

SUGARCANE RUSTS IN FLORIDA

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Abstract

SUGARCANE orange rust symptoms were first observed in Florida in June 2007 on cultivar CP 80-1743. The causal agent, *Puccinia kuehnii*, was subsequently verified morphologically and molecularly constituting the first confirmed report of sugarcane orange rust in the Western Hemisphere. Orange rust was distributed throughout the entire Florida sugarcane industry, primarily on cultivars CP 80-1743 and CP 72-2086. The objective of this research was to evaluate the reaction of the commercial cultivars to both sugarcane brown and orange rusts and to assess their effect on the CP-cultivar development program in Florida. The rust reactions of several widely grown commercial cultivars, newly released cultivars and clones in the selection program, parental clones and CP historical clones were determined in order to develop a suitable resistance strategy to address the new incursion of orange rust. Changes in the program to increase the level of rust-resistant progeny are detailed.

Introduction

Sugarcane brown and orange rusts are the two primary rust diseases of sugarcane (Magarey, 2000; Raid and Comstock, 2000). Both sugarcane rusts have had a significant impact on the Florida sugarcane industry. The development of sustained resistance to sugarcane brown rust caused by *Puccinia melanocephala* has not always been successful.

The brown rust pathogen, *P. melanocephala*, was unable to develop on a number of cultivars when they were released but they subsequently show severe symptoms. After several years of commercial production, brown rust developed on CP 70-1133, CP 72-1210 and CP 78-1247 rendering them unsuitable for further cropping. Additionally, CP 78-1628, a major cultivar on sand soils, has also become susceptible to brown rust since being released in 1991 (Tai *et al.*, 1991).

During the clonal selection program, rust reactions were traditionally determined solely on natural infection of clones in field plots. Unfortunately, this can lead to inconsistent results due to variability in disease levels between locations resulting in an inadequate rust resistance evaluation. Another possible reason for the change in resistance rating may be pathogen variation.

There have been reports of pathogenic races of *P. melanocephala* in Florida (Shine *et al.*, 2005) and *P. kuehnii* in Australia (Braithwaite, 2005). Some stages of the Canal Point cultivar development program are of necessity planted at a time of the year that does not favour rust infection in these canes (in the March through mid-May period when brown rust is most prevalent).

In June 2007, orange rust caused by *P. kuehnii* was detected in Florida (Comstock *et al.*, 2008). Orange rust was initially recognised because of the unusual amount of rust seen in the previously rust-

resistant cultivar CP 80-1743. On close examination, the rust pustules were lighter and more orange in colour than brown rust pustules. Furthermore, the urediniospores had a prominent apical thickening instead of the uniform walls observed in *P. melanocephala*.

These morphological characteristics, together with molecular sequencing information, confirmed the pathogen as *Puccinia kuehnii*, the orange rust pathogen (Comstock *et al.*, 2008). Subsequently, orange rust was confirmed in Guatemala, Costa Rica, Nicaragua, El Salvador, Mexico and Panama (Ovalle *et al.*, 2008; Chavarría *et al.*, 2009; Flores *et al.*, 2009). In Florida, orange rust associated yield losses in crops of CP 80-1743 were estimated at 40% in 2008 (Richard Raid, Pers. Comm.).

This is similar to losses reported in the Central district of Australia in the 1999-series plant crop (Magarey *et al.*, 2008). Orange rust has reduced yields of CP 72-2086 in some locations in Central America where environmental conditions are conducive for disease development. This is particularly true in the southern region of Costa Rica and the lower elevations of Guatemala, both warm humid areas. Losses of 8% were estimated in Guatemala on CP 72-2086 (W. Ovalle, Pers. Comm.).

CP 80-1743 occupied 22.8% of the industry acreage in Florida in 2007 and up to 40% in some sections of the industry. Initial surveys indicated that orange rust was distributed throughout the entire industry (where CP 80-1743 or other susceptible clones were grown). Yield losses dictated the need to withdraw CP 80-1743 from production. Germplasm in the Canal Point (CP) Cultivar Development Program was impacted, as a large portion of parental clones were susceptible, as too were a portion of the clones in the selection program and these too had to be eliminated.

A whorl inoculation technique was recently developed and is being used to determine rust reactions in Florida (Sood *et al.*, 2009). This procedure allows individual plants to be inoculated in the field with specifically selected inoculum. When windblown inocula are limited, screens can be completed using stored spores. Large numbers of clones can be screened using limited labour. Ratings are applied based on the type and severity of pustule development.

