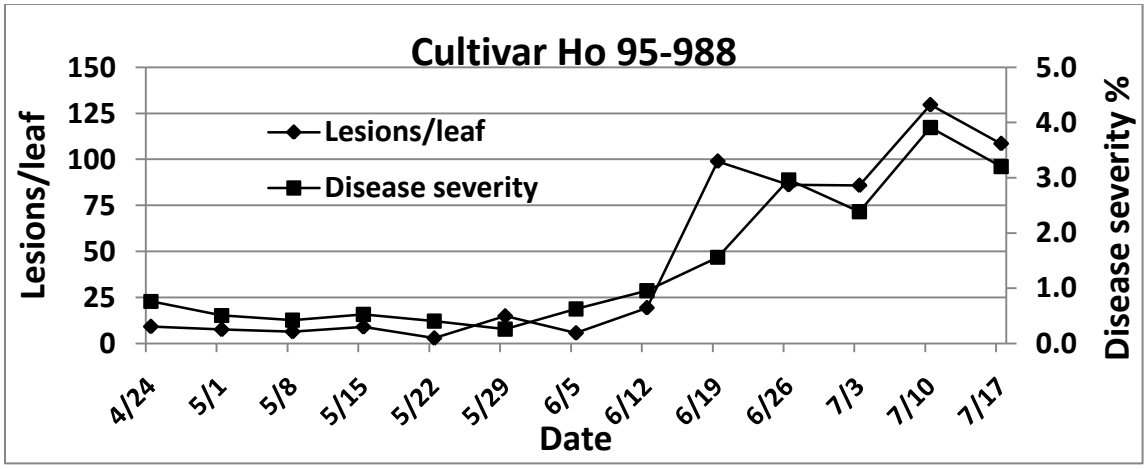

Figure 3.35. Brown rust lesions per 10-cm-section of leaf compared to disease severity determined by image analysis during the 2010 brown rust epidemic in cultivar Ho 95-988 at Shriever, LA.

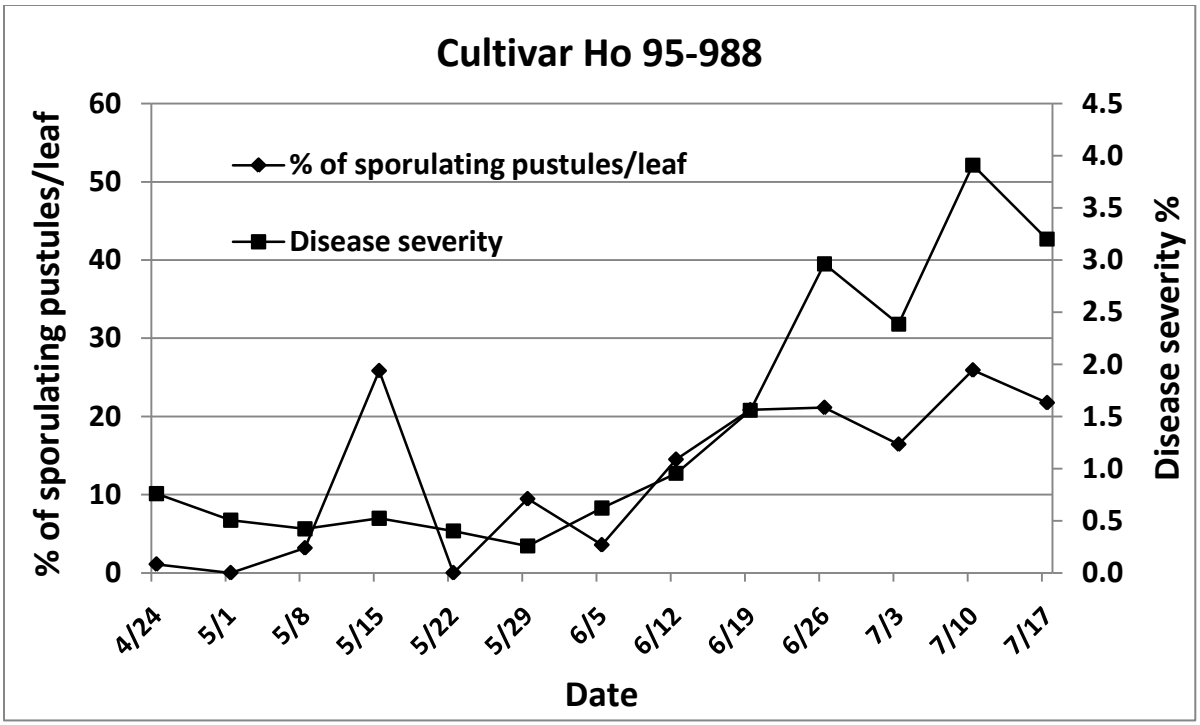

Figure 3.36. Percentage of sporulating pustules compared to disease severity during the 2010 brown rust epidemic in cultivar Ho 95-988, Shriever, LA.

