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Controlling sugarcane diseases in Florida: a challenge in constant evolution

Philippe C Rott[1], Claudia Kaye[2], Moramay Naranjo[3], James M Shine Jr[4], Sushma Sood[5], Jack C Comstock[5] and Richard N Raid[1]

[1]*University of Florida, IFAS, Plant Pathology Department, Everglades Research and Education Center, Belle Glade, FL 33430, USA*; pcrott@ufl.edu
[2]*US Sugar Corporation, Clewiston, FL 33440, USA*
[3]*Florida Crystals Corporation, Belle Glade, FL 33430, USA*
[4]*Sugar Cane Growers Cooperative of Florida, Belle Glade, FL 33430, USA*
[5]*USDA-ARS, Sugarcane Field Station, Canal Point, FL 33438, USA*

Abstract Diseases are limiting factors for the sugarcane crop in almost any sugarcane growing location. More than 40 diseases have been recorded in Florida, with brown rust, orange rust and yellow leaf currently impacting on sugarcane production. Ideally, these diseases should be controlled using resistant cultivars, but most cultivars grown in 2015-2016 in Florida are susceptible to either brown rust or orange rust. Several cultivars that were initially resistant to orange rust became susceptible when grown over large areas, suggesting a change in the rust pathogen populations. At the present time, control of the two rust diseases relies mainly on use of fungicides. Most cultivars are also susceptible to infection by *Sugarcane yellow leaf virus* (SCYLV) and it has been shown that this virus can reduce yields even in absence of disease symptoms. Use of healthy seed cane is only partially successful and the recently discovered new host and potential vector of SCYLV may explain the difficulty in controlling this virus in Florida. Identification of sustainable resistance sources and transfer of these resistances to new cultivars are critical for successful control of sugarcane diseases in Florida.

Key words Brown rust, disease control, fungicides, orange rust, yellow leaf, resistance

INTRODUCTION

Sugarcane is host to numerous pathogens that can affect production and cause yield losses. More than 100 pathogens, including bacteria, fungi and viruses, have been reported to cause diseases of sugarcane, and at least 40 of those are known to occur in Florida (Rott *et al*. 2000). In an effort to limit disease impact, new sugarcane cultivars (CP cultivars) are produced in the context of a tripartite program between the University of Florida (Everglades Research and Education Center at Belle Glade), USDA-ARS (Sugarcane Field Station at Canal Point), and the Florida Sugar Cane League (Sandhu *et al*. 2014). Over the last decade, this program has been highly successful in developing and releasing sugarcane cultivars resistant to major sugarcane diseases, including leaf scald (*Xanthomonas albilineans*), ratoon stunting (*Leifsonia xyli* subsp. *xyli*), brown rust (*Puccinia melanocephala*), smut (*Sporisorium scitamineum*), and mosaic (*Sugarcane mosaic virus*).However, for various reasons, three diseases remain as problems to the industry: yellow leaf caused by *Sugarcane yellow leaf virus* and which has been present in Florida for at least two decades; brown rust, which has been present in Florida since the late 1970s; and orange rust, caused by *P. kuehnii*, and first reported in Florida in 2007, less than 10 years ago. The objective of this paper is to illustrate how changes in host-pathogen interactions impact progress and control of these diseases in Florida.

BROWN RUST

The causal agent of brown rust was first reported in the Western Hemisphere in 1978 in the Dominican Republic. It was observed soon after in most sugarcane growing locations of the Americas, including Florida in 1979 (Purdy *et al.* 1983). Brown rust symptoms occur mainly on the leaves and include yellow flecks, reddish brown elongated lesions and pustules releasing fungal spores called urediniospores (Raid and Comstock 2000). The leaf tissue becomes necrotic in severely affected plants and these may show reduced stalk number and biomass resulting in reduced cane yield (Comstock *et al.* 1992). Appearance of brown rust in the Western Hemisphere caused tremendous losses, especially in cultivar B4362, which was widely cultivated throughout the Caribbean when rust epidemics initially occurred. In 1988, in Florida, yield loss estimates averaged 39% in cultivar CP78-1247 (Comstock *et al.* 1992). As a result, developing cultivars resistant to brown rust became a major objective of the CP breeding program.

