Comparison of Progress of Brown Rust and Orange Rust and Conditions Conducive for Severe Epidemic Development During the Sugarcane Crop Season in Florida

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Abstract

Brown rust (caused by *Puccinia melanocephala*) and orange rust (caused by *P. kuehnii*) are two major diseases of sugarcane in Florida. To better understand the epidemiology of these two rusts, disease severity and weather variables were monitored for two seasons in cultivars CL90-4725 (susceptible to brown rust and resistant to orange rust) and CL85-1040 (susceptible to orange rust and resistant to brown rust). Brown rust was most severe during mid-May to mid-July, whereas orange rust severity peaked during two periods: mid-May to early August and then November to December. Overall, disease severity was higher for orange rust than for brown rust. Maximum disease severity was correlated with the number of hours at night with an average temperature of

20 to 22.2°C for brown rust one season and orange rust both seasons. Slightly higher correlation was obtained when relative humidity above 90% was included in the number of hours at night with an average temperature of 20 to 22.2°C for brown rust but not orange rust, suggesting that leaf wetness is not a limiting factor for either disease in Florida. Epidemics of brown rust began at lower night temperatures (16.7 to 22.2°C) in one season, but epidemics of orange rust lasted longer under higher temperatures. The correlation of rust severity on recently emerged leaves with conducive temperatures recorded in 10-, 20-, or 30-day windows starting 7 days before disease assessment suggested that earlier inoculum production is needed to create severe epidemics that result in yield loss.

Brown rust (caused by *Puccinia melanocephala* H. & P. Sydow) and orange rust (caused by *Puccinia kuehnii* E. J. Butler) are two major diseases of sugarcane (*Saccharum* interspecific hybrids). Brown rust is present in almost all sugarcane growing areas around the world (Raid and Comstock 2000). India was the first country to report brown rust in 1950 (Patel et al. 1950), and then it spread to Southeast Asia, Africa, southern Asia, and Australia (Glynn et al. 2010). Brown rust epidemics occurred in Australia, Taiwan, the Caribbean islands, and the United States during the late 1970s (Dean and Purdy 1984). Ten to 50% yield reductions owing to brown rust have been reported in several sugarcane cultivars in Florida and Louisiana (Comstock et al. 1992; Hoy and Hollier 2009; Purdy et al. 1983; Raid and Comstock 2006).

Orange rust was considered a minor disease of sugarcane before occurrence of severe epidemics in Australia during 1999 to 2001. Yield losses reached up to 50%, causing an economic loss of 200 million Australian dollars during this period of orange rust epidemics (Magarey et al. 2001). Orange rust was first reported in the Western Hemisphere in 2007, when an outbreak of the disease occurred in the United States (Comstock et al. 2008). Within a few years, the disease

was found in sugarcane growing regions in South and Central America and the Caribbean (Barbasso et al. 2010; Chavarría et al. 2009; Comstock et al. 2008; Flores et al. 2009; Ovalle et al. 2008; Pérez-Vicente et al. 2010). In Florida, both brown and orange rust have posed a constant threat to sugarcane production since their first outbreaks in 1978 and 2007, respectively (Comstock et al. 2010). More than 90% of sugarcane cultivars grown during the 2015 to 2016 crop season in Florida were susceptible to either brown or orange rust (Raid et al. 2015).

The primary factors that influence disease progress of brown and orange rust are host genotype, environment (temperature and leaf wetness), and pathogen inoculum load (Barrera et al. 2012; Comstock and Ferreira 1986; Irej 1987; Purdy et al. 1983; Raid and Comstock 2000; Sandoval et al. 1983; Victoria et al. 1984). Maximum daily temperature was the variable most correlated with seasonal epidemic development and decline of sugarcane brown rust in Louisiana (Barrera et al. 2013). Successful infection of sugarcane by *P. melanocephala* occurred with a 7-h leaf wetness period at 17°C but not with a 4-h period (Barrera et al. 2012). Plant age is also considered a limiting factor for brown rust progress, because severe symptoms of this disease have mostly been observed on 4- to 7-month-old sugarcane: the highest brown rust severity was observed on 4-month-old, 4- to 6-month-old, and 5- to 7-month-old plants in Colombia, Hawaii, and Cuba, respectively (Comstock and Ferreira 1986; Sandoval et al. 1983; Victoria et al. 1990). This effect of plant age, however, has not been reported for orange rust, for which symptoms are observed in Florida from early season until harvest (Rott et al. 2017).

Germination of brown rust urediniospores is favored by temperatures between 15 and 30°C. Temperatures above 30°C greatly reduce germination capacity of these spores (Purdy et al. 1983; Sotomayor et al. 1983). At least 8 h of leaf wetness has been described as necessary for brown rust development in Florida (Raid and Comstock 2000). In a growth chamber study in Australia, 14 h of leaf wetness resulted in the highest levels of brown rust infection (Ryan and Egan 1989). In Louisiana, minimum daily temperature was the limiting factor for the onset of brown rust epidemics, and maximum daily temperatures were associated with the decline of disease severity:

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field epidemics started after approximately 23 to 25 days with at least 7 h of leaf wetness per day and when daily minimum temperatures exceeded 17°C, whereas epidemics declined when maximum daily temperatures exceeded 32°C (Barrera et al. 2013).

At least 97% relative humidity (RH) and temperatures between 17 and 24°C were found to be the most conducive conditions for orange rust in Australia (Magarey et al. 2004). In Florida, temperatures between 20 and 25°C, and more than 8 h of leaf wetness were necessary for development of sugarcane orange rust in a growth chamber experiment (Martins et al. 2010).

The recent rise of orange rust as a new disease has not only changed the status of sugarcane cultivars grown in Florida but has also demanded an efficient management strategy against sugarcane rusts (Rott et al. 2017). General trends of progress of brown and orange rust during the crop seasons have been reported in Florida (Raid and Comstock 2006; Rott et al. 2017). However, comparison of variation in disease progress based on numerical data is not available in the literature. Additionally, direct comparisons of progress of both diseases to investigate environmental conditions associated with disease progress at the same location have not been conducted so far. The objectives of this study were therefore to (i) regularly monitor, quantify, and compare severity of brown and orange rust during two consecutive crop seasons of sugarcane; (ii) identify the periods with highest disease levels; (iii) determine temperatures favorable for in vitro germination of spores of *P. kuehnii* and *P. melanocephala*; and (iv) determine the environmental conditions conducive for both sugarcane rust diseases in Florida.