3.4.10 Correlation Analyses for 2010 Epidemic

The results from correlation analyses for the 2010 brown rust epidemic were similar to the 2009 epidemics. Correlation coefficients for some variables were higher (Table 3.6), including leaf wetness on upper leaves, leaf wetness on lower leaves, rain, minimum temperature on lower leaves, and maximum relative humidity. Lower correlation coefficients also were obtained for shoot population and maximum temperature on lower leaves. The lower correlation observed for shoot population was expected as a result of a longer epidemic period during which the normal decrease in shoot population due to shading began.

Table 3.6. Correlation coefficients of all variables with disease severity during the 2010 brown rust epidemic.

Variable	Cultivar Ho 95-988	
	Correlation	p-value
Canopy cover	0.93	<0.01
Plant height	0.95	<0.01
Shoot population	0.11	0.15
Number of leaves/plant	0.62	<0.01
Leaf wetness lower leaves	0.85	0.17
Leaf wetness upper leaves	0.78	0.01
Temperature lower leaves (mean)	0.44	0.03
Temperature lower leaves (minimum)	0.63	<0.01
Temperature lower leaves (maximum)	-0.29	0.49
Temperature upper leaves (mean)	0.42	0.12
Temperature upper leaves (minimum)	0.83	0.04
Temperature upper leaves (maximum)	0.33	0.11
Rain	0.9	0.09
Ambient temperature (mean)	0.47	<0.01
Ambient temperature (maximum)	0.65	<0.01
Ambient temperature (minimum)	0.59	<0.01
Relative humidity (mean)	0.67	0.11
Relative humidity (maximum)	0.7	0.09
Relative humidity (minimum)	0.52	0.41

3.4.11 Hourly Correlation Analyses

The hourly correlation analysis for the 2010 epidemic in Ho 95-988 found higher correlation for different periods of time compared to the 2009 epidemics. The highest correlations for leaf wetness and relative humidity were obtained from 3:00-11:00 PM, and from 12:00-6:00 AM for all temperature variables (Table 3.7). The lower variation in environmental variables and the low disease severity during the epidemic probably accounted for these results.

Table 3.7. Comparison of environmental variable correlation coefficients for the more correlated time period during the day and the daily averages for Ho 95-988 during the 2009 brown rust epidemic.

Variable	Ho 95-988	
	Hourly	Daily
Leaf wetness on lower leaves	0.87	0.85
Leaf wetness on upper leaves	0.92	0.78
Relative humidity	0.71	0.67
Ambient temperature	0.59	0.46
Temperature on lower leaves	0.61	0.44
Temperature on upper leaves	0.73	0.42

3.4.13 Multiple Regression Analyses

All variables listed in Table 3.6 and plant height and canopy cover were evaluated in different combinations to produce regression models (Table 3.8). All four-variable models containing plant height, leaf wetness (either on lower or upper leaves, or relative humidity), and temperature (either on upper or lower leaves or ambient temperature), and canopy cover, had R^2 above 0.86, but not all were significant ($P \leq 0.05$). When three variables were combined, including leaf wetness (either on lower or upper leaves or relative humidity), temperature (either on upper leaves or lower leaves or ambient temperature), and canopy cover, all regression models had R^2 above 0.84; however, not all were significant. When plant height was used in the three-variable models, most had R^2 above 0.9 and were significant. Two variable models including leaf wetness (on upper leaves or lower leaves or relative humidity) and temperature (on upper leaves or lower leaves or ambient temperature) provided R^2 above 0.8.

Table 3.8. Multiple regression models combining different variables for the 2010 brown rust epidemic in cultivar Ho 95-988.