Initially, resistance to brown rust was determined by the absence of visible disease symptoms in the field, and multiple cultivars showing good rust resistance were released over the years. These proved to be of great benefit to the sugarcane growers. However, in Florida, brown rust has a history of overcoming disease resistance in cultivars that previously demonstrated resistance (Comstock *et al*. 2010). Important cultivars, such as CP72-1210, CP74-2005 and CP78-1628 that did not show symptoms of brown rust for several years following release, had to be withdrawn from commercial production because of the breakdown in resistance. Similarly, the promising cultivar CP78-1247 was resistant to brown rust from 1978 to 1988 when it suddenly showed extremely high rust susceptibility throughout south Florida. These frequent breakdowns of rust resistance were attributed to changes in the population structure of *P. melanocephala* and appearance of new and more virulent strains of the pathogen (Raid 1989). Two studies supported this hypothesis of development of pathogenic races. In 1984, based on description of sugarcane reaction to rust in field experiments under natural infection of sugarcane cultivars, Dean and Purdy (1984) reported the occurrence of at least two different races of *P. melanocephala* in Florida. In 1991-1992, Shine and collaborators observed disease progress in six different sugarcane cultivars inoculated with five isolates of the brown rust pathogen under controlled conditions. They showed that four pathogenic races were present (Shine *et al.* 2005).

Resistance to brown rust has been shown to be a highly heritable trait (Hogarth *et al*. 1993), but the mechanisms of resistance to this disease are so far unknown. However, molecular investigations into genetic basis of sugarcane resistance to brown rust resulted in identification of a major resistance gene called *Bru*1 (Costet *et al.* 2012a). The *Bru*1 gene was originally found and described in R570, a cultivar that was bred on Réunion Island (France) (Daugrois *et al*. 1996). Cultivar R570 was released for commercial production in 1978 and has been grown commercially or in germplasm collections in numerous geographical locations where brown rust exists (North and South America, Africa, Caribbean Islands, Mascarene Islands, etc.). At the present time, no breakdown of resistance to brown rust has been reported in this cultivar, making the *Bru*1 gene an excellent candidate for durable resistance. The *Bru*1 gene has subsequently been used in sugarcane breeding programs worldwide to improve brown rust resistance by marker-assisted selection. Recently, *Bru*1 was detected in 285 (27%) of 1,072 clones used as a sugarcane parental pool for crossing in Florida, and there is a continual increase in the frequency of *Bru*1 in the parental material used in the CP cultivar development program (Glynn *et al*. 2013).

Areas with commercial varieties possessing *Bru*1 have increased dramatically in Florida over the last decade, with 50% of the clones released between 2011 and 2014 by the CP program having the *Bru*1 gene (Zhao *et al*. 2015). Fortuitously, there has been no recent breakdown of brown rust resistance in Florida, unlike the pre-1988 period when the *Bru*1gene was rare in cultivars and pathogenic races were identified in sugarcane clones without this gene. However, even if *Bru*1 might be difficult (if not impossible) to be overcome by *P. melanocephala*, it is hazardous to rely on a single resistance gene. History has shown that the potential development of a new rust race exists, and selection for a new virulent race is increased when cultivars possessing a single resistant gene are planted in large contiguous surfaces. If the *Bru*1 gene breaks down, the sugarcane industry may face a catastrophic situation. Therefore, additional resistance genes need to be identified and used in breeding programs. Efforts should also be made to diversify the on-farm sources of resistance by planting cultivars with different brown rust resistance mechanisms. Another brown rust resistance gene called *Bru*2 was reported 10 years ago to occur in sugarcane variety MQ76-53, but durability of this gene and its genetic basis are still unknown (Raboin *et al*. 2006). *Bru*2 appears to be genetically different from *Bru*1 and it may constitute a very useful alternative source for resistance to brown rust. However, molecular markers to detect this gene in parental clones or in cultivated varieties are not currently available.