Materials and Methods

Sugarcane cultivars monitored in the field. Two sugarcane cultivars, CL90-4725 (highly susceptible to brown rust but resistant to orange rust) and CL85-1040 (highly susceptible to orange rust but resistant to brown rust), were selected for the study. In 2013, five commercial sugarcane cultivars were planted at three different dates (October 11, November 12, and December 11) in four blocks using a randomized complete block design at the Everglades Research and Education Center (EREC), Belle Glade, FL. Each plot of this experiment consisted of four rows of a single cultivar, and each row was 10.7 m long with 1.5-m interrow spacing and 3-m alleys between plots. The four rows were bordered with a single row of CL90-4725 on the east side and a single row of CL85-1040 on the west side of the plot. The five border rows (total of 53.5 m) per block of each of these two cultivars planted on November 12, 2013, were used to investigate rust progress in 2014 (2013 to 2014 crop season). On November 14, 2014, sugarcane was planted in two separate commercial fields at EREC: one field consisted of 27 sugarcane rows (each 274 m long) of cultivar CL90-4725, and the second field had 39 rows (each 152 m long) of cultivar CL85-1040.

Fig. 1. Simulated percentages of sugarcane leaf surface affected by rust. ©ISSCT; reproduced, by permission, from Purdy and Dean (1981).

Disease scoring of sugarcane plants. The two cane crops were exposed in the field to natural rust infections. With few exceptions, rust severity data were collected every 2 weeks: from January to December in 2014 and 2015, following planting of sugarcane in November 2013 and 2014, respectively. From April 17 to July 7, 2014, rust severity data were collected only every month. Rust symptoms were assessed by visual observation of the top visible dewlap (TVD) leaf and leaf +5 (fourth leaf below the TVD leaf) of each scored sugarcane stalk. The TVD leaf is the most recent fully emerged leaf of a sugarcane stalk. Because TVD leaves open every 7 to 10 days (Van Dillewijn 1952), rust symptoms that appear on TVD leaves represent the most recently developed infections at a given date of observation. Leaves +5 are older leaves, which have been exposed to rust for at least 3 to 4 weeks. Therefore, they reflect an accumulation of disease pressure that the sugarcane plant endured during its growth. Because of its size and growth, it is difficult to assess rust on the entire sugarcane plant. The two leaves selected for scoring in this experiment can therefore provide trends of rise and fall of overall rust severity on a sugarcane plant. Sugarcane has indeterminate growth, and new leaves continuously develop at the stalk apex. Additionally, because plants were randomly chosen at each scoring date, scored leaves differed from one scoring date to the next.

To determine rust severity on the TVD leaf and leaf +5, percentage of the leaf area covered by rust was assessed visually using a scale of simulated diseased leaf surface developed by Purdy and Dean (1981) (Fig. 1). As suggested by the scoring guideline, only the upper third area was considered for disease severity assessment of the TVD leaf, because lesions tend to accumulate on the upper third of this leaf. The entire leaf surface of leaf +5 was used to determine disease severity on the lower leaf of the plant. TVD leaves were scored for rust starting in January but scoring of leaf +5 began in March, because 3 to 4 months of growth was needed for sugarcane to exhibit at least five leaves.

Eight stalks from a 53.5 m total row length in each of the four blocks of the randomized field trial in 2014 and eight stalks from each of four different locations separated by at least 50 m in the commercial fields in 2015 were scored for rust. Rust severity data of the eight stalks per variety were averaged at each scoring date for each block. Each of the four locations of a commercial field was treated as a block. Areas under the disease progress curves (AUDPCs) were calculated for the percent of rust-affected area using mean rust severity of four blocks at each scoring date. AUDPC values were compared among cultivars for each crop season using the linear mixed effect procedure as implemented in nlme package (statistical software R) (Pinheiro et al. 2018; R Core Team 2016). Treatment means were separated by Tukey's method at $P = 0.05$ using the lsmeans package in statistical software R (Lenth and Love 2018; R Core Team 2016).

In vitro germination assay of brown and orange rust spores.

The effect of temperature on germination capacity of urediniospores of both rusts was determined in vitro using a completely randomized design. Leaves of sugarcane cultivars CL90-4725 and CL85-1040 exhibiting uredinia were collected in the field at Belle Glade. Urediniospores of *P. melanocephala* and *P. kuehnii* were collected in the laboratory by vacuuming the lower side of collected leaves with a glass spore collector attached to a vacuum pump. One half milligram of urediniospores was deposited on a glass slide covered with a 1-mm-thick layer of 1% water agar. One 50- μ l drop of deionized water supplemented with 0.002% nonanol (Sigma-Aldrich, St. Louis, MO) and 0.1% Tween 20 (Sigma-Aldrich) was added on top of the agar, and spores were spread evenly with a glass rod. Slides were then placed inside an empty pipette tip box containing water at the bottom to ensure a high humidity level necessary for spore germination and to avoid desiccation of agar. Slides were kept overnight in the dark at temperatures ranging from 20 to 33°C: 20, 25, 28, 29, 30, 31, 32, and 33°C. Percentage of germination was determined 18 to 20 h later by counting four sets of 100 spores observed on two slides (two sets per slide) using a microscope (100 $\times$ magnification). Urediniospores producing germ tubes longer than the urediniospore's size were considered germinated spores. In a first germination experiment, spore samples of *P. melanocephala* and *P. kuehnii* were tested together at each of the eight temperatures

mentioned above. In a second experiment, spore samples of both pathogens were simultaneously tested at 20, 29, 30, and 31°C. These four temperatures were selected because they were the most relevant for differentiation of both rust pathogens based on results obtained in the first germination experiment. For both experiments, each germination assay was performed three times using freshly collected spores. Mean germination and standard deviation at each temperature were calculated with the mean percent value of the four sets of 100 spores each. Data of in vitro germination assays with the four temperatures (experiment 2) were analyzed using a generalized linear model with binomial errors and logit link in which temperature was treated as a factor with four levels. The emmeans package in the statistical program R was used to fit the generalized linear model (Lenth et al. 2018; R Core Team 2016). Percent mean germination at the different temperatures was compared by Tukey's method at $P = 0.05$ using the multcomp package in statistical software R (Hothorn et al. 2017; R Core Team 2016).