The objectives of this paper are 1) to provide a brief history of the sugarcane orange rust outbreak in Florida, 2) present the impact of sugarcane orange rust on the commercial cultivars and parental clones, 3) to present resistance data on clones in the CP-Cultivar Development Program and compare the ratings of individual clones as they are advanced from one stage to the next.

Materials and methods

Rust rating scales

Both sugarcane brown and orange rusts were rated using two different rating scales depending on whether the plants were naturally infected in the field or were inoculated using a whorl inoculation technique under field conditions.

The natural infection scale is based on the presence and number of pustules using a 5 point scale as follows: 0 = no symptoms; 1 = one or a very few pustules; 2 = more than a few pustules; 3 = numerous pustules both on the lower leaves and to a lesser extent on the upper leaves and 4 = severe rust development with extensive coalescing of pustules and leaf necrosis due to rust. Ratings 0 and 1 are classified as resistant reactions, a rating of 2 is moderately susceptible and 3 and 4 are susceptible.

Field plot ratings require rust assessments on check varieties to validate the ratings. The artificial inoculation scale used to determine rust reactions after whorl inoculation is based entirely on pustule formation and presence and amount of sporulation occurring using the following scale: 1 = no visible symptoms; 2 = small yellow flecking with no sporulation and no pustule development; 3 = small pustules with limited urediniospore development (some sporulation); and 4 = pustules with abundant sporulation. Ratings 1 and 2 are resistant and ratings 3 and 4 are respectively, moderately susceptible and susceptible.

Whorl inoculation procedure

A whorl inoculation procedure was used to inoculate 3 to 6 month old field plants as previously described (Sood *et al.*, 2009). Briefly, inoculations are made using an automatic pipetter by placing 0.5 to 1.0 mL urediniospore suspension (10^4 urediniospores per mL for *P. kuehni* and 10^5 urediniospores per mL for *P. melanocephala*) into the spindle leaf whorl of individual stalks. Inoculated stalks are marked by cutting off one third of the leaf tips to identify what leaves to evaluate. Inoculations are made at 7:30 am to 9:00 am when the plants have dew on their leaves and prior to the peak temperature of the day. There were three inoculated plants in a single plot for Stage II clones, six inoculated plants for Stage III clones (3 plants in two plots) and 12 plants for Stage III increase and Stage IV clones (3 plants in 4 plots). Rust reaction ratings were made 4 weeks after inoculation using the whorl inoculation scale described above and the data were averaged.

Rust reactions based on natural infection

Rust reactions of both brown and orange rust (since 2007) reactions were based on natural infection using the 0 to 4 rating scale described above. The following germplasm was rated for their rust reactions: commercial cultivars, released cultivars (with < 1% of the acreage), clones in the program to increase seedcane for multiplication, clones in all stages of the selection program including Stage II (single location, unreplicated), Stage III (four locations), and Stage IV (10 locations), parental clones and 1060 domestic and foreign clones in the CP historical nursery. The number of ratings for each genotype varied from a single rating in the unreplicated plots to more than 6 for the replicated trials. The highest rating is designated the assigned reaction.

Rust reactions based on whorl inoculation

Clones in the following CP Series were tested using the whorl inoculation technique: CP 05 Series in 2008 (Stage III increase) and 2009 (Stage IV), CP 06 Series in 2008 (Stage III) and 2009 (Stage III increases) and CP 07 Series in 2008 (Stage II) and 2009 (Stage III). Data are summarised to indicate the percentage of resistant clones at each stage. Correlations of the ratings in these different stages were made using clones common to each of the above series.

Results and discussion

Rust in commercial sugarcane production

The rust ratings of the commercial cultivars grown in Florida are presented (Table 1). Cultivar CP 80-1743 was the most susceptible and withdrawal from commercial production is recommended. Its acreage decreased from 22.8% in 2007 to 19.6% in 2009 prior to harvest and planting. In 2007, many growers could not change their planting plans because the orange rust resistance reaction of available, alternative cultivars could not be finalised prior to planting. Since 2007, the acreage of CP 88-1762 and CP 89-2143 increased by 1.9 and 4.2% to 20.4 and 31.1% respectively.

Growers are reluctant to further increase CP 89-2143 and CP 88-1762 because these two cultivars are grown primarily on organic soils and the proportion of their acreage in some areas is very high (approaching 40%). Orange rust has recently been observed on CP 88-1762 that had not previously exhibited symptoms, suggesting a change in *P. kuehni* pathogenicity. This is a real concern because shifts in pathogenicity can severely affect commercial production, cultivar deployment, and restrict the progress in the cultivar development program.