Multiple regression models	Ho 95-988		Multiple regression models	Ho 95-988	
	R^2	p-value		R^2	p-value
4-variable models:					
LWL +TLL+SP+PH	0.99	<0.01	RH+TUL+SP+PH	0.86	0.53
LWU+TLL+SP+PH	0.98	<0.01	LWL +AT+SP+PH	0.99	<0.01
RH+ TLL +SP+PH	0.92	<0.01	LWU+AT+SP+PH	0.98	<0.01
LWL +TUL+SP+PH	1.00	0.01	RH+AT+SP+PH	0.92	<0.01
LWU+TUL+SP+PH	0.96	0.29			
3-variable models:					
LWL+ TLL +SP	0.90	<0.01	LWL+ TLL +PH	0.98	<0.01
LWU+ TLL +SP	0.98	<0.01	LWU+ TLL +PH	0.95	<0.01
RH+ TLL +SP	0.81	<0.01	RH+ TLL +PH	0.91	<0.01
LWL+TUL+SP	1.00	<0.01	LWL+TUL+PH	0.98	0.03
LWU+TUL+SP	0.96	0.06	LWU+TUL+PH	0.95	0.07
RH+TUL+SP	0.86	0.20	RH+TUL+PH	0.86	0.21
LWL+AT+SP	0.89	<0.01	LWL+AT+PH	0.98	<0.01
LWU+AT+SP	0.97	<0.01	LWU+AT+PH	0.96	<0.01
RH+AT+SP	0.78	0.01	RH+AT+PH	0.91	<0.01
2- variable models:					
LWL+ TLL	0.81	<0.01	RH+TUL	0.67	0.19
LWU+ TLL	0.90	<0.01	LWL+AT	0.81	<0.01
RH+ TLL	0.52	0.04	LWU+AT	0.92	<0.01
LWL+TUL	0.83	0.07	RH+AT	0.52	0.04
LWU+TUL	0.87	0.05			

Abbreviations: LWU=leaf wetness on upper leaves, LWL=leaf wetness on lower leaves TLL=temperature on lower leaves, TUL=temperature on upper leaves, SP=Shoot population, PH=plant height, AT=ambient temperature, and RH=relative humidity.

3.5 Determination of Disease Conducive and Limiting Conditions

Brown rust epidemic onset and progress requires the occurrence of leaf wetness and temperature conditions conducive for infection. Daily values from the 2009 and 2010 epidemics for moisture and temperature variables correlated with disease severity. Therefore, the actual conditions for these variables were evaluated to determine the occurrence of potentially conducive or limiting conditions for disease increase in the 2009 and 2010 epidemics. Results from controlled conditions experiments suggest a minimum of 7 h of leaf wetness and a temperature of 17 C are required for successful infection. An evaluation of the temperature data from the 2009 epidemics revealed that a weekly mean for daily maximum ambient temperature of 32 C or more preceded the decline of disease severity.

The number of days per week with more than 7 h of leaf wetness ranged from 0 to 7 from the last week of April until July during 2009 and 2010 (Fig. 3.37). The number of infection conducive days ranged from 5 to 7 days/wk from the beginning of May until the week of June 19 in both cultivars during 2009 then decreased during late June and early July. The number of conducive days per week was more variable during the 2010 epidemic. The number of conducive days ranged from 3 to 6 days/wk during May then ranged from 6 to 7 during June.

The weekly mean for minimum daily temperature exceeded 17 C the week of May 1 for the 2009 epidemics and May 8 for the 2010 epidemic. The number of days per week with a maximum ambient temperature below 32 C varied between the 2009 and 2010 epidemics (Fig. 3.38). During 2010, there were no days with a maximum daily temperature below 32 C for the week of May 29 then there were two weeks in early July

when there were 3 days with daily maximum temperatures below 32 C. There were no disease conducive days the week of June 19 for any of the three epidemics and for two weeks thereafter at both locations in 2009.

The cumulative number of infection conducive days occurring during each epidemic after the minimum temperature was reached until the onset of disease on young leaves was determined separately for leaf wetness and temperature (Table 3.9). The cumulative number of conducive leaf wetness days until epidemic onset was similar for all three epidemics, while the number of days until the onset of the 2009 LCP 85-384, 2009 Ho 95-988, and 2010 Ho 95-988 epidemics increased progressively, In contrast, the cumulative number of temperature conducive days was least for the epidemic in LCP 85-384 (2009) with the earliest start date and progressively greater for the Ho 95-988 2009 epidemic that began a week later and the 2010 epidemic that did not begin on the young leaves for another 3 wk.

Table 3.9. Cumulative leaf wetness and temperature conducive days prior to brown rust epidemic onset in 2009 and 2010 in two susceptible cultivars.