Weather variables and disease progress. Experimental plots and commercial fields investigated in this study were located within a 300-m radius of the Belle Glade weather station of the Florida Automated Weather Network (FAWN 2016). Hourly and daily weather data of temperature (°F), RH (%), and precipitation (mm) for 2014 and 2015 were retrieved from this weather station. Based on these data, weather variables such as hourly and daily minimums, maximums, averages, sums, and the number of hours of specific conditions were calculated using R statistical software. An empirical approach based on $RH \geq 90\%$ measured at 2 m height was used to estimate leaf wetness, as described in other studies (Kim et al. 2002; Rowlandson et al. 2015). This leaf wetness duration model has been verified in Florida for operational use in strawberry disease-warning systems (Montone et al. 2016). A total of 46 weather variables were created for different time periods prior to each TVD rust assessment day: 7 to 17, 7 to 27, and 7 to 37 days prior to each disease assessment (Supplementary Tables S1 and S2). Because one of the outcomes of these investigations is to develop disease forecasting systems for better chemical control of sugarcane rusts, time periods were started 7 days before disease assessments to give a week notice to farmers for fungicide applications. Sugarcane rusts have a latent period of 9 to 14 days between leaf infection and sporulation (Irey 1987; Martins et al. 2010). The three time periods were considered to account for one to multiple rust cycles during rust epidemics. Pearson correlation coefficients were calculated between rust severity of the TVD leaf and weather-based variables for 2014 and 2015 using SAS JMP version 13.2 (2017; SAS Institute, Cary, NC). These correlation coefficients were then used to analyze the relationship between weather variables and progress of brown and orange rust of sugarcane. All temperature-related variables were transformed into Celsius data after correlation analyses.

Results

Progress of sugarcane brown rust in cultivar CL90-4725. In 2014, severity of brown rust on the TVD leaf of CL90-4725 was below 5% until mid-March (Fig. 2A). Starting mid-March, disease severity on the TVD leaf increased and reached a maximum value of 17% from mid-May to mid-June. After mid-June, rust severity declined and was again below 5% at the end of July. It remained below this value until the end of the year. In 2015, although the pattern of progress of brown rust severity was similar to the one observed in 2014, it was delayed by 1.5 months: Percent rust severity on the TVD leaf was above 5% from May to the end of July. Brown rust severity reached a maximum value of 13% during May to mid-July. As in 2014, brown rust severity in 2015 was below 5% in August and remained below this level until the end of the year.

Brown rust severity was above 5% on leaf +5 at the first scoring date at the beginning of March 2014 (note: this leaf was not scored earlier because the sugarcane plant did not have five fully unfolded leaves before that date) (Fig. 2B). From March to the beginning of May 2014, the rust-affected area on this leaf progressively increased and reached a maximum value of 22% in May. Rust severity then progressively declined but remained above 10% until the end of July

2014. As with the TVD leaf, progress of rust severity on leaf +5 in 2015 was delayed by 1.5 months when compared with progress in 2014: values above 5% were only observed starting at the beginning of April 2015, and the highest disease severity (24%) was observed mid-July 2015. However, during both years, the rust-affected area rapidly declined on leaf +5 starting mid-July and remained below 5% until the end of year. No active sporulation of brown rust was observed after August 2014 and 2015 in cultivar CL90-4725, regardless of the leaf observed.

Progress of sugarcane orange rust in cultivar CL85-1040. In 2014, orange rust severity on the TVD leaf was low (<5%) until May (Fig. 3A). The rust-affected area on this leaf then rapidly increased and reached maximum values of 24% from June to mid-July. In July 2014, rust severity declined rapidly and was below 5% at the end of August. Orange rust severity started to increase again at the end of September and was above 10% at the beginning of December 2014. In 2015, the progress pattern of orange rust severity on the TVD leaf was similar to the one observed in 2014. However, disease severity at the end of the year was higher in 2015 than in 2014 (24 versus 11%).

In 2014, orange rust severity on leaf +5 was above 5% at the first scoring date at the beginning of March (note: this leaf was not scored earlier because the sugarcane plant did not have five fully unfolded leaves before that date) (Fig. 3B). From March to early May, the rust-affected area of this leaf progressively increased and reached 15% in May. Orange rust severity then rapidly increased and reached values above 20% from the end of May to mid-August, with peak

Fig. 2. Progress of sugarcane brown rust in plant cane crop of cultivar CL90-4725 in 2014 and 2015. **A**, Progress of percent rust-affected area on the upper third of the top visible dewlap (TVD) leaf (see also Fig. 1). **B**, Progress of percent rust-affected area on the fourth leaf below the TVD leaf (leaf +5). Each point at each scoring date represents the average value of four sets of eight stalks (32 leaves). The mean coefficients of variation of percent rust-affected area on the TVD leaf and leaf +5 were 25.8 and 16.4% in 2014, respectively, and 24.2 and 16.6% in 2015, respectively.

values above 40% in June and July 2014. Disease severity on leaf +5 rapidly decreased from mid-July to the beginning of September, when only 5% of the leaf area showed orange rust symptoms. In 2015, the progress pattern of orange rust severity on leaf +5 was similar to the one observed in 2014 with a few exceptions. Rust-affected area on this leaf reached 5% only in April 2015, and rust severity was always higher than 10% from September to December 2015.