CP 78-1628 was resistant to *P. melanocephala* when it was released and for several years afterward, but now is moderately susceptible to brown rust. In Florida, brown rust has a history of affecting previously resistant cultivars. Brown rust resistance in CP 70-1133 and CP 72-1210 appeared to 'breakdown' as the acreage expanded above 30% in Florida. Similarly brown rust resistance of LCP 85-384 'broke down' after its acreage approached 85%, contributing to its recent decline in Louisiana

(K. Gravois, pers. comm.). In Colombia, brown rust severity has increased on CC 85-92 and CC 84-75 that combined occupy 80% of the acreage (Angel *et al.*, 2008). The occurrence of new pathogenic races of either *P. melanocephala* or *P. kuehni* are a real threat to the Florida industry since races of brown rust have been previously reported (Shine *et al.*, 2005).

Orange rust development in 2009 has been slower than in 2008 since there was a severe freeze in March of this year and most of the sugarcane foliage was ‘burnt’ back by the freezing temperatures, thereby reducing the amount of *P. kuehni* inoculum. The rust epidemic was delayed 3 months compared to the previous year, and orange rust severity in September was at a level comparable to that in July in 2008. Disease ratings based on natural infection were therefore delayed in 2009.

Table 1—Rust reaction ratings based on natural infection and acreage of the major commercial cultivars grown in Florida in 2009.

| Cultivar | Brown Rust | Orange Rust | % acreage |
|------------|---------------------|------------------------------------|-------------------|
| CP 72-2086 | Mod. resistant (R1) | Susceptible (R3) | 3.9 |
| CP 78-1628 | Susceptible (R2-3) | Mod. resistant (R1) | 11.5 |
| CP 80-1743 | Mod. resistant (R1) | Susceptible (3) | 19.6 ^a |
| CP 84-1198 | Mod. resistant (R1) | Mod. Resistant (R1) | 3.7 |
| CP 88-1762 | Resistant (R0) | Mo. susceptible (R2) ^b | 21.4 |
| CP 89-2143 | Resistant (R0) | Mod. susceptible (R2) ^c | 32.6 |

^a In June 2007 when orange rust was detected CP 80-1743 occupied 22.8% of the commercial acreage.

^b CP 88-1762 previously had no orange symptoms but, in August 2009, orange rust was observed in multiple grower fields and it is now classified moderately susceptible.

^c Orange rust severity has been rated R3 in some locations in 2009.

Recently released cultivars, CP 00-1101 (Gilbert *et al.*, 2008), CP 00-1446 (Comstock *et al.*, 2009), CP 00-2180 (Glaz *et al.*, 2009), CP 01-1372 (Edme *et al.*, 2009) and CPCL 97-2730 (Milligan *et al.*, 2009) are either resistant or moderately resistant to rust (Table 2) and are rapidly being increased in Florida; however, the percent acreage of each cultivar in the industry still remains slightly below 1%. CP 80-1743 is not being replanted and the demand for these recently released resistant cultivars has been higher than the available seed cane for planting. The successful economic use of fungicides to control orange rust has enabled cropping for the normal crop cycle period, even in susceptible cultivars and has opened new control options to consider.

Table 2—Rust resistance ratings of recently released cultivars.

| Cultivar | Brown Rust (rating) | Orange Rust (rating) |
|--------------|---------------------|----------------------|
| CP 00-1101 | Resistant (R0) | Mod. resistant (R1) |
| CP 00-1446 | Mod. resistant (R1) | Mod. resistant (R1) |
| CP 00-2180 | Mod. resistant (R1) | Resistant (R0) |
| CP 01-1372 | Mod. resistant (R1) | Mod. resistant (R1) |
| CPCL 97-2730 | Resistant (R0) | Mod. resistant (R1) |
| CPCL 99-4455 | Mod. resistant (R1) | Resistant (R0) |

Rust reaction of parental clones

A high proportion of parental clones available for crossing in 2007 and 2008 were susceptible to orange rust (Table 3). The short time period since the orange rust incursion has limited significant changes in the resistance of parental populations. The proportion of resistant parental germplasm was

less than Australia where 70% of the germplasm was resistant (Magarey and Bull, 2009). Consequently, changes in the breeding population have been more difficult in Florida than Australia. Although resistant \times resistant and resistant \times susceptible crosses are desirable, some crosses of susceptible \times susceptible were made because of flower availability at the time of crossing. Some resistant progeny will be produced in these crosses. A more restrictive use of susceptible parental clones is being implemented.