Epidemic	Disease conducive days[a]	
	Leaf wetness	Temperature
LCP 85-384 (2009)	23	21
Ho 95-988 (2009)	25	28
Ho 95-988 (2010)	25	33

[a]Disease conducive days with more than 7 h leaf wetness or with a minimum temperature $\geq$17 C and a maximum < 32 C were counted after the first occurrence of daily minimum temperatures $\geq$ 17 C until the epidemic began on the young leaves.

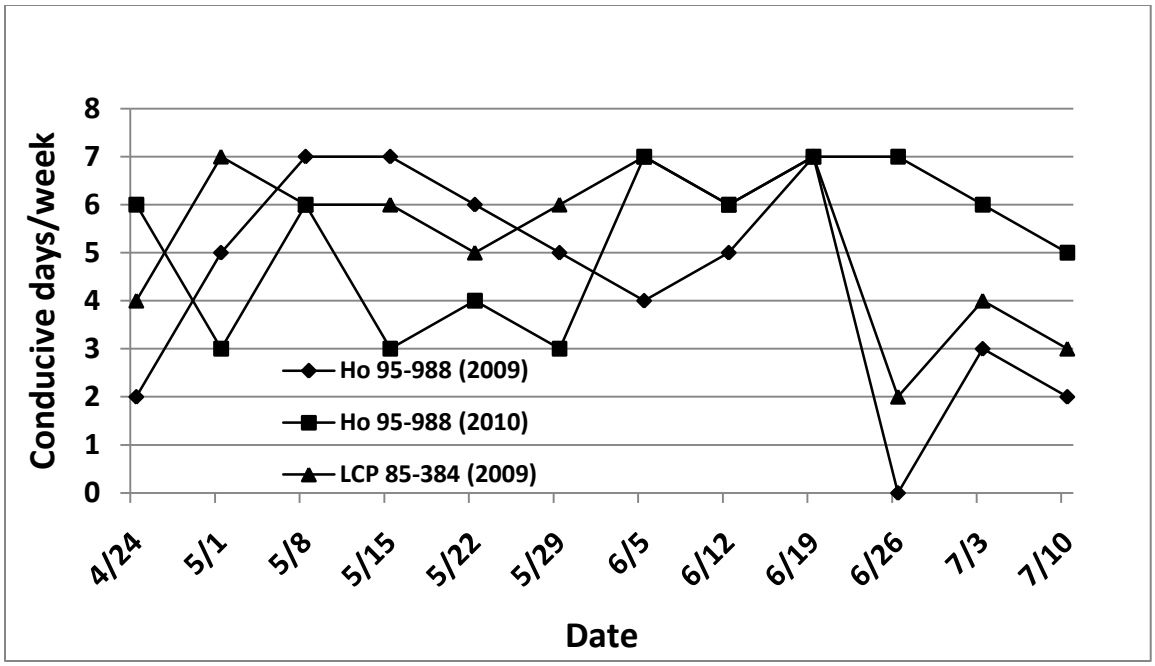

Figure 3.37. Number of days per week with 7 or more leaf wetness hours on upper leaves during the 2009 and 2010 brown rust epidemics in cultivars LCP 85-384 and Ho 95-988.