Comparison of disease progress between brown rust in CL90-4725 and orange rust in CL85-1040. In 2014, based on observations of the TVD leaf, the brown rust epidemic started 1.5 months earlier than the orange rust epidemic (Fig. 4A). Brown rust severity continuously increased from March through June, whereas orange rust severity started to increase only at the end of April. Peak disease severity of brown rust was reached in May and the beginning of June, whereas peak disease severity of orange rust was reached in June. Both rusts had about a 1-month peak period, but once peak severity values were reached for orange rust, they remained higher than those of brown rust until the end of the crop season. In 2015, the gradual progress of severity of both brown and orange rust occurred during the same months (April to June). Starting at the end of May 2015, severity values were always higher for orange rust than for brown rust. In both 2014 and 2015, there was one primary period of severe disease expression of brown rust from April to the end of July (Fig. 4A). In contrast, two periods of active disease expression were observed for orange rust: the first one from May to August and the second one toward the end of the year (Fig. 4A). During the rust peak periods in 2014 and 2015, disease severity on leaf +5 was up to 100% higher

for orange rust than for brown rust (Fig. 4B). The highest average percent of rust-affected leaf area was 47% for orange rust and 23% for brown rust.

AUDPCs were determined using the percentage of rust severity on the TVD leaf and leaf +5 for 2014 and 2015 (Table 1). AUDPC of the TVD leaf was significantly higher for orange rust than for brown rust in 2015 but not in 2014. However, during both years, AUDPC of orange rust of leaf +5 was significantly higher than AUDPC of brown rust of the leaf +5, indicating more severe symptoms or a longer duration of the orange rust epidemics (Table 1).

In vitro germination of urediniospores of *P. melanocephala* and *P. kuehni* at different temperatures. Urediniospores of *P. melanocephala* germinated in vitro at temperatures from 20 to 32°C and stopped germinating at 33°C, whereas urediniospores of *P. kuehni* germinated from 20 to 30°C. More than 50% of urediniospores of both fungal species germinated between 20 and 29°C. Germination of spores of *P. kuehni* dropped from 65% at 29°C to 18% at 30°C and was zero at 31°C (Table 2). At 31°C, 40% of spores of *P. melanocephala* still germinated.

Relationship between weather conditions and rust severity. Analysis of 46 weather variables revealed that brown rust severity in 2014 was best correlated to the number of hours averaging 20 to 22.2°C at night (8:00 p.m. to 8:00 a.m.) (Table 3). The highest correlation coefficient (0.60) was obtained for the period 7 to 37 days before disease assessment. The correlation coefficient between disease severity and the same temperature range at night while

Fig. 3. Progress of sugarcane orange rust in plant cane crop of cultivar CL85-1040 in 2014 and 2015. **A**, Progress of percent rust-affected area on the upper third of the top visible dewlap (TVD) leaf (see also Fig. 1). **B**, Progress of percent rust-affected area on the fourth leaf below the TVD leaf (leaf +5). Each point at each scoring date represents the average value of four sets of eight stalks (32 leaves). The mean coefficients of variation of percent rust-affected area on the TVD leaf and leaf +5 were 34.2 and 20.4% in 2014, respectively, and 19.7 and 16.5% in 2015, respectively.

Fig. 4. Comparison of progress of brown and orange rust in plant cane crop of sugarcane cultivars CL90-4725 and CL85-1040, respectively. **A**, Progress of percent rust-affected area on the upper third of the top visible dewlap (TVD) leaf (see also Fig. 1). **B**, Progress of percent rust-affected area on the fourth leaf below the TVD leaf (leaf +5). Each point at each scoring date represents the average value of four sets of eight stalks (32 leaves). The mean coefficients of variation of percent rust-affected area are given in captions to Figures 2 and 3.

RH $\geq$ 90% was slightly lower: 0.52 versus 0.60 (Table 3). In 2014, a moderate correlation (0.48) was also found between brown rust severity and the number of hours averaging 16.7 to 22.2°C at night (8:00 p.m. to 8:00 a.m.).

Analysis of the 46 weather variables showed that orange rust severity in 2014 and in 2015 was best correlated to the number of hours averaging 20 to 22.2°C at night (8:00 p.m. to 8:00 a.m.) while RH was higher than 90% (Table 3). The highest correlation coefficients (0.72 in 2014 and 0.60 in 2015) were obtained for the period 7 to 37 days before disease assessment. Similar high values of the correlation coefficient (0.70 in 2014 and 0.57 in 2015) were obtained when this variable was considered alone (i.e., without the 90% RH criterion) (Table 3). In contrast to brown rust, no correlation was found between orange rust severity and the number of hours averaging 16.7 to 22.2°C at night (8:00 p.m. to 8:00 a.m.), regardless of the year. However, a significant negative correlation ranging from 0.42 to 0.53 was observed for both brown and orange rust between rust severity and the number of hours per day with maximum temperature $>32.2^{\circ}\text{C}$ (Table 3).

Discussion

This study reports the first direct comparison of disease progress of brown rust and orange rust of sugarcane in the same location during two entire crop seasons. In order to separate the disease development of *P. melanocephala* and *P. kuehnii*, two cultivars with opposite resistance levels to each disease were used: cultivar CL90-4725 is highly susceptible to brown rust but resistant to orange rust, and CL85-1040 is highly susceptible to orange rust but resistant to brown rust.

Each sugarcane rust showed different progress patterns of disease severity. The brown rust epidemic varied between seasons owing to differences in the winter and spring temperatures affecting the crop and the disease. With warmer conditions, the brown rust epidemic began in late March 2014, and this resulted in an earlier peak period than in 2015. The epidemic then ceased during summer. In contrast, the orange rust epidemic began in early May in both seasons regardless of the weather in the preceding months. The orange rust epidemic then lasted longer into the summer than brown rust (in both years), and a second peak occurred late in the season. A build-up period appeared necessary for both rusts, and this period was more evident for brown rust than orange rust. A delayed start of the brown rust epidemic in 2015 in contrast to orange rust might reflect a longer inoculum build-up period for brown rust associated with a weaker sporulating capability of *P. melanocephala*.

In both sugarcane cultivars, the lower leaf (leaf +5) had higher disease severity than the TVD leaf, but the pattern of increase and decrease of disease severity on the TVD leaf and leaf +5 was similar. This difference is likely owing to longer exposure of the older leaf (leaf +5) to infection and therefore occurrence of more rust cycles.