Table 3—Summary of orange rust reactions of parental clones used for crossing in the CP program for Florida based on ratings taken in 2007 and 2008.

| Prefix ^a | Rust rating/number of clones | | | | Total |
|---------------------|------------------------------|-----------|------------|-----------|-------|
| | R0 | R1 or R1+ | R2 of R2 + | R3 of R3+ | |
| CL | 3 | 11 | 18 | 0 | 32 |
| CPCL | 5 | 15 | 32 | 4 | 56 |
| CP | 15 | 34 | 43 | 8 | 100 |
| Total | 23 | 60 | 93 | 12 | 188 |

^a Prefix CL indicates clones developed entirely by the breeding program of US Sugar Corporation. CPCL prefix indicates clones derived from seed resulting from crosses made by the breeding program of US Sugar Corporation and selected by the CP program at the USDA-ARS Sugarcane Field Station. CP indicates clones developed entirely by the CP program.

Of the 1060 clones in the CP historical nursery, 78 and 59% were classified as resistant to brown and orange rust, respectively. The nursery comprised primarily Florida (CP, CPCL, and CL), Louisiana (Ho, HoCP, L and LCP) and Texas (TCP) clones. Since the breeding and crossing programs for the US mainland sugar industries relies on these clones, the impact of orange rust is important.

Effect of rust on the CP cultivar development program

In 2007, the proportion of clones resistant ($R \leq 1.0$) to both brown and orange rust that were advanced to Stage III (third clonal stage of selection) was 47.8% (Table 4). In 2008, 63.8% of the clones rated resistant ($R < 1$) and advanced to Stage III (the first multi-location yield trial) were assigned a rating based on natural infection. Moreover, the mean rust rating of the entire population of Stage II clones decreased while the ratings of the check clones increased. The proportion of rust-resistant germplasm was less in Florida when orange rust was introduced than that in Australia in 2000, where 70% of the germplasm was resistant (Magarey and Bull, 2009). Guatemala also has a lower proportion of resistant germplasm in their selection program than Australia (W. Ovalle, pers. comm.). The lower proportion of resistant clones in the western hemisphere than Australia may be because, in Australia, *P. kuehni* had been present for years and there probably was some selection for resistance in contrast to western hemisphere where the pathogen was newly introduced.

Table 4—Rust ratings of Stage II clones advanced to Stage III, 2007 versus 2008.

| Rust rating | 2007 | 2008 |
|-----------------|------------|------------|
| 0 | 32 (23.5%) | 86 (63.8%) |
| 0.5–0.9 | 33 (24.3%) | 0 (0%) |
| 1.1–1.4 | 48 (35.3%) | 40 (29.6%) |
| 1.5–1.9 | 21 (15.4%) | 9 (6.7%) |
| 2.0 | 2 (1.5%) | 0 |
| Mean | 0.8 | 0.4 |
| Check mean | 0.8 | 1.6 |
| Population mean | 1.6 | 1.1 |

There are two trends concerning sugarcane brown and orange rusts in the CP cultivar development program: 1. a trend toward more resistance. In time a shift toward resistance is likely to be more dramatic as selection for rust resistance will be emphasised compared to other traits, and 2. a possible change in orange rust pathogenicity.

Pathogenic changes are evident since CP 88-1762 did not have orange rust symptoms in 2007 when the disease was detected. Growers and scientists made numerous observations of crops of these major cultivars and, if orange rust was infesting them in 2007, it had to be at an extremely low level. In 2009, orange rust pustules were observed on CP 88-1762 for the first time. Only pathogenicity and molecular characterisation will confirm if in fact there are distinct races of *P. kuehni* present in Florida.

Whorl inoculation ratings

The proportion of rust resistant clones in the CP 05, CP 06 and CP 07 series are presented in (Table 6); the proportion of resistant clones in the CP 05 through CP 07 clones was high. Since susceptible clones were not advanced, there was an increase in percentage of resistant clones in 2009 compared to 2008. There were good correlations between the ratings obtained in whorl-inoculated tests and those obtained using natural infection (Sood *et al.*, 2009). The correlations were excellent between ratings obtained from one year to the next with r^2 values ranging from 0.75 to 0.83.