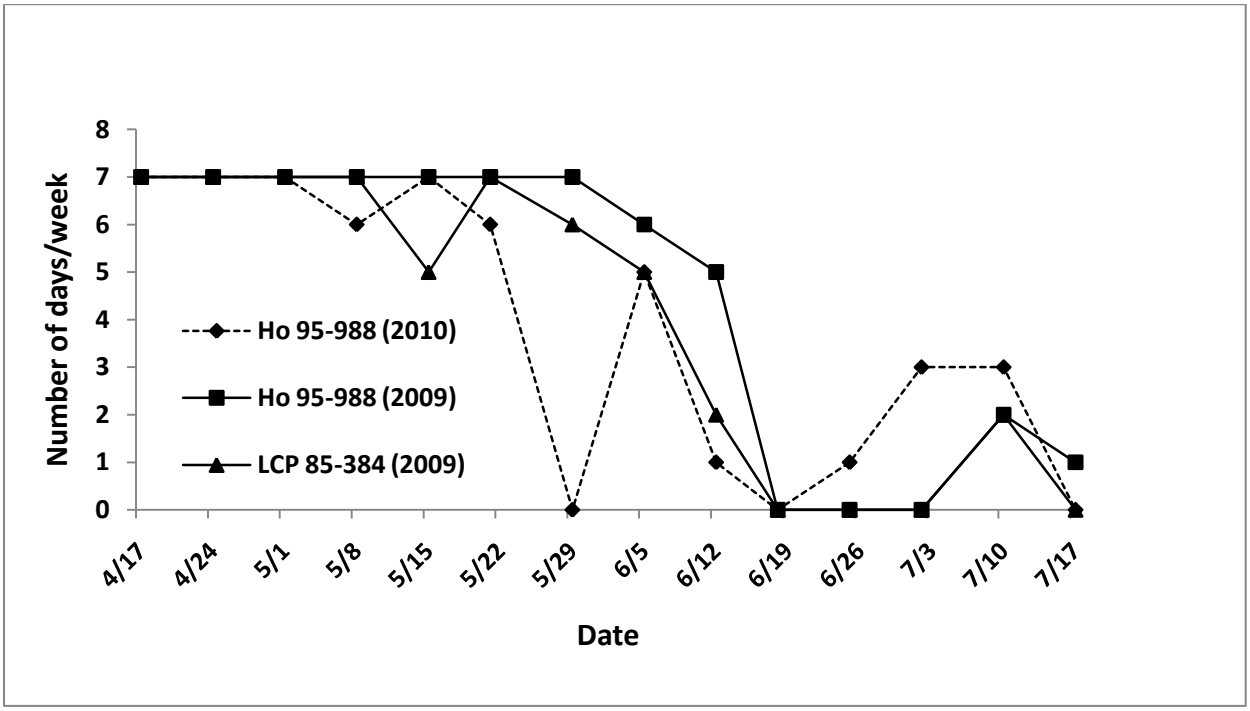

Figure 3.38. Days per week with maximum ambient temperature below 32 C during the 2009 and 2010 epidemics in cultivars LCP 85-384 and Ho 95-988.

3.6 Discussion

The severity of the 2009 brown rust epidemic in two different susceptible cultivars at different locations suggested that conditions were conducive for severe disease development. The disease progress curves were similar for the epidemics in both cultivars, and the correlation and multiple regression analyses identified similar crop development and environmental variables as the factors affecting disease increase.

The two cultivars included in the study vary in phenotypic characteristics but both are rated as highly susceptible to brown rust (LSU AgCenter, 2010). LCP 85-384 is characterized as having a smaller stalk diameter, high stalk population, and narrow upright leaves (Milligan *et al.,* 1994). Ho 95-988 differs from LCP 85-384 in having a larger stalk diameter, lower stalk population, broader leaves, and drooping leaf blade (Tew *et al.,* 2005). Despite these morphological differences, similar epidemics developed in both cultivars growing at different locations. This suggested that cultivar (genotype) and location are not major factors affecting the development of brown rust epidemics. If a commonality exists in the interaction of factors that result in severe epidemic development, this would facilitate the use of epidemiological information in the formulation of disease advisory or forecasting systems.

The same combination of factors affected disease increase in 2009 and 2010. However, disease severity was lower in the 2010 brown rust epidemic. This difference offered the opportunity to examine the impact of individual variables on disease increase and determine their relative importance and conducive or limiting values.

Epidemics of brown rust increase then decline within the first half of the sugarcane growing season. An analysis of all the factors involved can better elucidate

what determines the epidemic period and then the combination of factors during the epidemic period that determine severity. Conditions conducive for infection and disease increase must develop then the occurrence of non-conducive or limiting conditions result in the decline of the epidemic.

Plant height was highly correlated with disease increase and a component of the significant multiple regression models. This is understandable since plant height increased in parallel with the disease progress curves. However, height continued to increase as the brown rust epidemics declined. Plant growth reflects the occurrence of favorable environmental and fertility conditions. Plant height and disease increase are both affected by the same environmental variables: moisture and temperature.

Creation of a microclimate that allows the occurrence of environmental conditions favorable for infection of leaves is one possible way that crop development could be conducive to disease increase. The leaf number per shoot does not continuously increase, but increasing shoot population increases the number of leaves available for infection and could affect microclimate. However, crop canopy closure was only 10% for LCP 85-384 and 22% for Ho 95-988 at the beginning of the 2009 epidemics, and leaf temperature and wetness were generally similar during the epidemics for fully exposed, young leaves and lower, partially shaded leaves. These results suggest that crop development during the epidemic period is not a major determinant of brown rust severity.