Temperature and leaf wetness have been shown to be key factors for development of sugarcane rusts (Barrera et al. 2012, 2013;

Magarey et al. 2004; Raid and Comstock 2000). However, in south Florida, daily leaf wetness duration appears to be sufficient all year long for rust development (C. Fraisse, unpublished data) and should therefore not be a limiting factor for rust development. In our study, similar high correlation values were obtained between disease severity of brown rust or orange rust and nighttime temperatures (8:00 p.m. to 8:00 a.m.) with or without RH $\geq$ 90%. This suggests that, although RH resulting in leaf wetness plays a crucial role in disease development, it is not a limiting factor in Florida.

Based on growth chamber studies, Martins et al. (2010) reported that daily temperatures between 20 and 25°C were the most favorable temperatures for orange rust development. In our study, the most conducive environmental conditions for orange rust development were extended periods with nighttime (8:00 p.m. to 8:00 a.m.) temperatures between 20 and 22.2°C. This environmental condition was also found favorable for brown rust development in 2014 but not in 2015. Additionally, the best correlation coefficient values between rust severity and the number of hours at night with 20 to 22.2°C average temperatures were lower for brown rust than for orange rust (0.60 versus 0.72). A larger temperature range (16.7 to 22.2°C) also resulted in significant correlation coefficients with disease severity for brown rust in 2014 but not for orange rust. This could explain why brown rust can appear earlier than orange rust during the crop season.

These correlations between disease severity and nighttime temperatures were not observed for brown rust in 2015, when the onset of severe symptoms of this disease was delayed until mid-May, which was almost 1.5 months later than in 2014. Comparison of the weather data for the two years revealed that the month of February was cooler in 2015 than in 2014 (16.6 versus 20.0°C average daily temperature). These cooler temperatures most likely resulted in a suppression of infection and disease development. Furthermore, a frost event resulting in leaf damage was reported during the first week of March 2015 in the Everglades Agricultural Area (data not shown). Cooler winter and reduced healthy leaf canopy owing to frost may have reduced the rust inoculum levels and thus delayed the onset of brown rust symptoms in 2015. Additionally, these weather conditions resulted in retarded plant growth when compared with 2014. This could also have increased the delay for plants to develop canopy conditions suitable for rust. A similar case of 2.5-month delay of brown rust onset was reported in Florida in 1985 owing to freeze-associated damage (Irey 1987). Delayed starts of brown rust epidemics owing to winter freezes were previously also reported in Louisiana (Barrera et al. 2013). This shift of active periods of brown rust epidemics most likely affected the correlation coefficient between the temperature variable and disease severity.

Germination of rust spores is the initial step of rust infection (Miles et al. 2003). In our study, temperatures between 20 and 29°C all resulted in similar and high percentages of in vitro spore germination. However, according to Magarey et al. (2004), temperatures around 20°C (17 to 23°C) were the most favorable for spore germination

Table 1. Comparison of area under the disease progress curve (AUDPC) values of percent rust-affected area of the top visible dewlap (TVD) leaf and the fourth leaf below TVD leaf (leaf +5) of sugarcane cultivars CL90-4725 and CL85-1040 affected by rust^z

Year	Rust disease	Leaf	AUDPC value	Mean CV
2014	Brown rust	TVD	1,813 c	25.8
		Leaf +5	2,842 b	16.4
2014	Orange rust	TVD	2,464 bc	34.2
		Leaf +5	6,010 a	20.4
2015	Brown rust	TVD	1,460 c	24.2
		Leaf +5	2,618 b	16.6
2015	Orange rust	TVD	2,769 b	19.7
		Leaf +5	7,455 a	16.5

^z Brown rust severity was observed on sugarcane cultivar CL90-4725. Orange rust severity was observed on sugarcane cultivar CL85-1040. For each year, AUDPC values followed by the same letter are not significantly different at $P = 0.05$ according to Tukey's comparison test. CV = coefficient of variation.

Table 2. Percent germination of urediniospores of *Puccinia melanocephala* and *P. kuehnii* at four different temperatures

Temperature (°C)	Mean germination percent of urediniospores ^z	
	Brown rust	Orange rust
20	58 $\pm$ 2 b	64 $\pm$ 2 a
29	56 $\pm$ 2 b	65 $\pm$ 2 a
30	51 $\pm$ 1 c	18 $\pm$ 2 e
31	40 $\pm$ 2 d	0 f

^z Brown rust urediniospores were collected from sugarcane cultivar CL90-4725. Orange rust urediniospores were collected from sugarcane cultivar CL85-1040. Each value represents average data (mean $\pm$ standard deviation) of three independent experiments. Subsamples of each spore sample were exposed at the same time to all four temperatures. Values in both columns followed by the same letter are not significantly different at $P = 0.05$ according to Tukey's comparison test.

of the orange rust pathogen. Different strains of the pathogen or different experimental spore germination conditions such as the use of agar media for spore germination in our experiments versus the use of only free water in Magarey's experiments may explain these differences.

In our study, brown rust spores were able to germinate in vitro at higher temperatures (up to 32°C) than those of orange rust (absence of germination at 31°C). Additionally, according to Sotomayor et al. (1983), the optimum temperature range for germination of urediniospores and formation of appressoria by *P. melanocephala* in Florida was between 15 and 30°C. In India and Taiwan, optimal germination of the brown rust pathogen occurred between 21 and 26°C (Hsieh et al. 1977; Sahni and Chona 1965). If we assume that high summer temperatures are mainly responsible for rust decline, then brown rust decline should occur later (and not earlier) than orange rust. Therefore, in vitro spore germination data are not sufficient to explain the difference between brown rust and orange rust decline during summer months in Florida. Additionally, brown rust and orange rust progress was negatively correlated with the number of hours per day with maximum temperatures higher than 32.2°C, suggesting that high summer temperatures negatively affected the development of the two diseases. Similar observations were made for brown rust in Louisiana, where severe field epidemics declined once maximum ambient daily temperature was 32°C or higher (Barrera et al. 2013). In the Dominican Republic, lower levels of sugarcane brown rust were observed when temperatures exceeded 35°C (Liu and Bernard 1979). High temperatures most likely also affect other life-cycle components besides the spore germination. Other life-cycle components that could be influenced by high temperatures include colonization of the pathogen in planta, lesion/uredinia development, and sporulation. Optimal temperatures for infection and optimal temperatures for growth are different for wheat rust pathogens such as *P. tritici*, *P. striiformis*, and *P. graminis* (Kolmer et al. 2009). Optimal infection temperature for *P. graminis* f. sp. *tritici* (causal agent of wheat stem rust) is 15 to 24°C, whereas optimal growth temperature is 25 to 35°C. This issue remains to be investigated for sugarcane rusts.