While the CP breeding program has been successful in releasing cultivars resistant to brown rust, the number of cultivars susceptible to brown rust grown commercially has recently increased in Florida (Rice *et al*. 2015). This situation is not the result of the breakdown of varietal resistance to brown rust, but rather is due to successful management of sugarcane orange rust with fungicides (see below). Because brown rust can be controlled by only one or two applications of fungicides (also used to control orange rust), growers are willing to grow high performing or outstanding varieties even if these are susceptible to brown rust. Consequently, CP96-1252 was the leading cultivar and covered 17% of the cultivated acreage during the 2014-2015 crop season, although it can show severe brown rust symptoms and exhibit high yield losses (>30%) if left untreated during rust epidemics.

ORANGE RUST

Puccinia kuehnii, the causal agent of orange rust, was first reported in the Western Hemisphere in Florida near Belle Glade in 2007 (Comstock *et al*. 2008). The pathogen quickly became established throughout North, Central and South America. In the continental USA, orange rust has been reported in Florida and Louisiana but not in Texas. The source of introduction of the orange rust pathogen into the Western Hemisphere is unknown but it has been hypothesized that, similar to brown rust before it, conducive transoceanic wind currents facilitated introduction from Africa.

As with brown rust, orange rust symptoms occur mainly on the leaves. Symptoms include yellow flecks, reddish brown elongated lesions, and oval shaped orange-brown pustules releasing urediniospores. On highly susceptible varieties, abundant pustules develop and coalesce on the lower leaf surface, causing tissue death. Severely affected plants exhibit both reduced stalk numbers and biomass. Losses in Florida have been estimated at 10% overall, but reductions in cane yields of up to 43% have been determined on cultivar CP80-1743 (Rott *et al.* 2014). This latter cultivar was significantly impacted by orange rust, as it occupied nearly 25% of the total area when the initial outbreak occurred in 2007. Consequently, cultivar CP80-1743 has been withdrawn from commercial production and replaced by less susceptible cultivars. However, several cultivars, such as CP88-1762 and CP89-2143 that were initially classified as resistant to orange rust, based on an absence of disease symptoms during 2007 and 2008 field surveys, rapidly showed disease symptoms following an increase in area planted. CP89-2143 was rated resistant during the entire crop season in 2010 but was rated susceptible 2 years later (BASF and Glades Crop Care, unpublished data).

These apparent changes in disease susceptibility were attributed to changes in the pathogen and development of new races. A detached leaf bioassay to study pathogenic specialization of the orange rust pathogen was recently developed and successfully used to reproduce disease symptoms under controlled conditions (Hincapie *et al.* 2015). Additionally, leaf pieces of cultivars CL85-1040, CP80-1743, and CP89-2143 exhibited significantly more sporulating rust pustules when inoculated with spores collected from CP89-2143 than when inoculated with spores collected from CP85-1040, a cultivar that was susceptible as soon as orange rust first occurred in Florida. These data are the first evidence of occurrence of at least two different strains of *P. kuehnii* in Florida, and support the shift from resistance to susceptibility of CP89-2143 in 2010-2012 (Hincapie *et al.* 2015).

Following the severe outbreak of orange rust on cultivar Q124 during the late 1990s in Australia, this cultivar was easily replaced by new resistant cultivars (Magarey *et al.* 2011). Although the resistance breakdown of Q124 was attributed to development of a new race of the pathogen, in Australia, this appeared to be a single event. This seems not to be the case in Florida, where successive breakdowns of cultivar resistance suggest a constant evolution and specialization of *P. kuehnii*. Consequently, cultivation of orange rust resistant cultivars has had only limited success thus far, and orange rust susceptible cultivars account currently for over 70% of Florida's sugarcane holdings (Rice *et al*. 2015). Breeding and screening for orange rust appears to be a long and tedious task in this part of the Western Hemisphere. The reasons for this situation are unknown. They may be related to occurrence of environmental conditions conducive for disease progress in southern Florida nearly year round, with daily mean temperatures higher than 20°C and nocturnal leaf wetness durations of at least 8 hours. Consequently, and until significant progress in breeding has occurred, control of orange rust will rely on chemical control.