For most fungal pathogens, successful infection depends on the occurrence of a minimum duration of wetness under favorable temperatures (Huber *et al.,* 1992). Successful brown rust infection was previously considered to require at least 8 h of

wetness (Raid and Comstock, 2000). The results of the controlled conditions experiments presented in Chapter 1 indicated that successful infection of Ho 95-988 leaves required exposure to at least 7 h of leaf wetness.

The field study results suggested that the climate in Louisiana resulted in the occurrence of dew formation sufficient to provide brown rust conducive leaf wetness conditions by mid- to late-April that then persist during the epidemic period. During the 2009 epidemics, there were 5 or more days per week with 7 or more hours of leaf wetness from the last week of April until mid-June. The occurrence of 3 wk with only 3-4 conducive days during May might have delayed the 2010 epidemic start. This observation is also supported by the similar number of leaf wetness conducive days prior to the onset of epidemics in both years. The results suggest there may be a requirement for cumulative leaf wetness conducive days before an epidemic occurs on the young leaves, and that leaf wetness is the most important determining factor for brown rust severity during the epidemic period in Louisiana.

Conditions highly favorable for dew formation frequently exist during spring in Louisiana; however, the occurrence of rain is more variable. The effect of rain on brown rust epidemics has not been conclusively determined previously. A detrimental effect has been suggested, as rain has the potential to remove spores from the leaf surface (Raid and Comstock, 2006). The 2010 epidemic with lower disease severity was characterized by higher amounts of rain during the epidemic.

Temperature can affect the availability of primary inoculum and the number of secondary infection cycles during an epidemic. Thus, it can affect the time of epidemic onset, the rate of disease increase, and the eventual decline of the epidemic. If leaf

wetness is often adequate for infection during an epidemic, the effect of temperature then becomes an important determinant for its length and severity.

Sugarcane is grown in Louisiana at the northern limit of its cultivation range at 30^{o} N latitude. Variation in the occurrence of freezing conditions during winter can affect the overwintering of *P. melancephala* by determining the extent of survival of living leaf tissue infected by the fungus. Brown rust lesions were first observed on lower leaves by April 4 following the mild 2008-2009 winter, whereas disease was not detected until April 17 and only at the southernmost location in 2010 following a severe winter. An epidemic did not develop on young leaves of both susceptible cultivars during 2010 at St. Gabriel, the northernmost location in the study. Additional comparisons of hours below freezing during winter and the onset of brown rust epidemics may allow the development of a guideline for inclusion in a disease severity advisory.

The date of first infection would be affected by the availability of primary inoculum and the development of temperatures conducive for infection as the season changes and the sugarcane growing season begins. Under controlled conditions, temperatures conducive for infection range from 17-27 C (Chapter 2). The mean for a minimum temperature of 17 C did not occur until the week ending May 1 in 2009 and May 8 in 2010. Temperature can affect when a brown rust epidemic starts and determine when it begins to decline. Temperature eventually becomes limiting for continued disease increase. However, it is uncertain how this is accomplished. Temperatures during the night when leaf wetness is present could affect the success of the infection process, but the daily minimum temperature during the epidemic period was within the range favorable for infection during the 2009 and 2010 epidemics. A comparison of the data

sets suggested that a daily ambient maximum temperature of 32 C or above was inhibitory to disease increase. The temperature at the leaf surface can be higher than ambient temperature, and maximum daily temperatures exceeded 40 C during the decline of the 2009 epidemics and during the course of the less severe 2010 epidemic. Lower maximum temperatures in early July may have allowed the continuation of the 2010 epidemic at a low level.

High temperatures can adversely affect urediniospore germination, appressorium formation, and establishment of infection (Sotomayor *et al.,* 1983). Urediniospores lose the ability to germinate after 5 days exposed to 30 C (Sandoval *et al.,* 1980) and rapidly lose viability during hot weather with temperatures above 35 C (Purdy *et al.,* 1983). The detrimental effect of high temperatures on urediniospore viability under field conditions could constitute an important determining factor for the decline of epidemics. The results for sporulation from the study epidemics suggest that high temperatures might adversely affect urediniospore development as well.

The negative effect of high temperatures on brown rust epidemics has been reported previously. Lower levels of disease were observed in epidemics in Florida when temperatures exceeded 30 C (Comstock and Raid, 2000). Similarly, lower amounts of urediniospores were collected by spore traps following maximum daily temperatures above 30 C (Irey, 1987).

Longer periods of leaf wetness can expand the range of temperatures conducive for infection (Chapter 2). Field evidence for this can be found in the 2010 epidemic. Weeks with daily leaf wetness period means of approximately 14 h occurred the week before the two epidemic peaks during July. The abundant leaf wetness may have

allowed the epidemic to continue at a low level despite the occurrence of unfavorable high temperatures.