Based on the in vitro germination results, we would also expect brown rust to increase again at the end of the year, when temperatures are once again favorable for disease development. However, this is not what was observed in the field. In tropical areas with extended periods of favorable conditions, brown rust appears to be a disease of young sugarcane (4 to 7 months) (Comstock and Ferreira 1986; Sandoval et al. 1983; Victoria et al. 1990), which would explain why brown rust is absent in the fall and winter when temperatures are lower than in summer and similar to those in spring. Similar age-related disease resistance was reported in other host-pathogen systems, such as *P. tritici* in wheat (Pretorius et al. 1988), *Peronospora tabacina* in tobacco (Reuveni et al. 1986), and *Pseudomonas syringae* in *Arabidopsis* (Kus et al. 2002). Changes of susceptibility of a host to a pathogen as it ages can be caused by changes in the

physiology of the plant or by expression of specific resistance genes that are plant stage dependent. Expression of adult resistance genes is common in wheat (Börner et al. 2000; Herrera-Foessel et al. 2014; Singh and Gupta 1992), and a similar situation may occur in sugarcane for brown rust when the stalks mature and accumulate sugar.

In contrast to brown rust epidemics, orange rust epidemics in Florida appear to be mainly driven by temperature, because the disease can be observed at two different periods of the year on young (5 to 9 months old) and mature (10 to 12 months old) sugarcane. In Australia, temperatures favoring in vitro germination of *P. kuehni* spores were consistent with field conditions of disease development, suggesting that temperature is also a key factor in disease progress (Magarey et al. 2004). Therefore, plant age does not appear to be a limiting factor for orange rust progress. Additional experiments such as planting sugarcane in the field at different times of the year need to be undertaken to investigate importance of age/physiological effect of sugarcane in brown and orange rust development.

Correlation between disease severity of sugarcane rusts and nighttime temperatures when leaf wetness occurs was observed at different time periods preceding disease assessment on recently emerged leaves. The highest correlation coefficients were always obtained with the longest time interval (7 to 37 days before disease assessment), thus suggesting that earlier build-up of inoculum is needed to create severe epidemics that will result in yield loss. These weather conditions in a period of time before the emergence of the TVD leaf are therefore important in allowing the creation of a severe epidemic on the young sugarcane leaves. Similar observations of inoculum build-up have been observed and taken into consideration for plant disease forecasting, such as for late blight of potato and cereal airborne diseases (De Vallavieille-Pope et al. 2000; Harrison 1992).

In commercial sugarcane production, more foliar fungicide applications are performed to control orange rust (three to four treatments) than brown rust (one to two treatments) in Florida (Rott et al. 2016). In our study, epidemics of brown and orange rust had similar durations during the spring and summer months. However, disease severity was much higher (up to 100%) for orange rust compared with brown rust during the peak period of the two diseases in 2014 and 2015. Because the same chemicals are used to control both rusts, this may explain why more treatments are necessary to control orange rust than brown rust. Alternatively, as discussed above, it may also be possible that because *P. kuehni* has a higher sporulating ability than *P. melanocephala*, higher concentrations of chemicals or additional treatments are needed to reduce inoculum levels during epidemics.

Identification of a favorable temperature range at night when leaf wetness is not a limiting factor for rust epidemics is a first step in developing forecasting systems for both brown and orange rust. A more comprehensive modeling study to investigate the influence of several factors on orange and brown rust is underway. Such systems might include weather variables and the physiological stage of the sugarcane plant, as well as disease severity data from other sugarcane

Table 3. Weather conditions conducive for severe epidemics of sugarcane brown rust and orange rust as determined by Pearson's correlation coefficient between weather variables and rust severity on the top visible dewlap leaf^z

Weather variable	Brown rust						Orange rust					
	7 to 17 DBA		7 to 27 DBA		7 to 37 DBA		7 to 17 DBA		7 to 27 DBA		7 to 37 DBA	
	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015	2014	2015
Nighttime hours with an AT of 20 to 22.2°C when RH was ≥90%	0.45*	0.21	0.50*	0.27	0.52*	0.41	0.43	0.38	0.60**	0.46*	0.72**	0.60**
Nighttime hours with an AT of 16.7 to 22.2°C when RH was ≥90%	0.27	-0.06	0.39	0.02	0.45*	0.11	-0.01	0.00	0.18	0.007	0.26	0.15
Nighttime hours with an AT of 20 to 22.2°C	0.48*	0.17	0.58**	0.24	0.60**	0.37	0.40	0.36	0.58**	0.44*	0.70**	0.57*
Nighttime hours with an AT of 16.7 to 22.2°C	0.26	-0.08	0.43*	0.01	0.48*	0.10	-0.06	-0.01	0.15	0.06	0.23	0.13
Number of hours per day with maximum temperature >32.2°C	-0.21	-0.29	-0.44*	-0.53*	-0.38	-0.48*	-0.28	-0.11	-0.45*	-0.42*	-0.47*	-0.42*

^z DBA = days before disease assessment; nighttime hours = number of hours at night (8 p.m. to 8 a.m.); AT = average temperature; and RH = relative humidity.

* indicates *P* values < 0.05; ** indicates *P* values < 0.01.

cultivars susceptible to rust. This would increase the accuracy of prediction of sugarcane rust epidemics and subsequently improve the management of these diseases in Florida.