Immediately after the arrival of orange rust in Florida, and with host-plant resistance as a medium to long term goal, fungicides were investigated as an interim means of orange rust control. A series of trials were conducted and fungicide treatments that included three major classes of fungicides, alone and in combination and/or alternation were identified: the carboxamides (FRAC group 7), the strobilurins (FRAC group 11), and the triazoles (FRAC group 3) (Raid *et al.* 2016). Based upon yield loss data in the absence of fungicides, the loss of revenue due to orange rust has been estimated as exceeding USD 206 million (Rott, unpublished data). For this reason, and in order to apply fungicides as efficiently as possible, both orange rust and brown rust are now routinely monitored by Glades Crop Care (www.gladescropcare.com). Monitoring starts as soon as the rusts begin to appear in plant cane (usually in March-April) and continues through Fall (beginning of harvest season). A total of about 40 untreated fields/sites are selected every year by the sugarcane growers. These fields, which represent the 166,000 ha of sugarcane grown in the Everglades Agricultural Area, are surveyed and evaluated for rust severity every 2 weeks. Based on the information provided, growers can decide whether or not or when to apply fungicides. Three to four fungicide applications are needed per crop season to keep orange rust under an economical threshold, whereas only one or two applications are needed to control brown rust, which has a narrower window of susceptibility. Additional investigations are in progress to develop a decision support model for sugarcane rusts in Florida based on environmental conditions. Such a decision tool will help in forecasting disease progress and optimize application of fungicides.

YELLOW LEAF

Yellow leaf (previously known as yellow leaf syndrome or YLS) was first recognized in Florida in 1993, but the disease had most likely been present in the state for several years (Comstock *et al.* 1994). Symptoms included a bright yellow coloration on the abaxial side of the leaf midrib and necrosis starting from the leaf tip and spreading outward from the leaf midrib. Symptoms were most evident on mature sugarcane stalks. Four years later, in 1997, incidence of *Sugarcane yellow leaf virus* (SCYLV) exceeded 90% in a majority of Florida's commercial sugarcane cultivars, and 43 of 46 parental clones used in the crossing program for Florida tested positive for the virus (Comstock *et al*. 1998). These high levels of sugarcane infection suggested that resistance to yellow leaf was rare in the Florida sugarcane germplasm. Additionally, only 17 of the 46 parental clones displayed yellow leaf symptoms, suggesting that symptoms were not a reliable means for resistance screening. Yield loss experiments conducted from 2001 to 2004 showed that yield parameters (such as number of stalks, stalk weight and sucrose per plot) were significantly higher in SCYLV-free plots than in virus-infected plots (Comstock and Miller 2004). On average, across five cultivars, stalk weights were reduced by 11% in diseased plots versus healthy plots. However, even higher yield losses have been reported in other countries. For example, cultivar SP71-6163 suffered up to 25% yield losses in Brazil (Rott *et al*. 2008).