Yield loss due to brown rust is a function of the length of the epidemic and the level of severity reached on the young leaves (Hoy and Hollier, 2009). Disease severity on young leaves of LCP 85-384 was 15% by April 15 during 2004 when the documented yield loss was higher than in other annual epidemics that began later. An understanding of factors affecting the onset and decline of epidemics and maximum disease severity reached during the epidemic might allow the development of a disease advisory that would predict onset of the epidemic season and the potential severity.

This study provided data sets for environmental variables and disease progress that allowed the more accurate delineation of conditions conducive or limiting for brown rust increase that determine seasonal epidemic severity. The results suggest that conducive conditions consisting of a minimum daily temperature of 17 C and 7 h of leaf wetness will allow initiation of the epidemic depending on the availability of primary inoculum. However, the epidemic will not begin on the young leaves until 23 to 25 days with conducive leaf wetness have occurred. Continuing leaf wetness and temperature conditions favorable for disease will allow the epidemic to persist and increase in severity on the continuously emerging leaves. The epidemic will continue until daily temperature maximums consistently exceed 32 C in late May or by mid-June, and the epidemic will then decline. Since moisture and temperature conditions during the epidemic period are generally favorable for infection, the length of the epidemic period is an important determinant of severity and potential yield loss in a susceptible cultivar.

The data obtained in this study suggest that the development of a disease advisory based primarily on ambient temperature considered as winter severity, the first occurrence of favorable conditions, and the transition to limiting conditions could predict potential for a severe epidemic and the time period when fungicide application might be needed. The predictive value of cumulative disease conducive leaf wetness days for epidemic onset needs further evaluation, but this parameter has the potential to provide the basis for a forecaster that would allow the economic use of fungicides for brown rust management.

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Appendix. Correlation analysis performed for the 2009 and 2010 epidemics

Table A1. Hourly correlation analysis for the environmental variables originating from cultivar Ho 95-988 during the 2009 epidemic.

Time	Leaf wetness on lower leaves	Leaf wetness on upper leaves	Temperature on upper leaves	Temperature on lower leaves	Ambient temperature	Relative humidity
0:00	0.33	0.14	0.15	0.25	0.16	0.27
1:00	0.34	0.2	0.13	0.21	0.14	0.32
2:00	0.42	0.25	0.14	0.23	0.13	0.34
3:00	0.45	0.23	0.15	0.2	0.16	0.33
4:00	0.38	0.17	0.17	0.2	0.16	0.3
5:00	0.37	0.17	0.21	0.18	0.19	0.29
6:00	0.29	0.17	0.25	0.23	0.21	0.31
7:00	0.26	0.02	0.32	0.26	0.31	0.29
8:00	-0.43	-0.55	0.25	0.14	0.32	-0.08
9:00	-0.24	-0.25	0.22	0.22	0.32	-0.17
10:00	-0.34	-0.3	0.31	0.38	0.35	-0.25
11:00	0.05	0.19	0.18	0.21	0.32	-0.22
12:00	-0.4	-0.4	0.24	0.02	0.34	-0.26
13:00	0.11	0.2	0.13	0.1	0.32	-0.21
14:00	0.26	0.26	0.01	-0.11	0.27	-0.07
15:00	0.26	0.22	0.34	0.32	0.28	-0.06
16:00	-0.15	0.09	0.3	0.73	0.29	0.01
17:00	-0.32	-0.07	0.26	0.25	0.3	-0.02
18:00	0.03	0.09	0.69	0.34	0.32	-0.1
19:00	0.2	0.18	0.39	0.11	0.3	-0.1
20:00	0.39	0.41	0.1	-0.01	0.23	0.01
21:00	0.22	0.21	0.09	-0.06	0.16	0.17
22:00	0.41	0.24	0.11	-0.04	0.11	0.25
23:00	0.48	0.17	0.09	-0.07	0.08	0.3

Table A2. Hourly correlation analysis for the environmental variables with disease severity in cultivar LCP 85-384 during the 2009 epidemic.