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Literature Cited

- Barbasso, D., Jordão, H., Maccheroni, W., Boldini, J., Bressiani, J., and Sanguino, A. 2010. First report of *Puccinia kuehnii*, causal agent of orange rust of sugarcane, in Brazil. *Plant Dis.* 94:1170.
- Barrera, A., Hoy, J., and Bin, L. 2012. Temperature and leaf wetness effects on infection of sugarcane by *Puccinia melanocephala*. *J. Phytopathol.* 160: 294-298.
- Barrera, W., Hoy, J., and Bin, L. 2013. Effects of temperature and moisture variables on brown rust epidemics in sugarcane. *J. Phytopathol.* 161:98-106.
- Börner, A., Röder, M. S., Unger, O., and Meinel, A. 2000. The detection and molecular mapping of a major gene for non-specific adult-plant disease resistance against stripe rust (*Puccinia striiformis*) in wheat. *Theor. Appl. Genet.* 100:1095-1099.
- Chavarría, E., Subirós, F., Vega, J., Ralda, G., Glynn, N. C., Comstock, J. C., and Castlebury, L. A. 2009. First report of orange rust of sugarcane caused by *Puccinia kuehnii* in Costa Rica and Nicaragua. *Plant Dis.* 93:425.
- Comstock, J. C., and Ferreira, S. A. 1986. Sugarcane rust: Factors affecting infection and symptom development. *Proc. Int. Soc. Sugar Cane Technol.* 19:402-410.
- Comstock, J. C., Glynn, N. C., and Davidson, R. W. 2010. Sugarcane rusts in Florida. *Proc. Int. Soc. Sugar Cane Technol.* 27:1-9.
- Comstock, J. C., Shine, J. M., and Raid, R. N. 1992. Effect of sugarcane rust on growth and biomass. *Plant Dis.* 76:175-177.
- Comstock, J. C., Sood, S. G., Glynn, N. C., Shine, J. M., McKemy, J. M., and Castlebury, L. A. 2008. First report of *Puccinia kuehnii*, causal agent of orange rust of sugarcane, in the United States and Western hemisphere. *Plant Dis.* 92:175.
- Dean, J. L., and Purdy, L. H. 1984. Races of sugarcane rust fungus, *Puccinia melanocephala*, found in Florida. *Sugar Cane* 1984:15-16.
- De Vallavieille-Pope, C., Giosue, S., Munk, L., Newton, A., Niks, R., Østergård, H., Pons-Kühnemann, J., Rossi, V., and Sache, I. 2000. Assessment of epidemiological parameters and their use in epidemiological and forecasting models of cereal airborne diseases. *Agronomie* 20:715-727.
- Flores, R. C., Loyo, J. R., Ojeda, R. A., Rangel, O. C. A., Cerón, F. A., Márquez, W., Guerra-Moreno, A. S., Hernandez-Ibarra, H. M., González, R. E., Castlebury, L. A., Dixon, L. J., Glynn, N. C., Comstock, J. C., Flynn, J., and Amador, J. 2009. First report of orange rust of sugarcane caused by *Puccinia kuehnii* in Mexico, El Salvador, and Panama. *Plant Dis.* 93:1347.
- Florida Automated Weather Network (FAWN). 2016. Report generator. <https://fawn.ifas.ufl.edu/data/reports/>. Accessed September 24, 2016.
- Glynn, N. C., Dixon, L. J., Castlebury, L. A., Szabo, L. J., and Comstock, J. C. 2010. PCR assays for the sugarcane rust pathogens *Puccinia kuehnii* and *P. melanocephala* and detection of a SNP associated with geographical distribution in *P. kuehnii*. *Plant Pathol.* 59:703-711.
- Harrison, J. G. 1992. Effects of the aerial environment on late blight of potato foliage—A review. *Plant Pathol.* 41:384-416.
- Herrera-Foessel, S. A., Singh, R. P., Lillemo, M., Huerta-Espino, J., Bhavani, S., Singh, S., Lan, C., Calvo-Salazar, V., and Lagudah, E. S. 2014. Lr67/Yr46 confers adult plant resistance to stem rust and powdery mildew in wheat. *Theor. Appl. Genet.* 127:781-789.
- Hothorn, T., Bretz, F., Westfall, P., Heiberger, R. M., Schuetzenmeister, A., and Scheibe, S. 2017. multcomp: Simultaneous inference in general parametric models. R package version 1.4-8. <http://multcomp.R-forge.R-project.org>.
- Hoy, J. W., and Hollier, C. A. 2009. Effect of brown rust on yield of sugarcane in Louisiana. *Plant Dis.* 93:1171-1174.
- Hsieh, W.-H., Lee, C.-S., and Chan, S.-L. 1977. Rust disease of sugarcane in Taiwan: The causal organism *Puccinia melanocephala* Sydow. *Taiwan Sugar* 24:416-419.
- Irey, M. 1987. Effect of the environment on sugarcane rust epidemics in Florida. *J. Am. Soc. Sugar Cane Technol.* 7:30-35.
- Kim, K. S., Taylor, S. E., Gleason, M. L., and Koehler, K. J. 2002. Model to enhance site-specific estimation of leaf wetness duration. *Plant Dis.* 86: 179-185.
- Kolmer, J., Chen, X., and Jin, Y. 2009. Diseases which challenge global wheat production—The wheat rusts. Pages 89-124 in: *Wheat: Science and Trade*. B. F. Carver, ed. Wiley, Hoboken, NJ.
- Kus, J. V., Zaton, K., Sarkar, R., and Cameron, R. K. 2002. Age-related resistance in *Arabidopsis* is a developmentally regulated defense response to *Pseudomonas syringae*. *Plant Cell* 14:479-490.
- Lenth, R., and Love, J. 2018. lsmmeans: Least-squares means. R package version 2.27-62. <https://cran.r-project.org/web/packages/lsmmeans/lsmmeans.pdf>
- Lenth, R., Love, J., and Herve, M. 2018. emmeans: Estimated marginal means, aka least-squares means. R package version 1.1.3. <https://github.com/rvnlent/emmeans>
- Liu, L. J., and Bernard, F. 1979. Sugarcane rust in the Dominican Republic. *Sugarcane Pathol. Newsl.* 22:5-7.
- Magarey, R. C., Croft, B. J., and Willcox, T. G. 2001. An epidemic of orange rust on sugarcane in Australia. *Proc. Int. Soc. Sugar Cane Technol.* 23:410-416.