Table 6—The percent resistant CP 05, CP 06 and CP 07 Series clones as determined by the whorl inoculation procedure.

| | % Resistant clones | | | |
|---------------------------|--------------------|------|-------------|------|
| | Brown Rust | | Orange Rust | |
| CP Series | 2008 | 2009 | 2008 | 2009 |
| CP 05 Series ^a | 91 | 100 | 86 | 100 |
| CP 06 Series ^b | 86 | 98 | 83 | 91 |
| CP 07 Series ^c | 78 | 97 | 93 | 99 |

^a Comparison of rust reactions of CP 05 Series clones in Stage II increase and the clones advanced to Stage IV for 2008 and 2009, respectively.

^b Comparison of rust reactions of CP 06 Series clones in Stage III and the clones advanced to Stage III increase for 2008 and 2009, respectively.

^c Comparison of rust reactions of CP 07 Series clones in Stage II and the clones advanced to Stage III for 2008 and 2009, respectively.

Conclusions

Orange rust appeared suddenly and has dramatically affected the production of sugarcane in Florida and several Central American countries. The disease impacted both total sugarcane production and the retention of cultivars in the cultivar development programs. A major cultivar, CP 80-1743, is being withdrawn from commercial production and a number of potential Stage IV clones were eliminated solely for their rust reactions.

In commercial fields, fungicide applications increased sugar productivity from a range of 7.9 to 26% on CP 80-1743 in 2008, indicating partial losses due to orange rust (James Shine, Raul Perdomo and Michael Irej, pers. comm.) Where there was better control of orange rust with more frequent fungicide applications in experimental plots, the losses were higher (up to 40%) reflecting the maximum loss due to the disease (Richard Raid, pers. comm.). The parental population and the crossing strategy had to be changed to reflect the need for rust resistance. Changes in *P. kuehni* pathogenicity mean that constant monitoring of the susceptibilities of cultivars in commercial

production and in sugarcane breeding programs is required. At this time, there is not sufficient molecular and varietal reaction data to determine the origin of *P. kuehnii* in the western hemisphere. Since orange rust is dispersed by windblown urediniospores, it may spread and potentially may impact most sugarcane producing areas in the western hemisphere.

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LES DIFFÉRENTES ROUILLES DE LA CANNE À SUCRE EN FLORIDE

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MOTS-CLÉS: Rouille Brune de la Canne a Sucre,
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Résumé

LES SYMPTOMES de la rouille orangée ont été observés pour la première fois en Floride en juin 2007 sur le cultivar CP 80-1743. L’agent causal, *Puccinia kuehni*, a été subséquentment vérifié morphologiquement et moléculairement ce qui a constitué le premier rapport de la rouille orangée dans l’hémisphère ouest. La maladie était prévalente à travers l’industrie de la canne à sucre en Floride, principalement sur les cultivars CP 80-1743 et CP 72-2086. L’objectif de cette recherche a été d’évaluer la réaction des cultivars industriels à la rouille brune et à la rouille orangée afin de déterminer leur impact sur le programme de développement des cultivars CP en Floride. Les réactions aux différentes rouilles de plusieurs cultivars plantés sur une grande échelle, les cultivars nouvellement homologués, les clones en sélection, les clones parentaux et les clones CP historiques ont été évalués afin de développer une stratégie de lutte basée sur la résistance pour contrecarrer l’arrivée de la rouille orangée. Les changements au programme pour augmenter le niveau de résistance à la rouille sont élaborés.

ROYAS DE LA CAÑA DE AZÚCAR EN LA FLORIDA

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PALABRAS CLAVE: Roya Café, Roya Naranja,
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Resumen

LOS SÍNTOMAS de roya de la caña de azúcar de naranja se observaron por primera vez en la Florida en Junio de 2007, sobre el cultivar CP 80-1743. El agente causal, *Puccinia kuehnii*, fue verificado morfológica y molecularmente lo que se constituyó en el primer informe sobre presencia de la roya naranja de la caña de azúcar en el Hemisferio Occidental. La roya naranja está distribuida en toda la industria azucarera de la Florida, principalmente en las variedades CP 80-1743 y CP 72-2086. El objetivo de esta investigación fue evaluar la reacción de los cultivares comerciales tanto a la roya café como a la naranja de caña de azúcar y evaluar su efecto en el programa de desarrollo de cultivares CP en la Florida. Las reacciones de infección de varios cultivares comerciales ampliamente cultivados, de cultivares recién liberados y clones del programa de selección así como clones progenitores y clones históricos CP se evaluaron con el fin de desarrollar una estrategia de resistencia adecuada para hacer frente a la amenaza de la roya naranja en la industria azucarera de la Florida. Los cambios en el programa de mejoramiento para aumentar el nivel de resistencia en la progenie se presentan en detalle.