Due to limited sources of host plant resistance, and because mechanical inoculation of sugarcane with SCYLV is not feasible for rapid screening of breeding material, an alternative control measure was investigated in Florida. Meristem culture has been reported to be very successful in eliminating the virus from infected plants (Chatenet *et al*. 2001). Therefore, virus-free tissue-cultured plantlets were produced using Kleentek® technology, and these plantlets were used to establish disease-free nurseries for commercial sales starting in 2000. Sugarcane plants produced in these nurseries were then used to propagate seed cane for the subsequent establishment of new commercial fields. However, recent surveys revealed that use of virus-free planting material is only partially effective in controlling yellow leaf because material propagated in nurseries or commercial fields becomes re-infected by the virus within a few years (Kaye *et al*. unpublished data ; Naranjo *et al*. unpublished data). Observations made 20 years ago by the breeding program in Canal Point support this rapid re-infection of disease-free material: in 1997, incidence of SCYLV in 7-months-old transplanted seedlings from new crosses was only 1.6%, while incidence of the virus in stage II clones was 38% (Comstock *et al*. 1999). A metagenomics study performed with samples collected in 2014 from sugarcane cultivars and *Saccharum* germplasm in Florida revealed that more than 80% of samples were infected by SCYLV (Fernandez *et al*. 2015). Currently, overall yield reductions due to yellow leaf have been estimated to be 10-15% by the Florida sugarcane industry.

In order to better understand the epidemiology of yellow leaf, and especially the rapid spread of the virus in the Everglades Agricultural Area, several investigations were recently undertaken. Worldwide, four aphid species have been reported to be vectors of SCYLV: *Ceratovacuna lanigera* (sugarcane woolly aphid), *Melanaphis sacchari* (sugarcane aphid), *Rhopalosiphum maidis* (corn leaf aphid), and *R. rufiabdominalis* (rice root aphid) (Rott *et al*. 2008). The last three of these aphids are all present in Florida, but *M. sacchari* is considered to be the most widespread and efficient vector of SCYLV in sugarcane fields. Although infected sugarcane fields are present all year round in Florida, it was also hypothesized that a secondary host may play a role in dissemination of SCYLV. Under controlled conditions, several other grass species have been successfully infected with SCYLV (ElSayed *et al*. 2015), but only one was actually reported as a natural host of the virus: barley (*Hordeum vulgare*) in Tunisia (Bouallegue *et al*. 2014). Interestingly, barley plants that were found infected by SCYLV in this North African country were grown in fields that were about 100 km from sugarcane fields, suggesting that the inoculum source was not sugarcane but an unidentified wild grass (Maryem Bouallègue, personal communication).

Several grass species in close proximity to Florida sugarcane fields were sampled in 2015 and tested for SCYLV using serological and molecular diagnostic assays. All samples tested negative with the exception of *Sorghum almum* (Columbus grass). This species was found infected by SCYLV based on the reaction with SCYLV specific antibodies and the nucleic acid sequence of the virus coat protein (Espinoza Delgado *et al.* 2016). In the USA, *S. almum* is considered to be a federal and state noxious weed, and it is widely distributed in sugarcane growing areas in Florida. Interestingly, *S. almum* was also infested with high population levels of *M. sacchari* (> 100 individuals per plant), the sugarcane aphid, that also tested positive for SCYLV by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) (Wei *et al*. unpublished data). The importance of this new secondary host in epidemiology of yellow leaf of sugarcane remains to be established. However, if SCYLV can be efficiently transmitted from *S. almum* to sugarcane via aphids, control of this new grass host and/or the aphid populations, especially in areas where seed cane is produced, may contribute to reduction of incidence of SCYLV in commercial sugarcane fields in Florida.

CONCLUSION AND PERSPECTIVES

The sugarcane industry in Florida has succeeded in addressing several disease challenges over the past several decades, but will undoubtedly face new ones in the future. New strains of existing pathogens will develop or new diseases will be introduced for the first time. For example, it is estimated that it is only a question of time before tawny rust, a new rust disease that has recently been identified in South Africa (Martin *et al*. 2015), will cross the Atlantic Ocean and appear in the Western Hemisphere. Based on optimum temperatures for spore germination, we predict that tawny rust progress during the sugarcane crop season in Florida will resemble brown rust more than orange rust. Given the experience the local industry has acquired in controlling rusts with fungicides and developing resistant cultivars, it is expected that outbreaks of tawny rust could be effectively controlled upon arrival.