- Magarey, R. C., Nielsen, W. A., and Magnanini, A. J. 2004. Environmental requirements for spore germination in three sugarcane leaf pathogens. *Proc. Int. Soc. Sugar Cane Technol.* 33:307-315.
- Martins, T. D., Raid, R. N., Burnquist, W. L., Urashima, A. S., Filho, A. B., and Comstock, J. 2010. Influence of temperature and leaf wetness duration on orange rust of sugarcane. *Phytopathol.* 100:S78-S79.
- Miles, M. R., Frederick, R. D., and Hartman, G. L. 2003. Soybean rust: Is the U.S. soybean crop at risk? Online publication. APS Feature Story 06/2003. American Phytopathological Society, St. Paul, MN. <http://www.apsnet.org/publications/apsnetfeatures/Pages/SoybeanRust.aspx>
- Montone, V. O., Fraisse, C. W., Peres, N. A., Sentelhas, P. C., Gleason, M., Ellis, M., and Schnabel, G. 2016. Evaluation of leaf wetness duration models for operational use in strawberry disease-warning systems in four US states. *Int. J. Biometeorol.* 60:1761-1774.
- Ovalle, W., Comstock, J. C., Glynn, N. C., and Castlebury, L. A. 2008. First report of *Puccinia kuehnii*, causal agent of orange rust of sugarcane, in Guatemala. *Plant Dis.* 92:973.
- Patel, M. K., Kamat, M. N., and Padhye, Y. A. 1950. A new record of *Puccinia* on sugarcane in Bombay. *Curr. Sci.* 19:121-122.
- Pérez-Vicente, L., Martín-Triana, E. L., Barroso, F., Martínez-de la Parte, E., Borrás-Hidalgo, O., and Hernández Estévez, I. 2010. Definitive identification of orange rust of sugarcane caused by *Puccinia kuehnii* in Cuba. *Plant Pathol.* 59:804.
- Pinheiro, J., Bates, D., DebRoy, S., and Sarkar, D. 2018. nlme: Linear and nonlinear mixed effects models. R package version 3.1-137. <https://cran.r-project.org/web/packages/nlme/nlme.pdf>
- Pretorius, Z. A., Rijkenberg, F. H. J., and Wilcoxson, R. D. 1988. Effects of growth stage, leaf position, and temperature on adult-plant resistance of wheat infected by *Puccinia recondita* f.sp. *tritici*. *Plant Pathol.* 37:36-44.
- Purdy, L. H., and Dean, J. L. 1981. A system for recording data about the sugarcane rust/host interactions. *Sugarcane Pathol. Newsl.* 27:35-40.
- Purdy, L. H., Liu, L. J., and Dean, J. L. 1983. Sugarcane rust, a newly important disease. *Plant Dis.* 67:1292-1296.
- R Core Team. 2016. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Raid, R. N., and Comstock, J. C. 2000. Common rust. Pages 85-89 in: *A Guide to Sugarcane Diseases*. P. Rott, R. A. Bailey, J. C. Comstock, B. J. Croft, and A. S. Saumtally, eds. La Librairie du Cirad, Montpellier, France.
- Raid, R. N., and Comstock, J. C. 2006. Sugarcane rust disease. UF/IFAS Ext. SS-AGR-207. University of Florida, Gainesville. <http://ufdcimages.uflib.ufl.edu/IR/00/00/30/39/00001/SC00700.pdf>
- Raid, R. N., Rott, P. C., and Comstock, J. C. 2015. Varietal resistance to sugarcane rusts in Florida: A historical perspective and future prospects. Page 6 in: *Abstract of the XIth Pathology and IXth Entomology Workshops*, September 14-18, Guayaquil, Ecuador.
- Reuveni, M., Tuzun, S., Cole, J. S., Siegel, M. R., and Kuc, J. 1986. The effects of plant age and leaf position on the susceptibility of tobacco to blue mold caused by *Peronospora tabacina*. *Phytopathol.* 76:455-458.
- Rott, P., Sood, S., Comstock, J. C., Raid, R. N., Glynn, N. C., Gilbert, R. A., and Sandhu, H. S. 2017. Sugarcane Orange Rust. UF/IFAS Ext. SS-AGR-378. University of Florida, Gainesville. <http://edis.ifas.ufl.edu/sc099>
- Rott, P. C., Kaye, C., Naranjo, M., Shine, J., Jr., Sood, S., Comstock, J. C., and Raid, R. N. 2016. Controlling sugarcane diseases in Florida: A challenge in constant evolution. *Proc. Int. Soc. Sugar Cane Technol.* 29:595-600.
- Rowlandson, T., Gleason, M., Sentelhas, P., Gillespie, T., Thomas, C., and Hornbuckle, B. 2015. Reconsidering leaf wetness duration determination for plant disease management. *Plant Dis.* 99:310-319.
- Ryan, C. C., and Egan, B. T. 1989. Rust. Pages 182-210 in: *Disease of Sugarcane: Major Diseases*. C. Ricaud, B. T. Egan, A. G. Gillaspie, Jr., and C. G. Hughes, eds. Elsevier Science Publishers, Amsterdam, the Netherlands.
- Sahni, M. L., and Chona, B. L. 1965. Studies on sugarcane rust in India. *Indian Phytopathol.* 18:191-203.
- Sandoval, I., Picornell, V., Chaves, R., and Ramos, N. 1983. *Puccinia melanocephala* H&P. Syd.: Biological and ecological aspects. *Proc. Int. Soc. Sugar Cane Technol.* 18:845-853.
- Singh, R. P., and Gupta, A. K. 1992. Expression of wheat leaf rust resistance gene *Lr34* in seedlings and adult plants. *Plant Dis.* 76:489-491.
- Sotomayor, I. A., Purdy, L. H., and Trese, A. T. 1983. Infection of sugarcane leaves by *Puccinia melanocephala*. *Phytopathol.* 73:695-699.
- Van Dillewijn, C. 1952. Botany of Sugarcane. Chronica Botanica Co., Waltham, MA.
- Victoria, J. I., Moreno, C., and Cassalet, C. 1990. Genotype-environment interaction and its effect on sugarcane rust incidence. *Sugar Cane* 4:13-17.
- Victoria, J. I., Ochoa, B. O., and Gómez, J. F. 1984. La Roya de la caña en el valle del Cauca: Diseminación y efecto en la producción. Pages 209-218 in: *Memorias del I congreso de la Sociedad Colombiana de Técnicos de la Caña de Azúcar*, Tecnicaña.