Time	Leaf wetness on lower leaves	Leaf wetness on upper leaves	Temperature on upper leaves	Temperature on lower leaves	Ambient temperature	Relative humidity
0:00	0.41	0.03	0.34	0.22	0.31	-0.01
1:00	0.43	-0.05	0.31	0.2	0.27	0.09
2:00	0.43	-0.04	0.29	0.17	0.24	0.12
3:00	0.39	0	0.27	0.14	0.22	0.15
4:00	0.35	-0.01	0.26	0.12	0.21	0.16
5:00	0.27	0.04	0.23	0.12	0.2	0.17
6:00	0.26	0.04	0.21	0.09	0.15	0.16
7:00	0.33	0.01	0.37	0.34	0.31	0.21
8:00	-0.57	-0.3	0.43	0.54	0.43	-0.27
9:00	-0.64	-0.27	0.46	0.69	0.5	-0.56
10:00	-0.45	-0.16	0.45	0.61	0.53	-0.59
11:00	-0.09	-0.16	0.48	0.54	0.5	-0.54
12:00	0.14	0.09	0.39	0.39	0.49	-0.51
13:00	-0.15	-0.13	0.48	0.45	0.5	-0.44
14:00	-0.13	-0.17	0.54	0.46	0.52	-0.42
15:00	-0.01	-0.18	0.69	0.58	0.57	-0.45
16:00	-0.33	-0.32	0.61	0.52	0.58	-0.47
17:00	-0.24	-0.21	0.69	0.71	0.61	-0.47
18:00	-0.36	-0.4	0.67	0.69	0.63	-0.56
19:00	-0.4	-0.49	0.59	0.77	0.64	-0.5
20:00	-0.46	-0.38	0.58	0.58	0.6	-0.48
21:00	-0.45	-0.22	0.47	0.44	0.49	-0.39
22:00	-0.15	-0.15	0.44	0.38	0.44	-0.18
23:00	0.21	-0.07	0.41	0.34	0.39	-0.09

Table A3. Hourly correlation analysis for the environmental variables with disease severity in cultivar Ho 95-988 during the 2010 epidemic.

Time	Leaf wetness on lower leaves	Leaf wetness on upper leaves	Temperature on upper leaves	Temperature on lower leaves	Ambient temperature	Relative humidity
0:00	0.31	0.61	0.57	0.79	0.55	0.57
1:00	0.35	0.63	0.59	0.78	0.56	0.51
2:00	0.30	0.57	0.61	0.78	0.58	0.47
3:00	0.31	0.52	0.60	0.81	0.59	0.47
4:00	0.28	0.49	0.61	0.81	0.59	0.47
5:00	0.21	0.36	0.65	0.82	0.61	0.45
6:00	0.27	0.28	0.64	0.84	0.62	0.48
7:00	0.31	0.14	0.55	0.62	0.58	0.50
8:00	0.80	0.62	0.50	0.06	0.54	0.59
9:00	0.28	-0.01	0.38	0.17	0.58	0.58
10:00	0.45	0.50	0.19	-0.02	0.58	0.51
11:00	0.49	0.20	0.05	0.29	0.58	0.51
12:00	0.33	0.13	-0.08	0.27	0.55	0.50
13:00	0.60	0.74	-0.13	0.37	0.48	0.54
14:00	0.80	0.28	-0.09	0.31	0.44	0.62
15:00	0.85	0.79	-0.22	-0.11	0.36	0.65
16:00	0.75	0.59	0.11	0.48	0.36	0.69
17:00	0.94	0.94	-0.21	-0.28	0.22	0.77
18:00	0.86	0.91	-0.05	-0.30	0.36	0.73
19:00	0.80	0.86	0.34	0.10	0.44	0.71
20:00	0.75	0.63	0.60	0.53	0.55	0.69
21:00	0.76	0.90	0.61	0.65	0.56	0.72
22:00	0.68	0.92	0.59	0.72	0.57	0.68
23:00	0.48	0.75	0.60	0.79	0.58	0.65

Vita

Wilmer Barrera was born in Rosario de Mora, El Salvador, in December, 1983. He was raised and attended primary school at this municipality. He then completed high school studies at Instituto Nacional Profesora Berta Filedia Cañas in Planes de Renderos, San Salvador. In 2001, he was given a scholarship by EARTH University in Limón, Costa Rica, to complete a bachelor's degree in agricultural sciences. After his graduation at this university he was an intern for Sakata Seed America Inc., in Fort Myers, Florida. In 2007, he moved to Cocle, Panama, to perform duties as field supervisor for Ramafrut Internacional S.L. In the fall 2008, he was admitted to Louisiana State University to earn a master's degree in the Department of Plant Pathology and Crop Physiology with the guidance of Dr. Jeffrey Hoy.