As use of fungicides should only be a temporary solution to control rusts, development of molecular makers will certainly be a key issue to rapidly screen for resistance to these diseases. If molecular markers are currently available for brown rust, additional markers are needed to screen for resistance to other diseases currently occurring in Florida such as orange rust and yellow leaf. Recent findings look very promising. Yang *et al*. (2016) are close to development of marker-assisted breeding for orange rust resistance, and putative resistance genes have recently been identified for resistance to yellow leaf (Costet *et al.* 2012b; Debibakas *et al*. 2014; Izquierdo *et al*. 2013). This could lead to identification of useful markers in breeding programs worldwide. In the interim, foreign germplasm resistant to SCYLV is being imported with the aim of increasing resistance sources to yellow leaf in Florida.

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La lutte contre les maladies de la canne à sucre en Floride: un défi en constante évolution

Résumé. Les maladies sont des facteurs limitants dans presque toutes les zones de culture de la canne à sucre. Plus de 40 maladies ont été décrites en Floride où la rouille brune, la rouille orangée et la feuille jaune impactent actuellement la production de canne à sucre. Idéalement, ces maladies devraient être contrôlées par l'utilisation de cultivars résistants, mais la plupart des variétés cultivées en 2015-2016 en Floride sont sensibles soit à la rouille brune, soit à la rouille orangée. Plusieurs cultivars qui étaient initialement résistants à la rouille orangée sont devenus sensibles quand ils ont été cultivés sur de grandes surfaces, suggérant un changement dans les populations de l'agent pathogène. La lutte contre ces deux rouilles repose donc essentiellement sur l'utilisation de fongicides. La plupart des cultivars sont aussi sensibles à l'infection par le *Sugarcane yellow leaf virus* (SCYLV), et il a été démontré que ce virus peut réduire les rendements en l'absence des symptômes de la maladie. L'utilisation de boutures saines à la plantation est seulement partiellement efficace, et la découverte récente d'un nouvel hôte et d'un vecteur potentiel du SCYLV pourrait expliquer les difficultés rencontrées pour lutter contre ce virus en Floride. L'identification de sources de résistance durable et le transfert de ces résistances à de nouveaux cultivars sont essentiels pour lutter efficacement contre les maladies de la canne à sucre en Floride.

Mots-clés: Feuille jaune, fongicides, lutte contre les maladies, résistance, rouille brune, rouille orangée

El control de las enfermedades de la caña de azúcar en Florida: un reto en constante evolución

Resumen. Las enfermedades son factores limitantes en casi todas las áreas donde se cultiva caña de azúcar. Más de 40 enfermedades han sido reportadas en Florida, con la roya marrón, la roya naranja y la hoja amarilla actualmente impactando sobre la producción de caña de azúcar. Idealmente, estas enfermedades deben ser controladas usando cultivares resistentes, no obstante la mayoría de las variedades plantadas en Florida en 2015-2016 son susceptibles a la roya marrón o a la roya naranja. Muchas variedades que inicialmente eran resistentes a la roya naranja se convirtieron en susceptibles cuando fueron plantadas a gran escala, sugiriendo un cambio en las poblaciones del patógeno. En estos momentos el control de ambas royas se realiza fundamentalmente con el uso de fungicidas. La mayoría de las variedades también son susceptibles a la infección por *Sugarcane yellow leaf virus* (SCYLV) y se ha demostrado que este virus puede reducir los rendimientos aún en ausencia de síntomas de la enfermedad. El uso de semilla de caña sana es sólo parcialmente eficaz y el reciente descubrimiento de un nuevo huésped y de un vector potencial de SCYLV podría explicar la dificultad en el control de este virus en la Florida. La identificación de fuentes sostenibles de resistencia y la transferencia de éstas a los nuevos cultivares son esenciales para el éxito en el control de las enfermedades de la caña de azúcar en la Florida.

Palabras clave: control de enfermedades, fungicidas, hoja amarilla, resistencia, roya marrón, roya naranja