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Original article

Severity of *Colletotrichum falcatum* Went in sugarcane resistance patterns in Cuba

Severidad de *Colletotrichum falcatum* Went en patrones de resistencia de caña de azúcar en Cuba

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ABSTRACT: Red stem rot is a disease widely distributed worldwide and causes significant losses in sugarcane plantations. The objective of this study was to determine the severity of *Colletotrichum falcatum* in sugarcane resistance patterns and their stability against disease. Plots were established in the experimental block of the Sugarcane Research Institute located in Mayabeque from October 2014 to 2019 with the disease resistance patterns. Artificial inoculation of the fungus was carried out at 12 months of age in 25 randomly selected stems of each cultivar. The evaluation was carried out four months later. Simple classification analysis of variance (ANOVA) and Tukey's means comparison test ($p < 0.05$) were performed to compare the percentages of disease severity in the evaluated cultivars and years; as well as a simple linear regression to relate the percentages of disease severity with rainfall, maximum, minimum and average temperatures, as well as relative humidity ($p < 0.01$). The severity of the disease showed significant differences, with a wide contrast between highly resistant and highly susceptible varieties. The highest percentages of severity were reached in 2014, 2015, and 2018; however, these rates bear little relation to the values of the meteorological variables studied during this period. This time of year is confirmed as the most favorable time for inoculation with *C. falcatum* to evaluate its behavior against red rot.

Key words: Disease, fungus, red rot, reaction.

RESUMEN: La pudrición roja del tallo es una enfermedad ampliamente distribuida en el mundo y ocasiona grandes pérdidas en las plantaciones cañeras. El objetivo de este trabajo fue determinar la severidad de *Colletotrichum falcatum* en patrones de resistencia de caña de azúcar y su estabilidad ente la enfermedad. Las parcelas se establecieron en el bloque experimental del Instituto de Investigaciones de la Caña de Azúcar ubicado en Mayabeque en el mes de octubre de los años del 2014 al 2019 con los patrones de resistencia a la enfermedad. La inoculación artificial del hongo se efectuó a los 12 meses de edad en 25 tallos elegidos al azar en cada cultivar. La evaluación se realizó cuatro meses después. Se efectuó análisis de varianza (ANOVA) de clasificación simple y prueba de comparación de medias por Tukey ($p < 0,05$), para comparar los porcentajes de severidad de la enfermedad en los cultivares evaluados y los años; así como una regresión lineal simple para relacionar los porcentajes de severidad de la enfermedad con las precipitaciones, temperaturas máximas, mínimas y medias, así como la humedad relativa ($p < 0,01$). La severidad de la enfermedad mostró diferencias significativas, con un amplio contraste entre el altamente resistente y el altamente susceptible. En los años 2014, 2015 y 2018 se alcanzaron los mayores porcentajes de severidad; aunque tienen poca relación con los valores de las variables meteorológicas estudiadas en este período. Se ratifica a esta época del año como la propicia para la inoculación con *C. falcatum* para evaluar su comportamiento ante la pudrición roja.

Palabras clave: Enfermedad, hongo, pudrición roja, reacción.

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INTRODUCTION

Red rot of the stalk, caused by *Colletotrichum falcatum* Went (conidial state) [= *Glomerella tucumanensis* (Spegazzini) Von Arx and E. Müller], is a widely distributed disease in areas where sugarcane (*Saccharum* spp.) is cultivated in the tropics (1). In Cuba, it is among the diseases detected since 1915, with a wide dispersion in sugarcane plantations of sugar companies (2,3).

The disease causes severe damage to sugarcane plants (4,5), which affects the commercial lifespan of many elite cultivars of this grass. In the northwestern region of São Paulo, Brazil, an 18.6 % decrease in agricultural yield has been estimated due to the effects of red rot in sugarcane plantations (6). It also influences juice quality, reducing sucrose content, purity, Brix degrees, and commercial sugar (7).

Symptoms of red rot can occur on both leaves and stalks of sugarcane (8). On leaves, they are of lesser importance, producing elongated spots on the upper side of the midrib, initially intense red in color, later turning reddish-brown (9,10). Lesions may extend along the entire rachis in both directions, eventually occupying the entire midrib (11). On stalks, they cause a color change of internal tissues to red, with dry rot at the tips (12,13). Internal putrefaction of plants is characterized by the presence of transverse white bands in tissues infected by *C. falcatum* (14-16). Furthermore, it causes discoloration of the foliage and stalk rind, leading to plant death and the death of new shoots in the stool (17,18).

The high genetic variability of *C. falcatum* increases the virulence of the fungus on sugarcane plants (19,20). This situation causes the loss of cultivar resistance due to the extensive commercial plantations of this grass, which increase exposure to this fungus, thus jeopardizing crop durability (21). Based on the aforementioned considerations, the management of this disease through resistant cultivars is difficult (22). Likewise, some authors point to a genotype-environment-pathogen interaction that influences the incidence and severity of red rot and warn about the erosion of resistance in commercial cultivars (23,24).

In Pakistan, the differential reaction of cultivars to red rot in sugarcane germplasm and changes in symptom severity have been reported (25); while this same situation is indicated in clones and commercial cultivars in India (26,27). Consequently, it is necessary to confirm the stability of cultivar behavior against the disease. Therefore, the objective of this study is to determine the severity of *Colletotrichum falcatum* on sugarcane resistance patterns.

MATERIALS AND METHODS

The study was conducted in the experimental block of the Sugarcane Research Institute on a typical Red Ferrallitic soil (28), located at the georeferenced coordinates North Latitude: 22° 47' 12'' and West Longitude: 82° 23' 13'', in the municipality of Quivicán, Mayabeque province, Cuba.

The experiments were established in plots consisting of a single row 7.5 m in length and 1.6 m in width, without replicates. Five cultivars categorized as resistance patterns to sugarcane red rot were planted ('PR980', highly resistant; 'Ja60-5', resistant; 'My5514', moderately resistant; 'B4362', susceptible; and 'C137-81', highly susceptible) (29) during the months of October from 2014 to 2019. No fertilizer applications were made, and only three irrigations were carried out with norms of 250 m^3 ha^{-1} every seven days after planting to ensure plant sprouting.

The third basal internode of 25 randomly selected stalks from each cultivar at 13 months of age (early November) was perforated with a 0.2 cm diameter, 2.0 cm deep punch. Into the hole, 0.2 mL of a conidial suspension of *C. falcatum* at a concentration of 5 × 10^5 CFU mL^{-1} was deposited using a syringe. The inoculum was obtained from diseased plants of 'C137-81' under natural conditions from the aforementioned experimental block. The inoculum was isolated in Petri dishes at the plant protection laboratories of the Basic Business Units of the Sugarcane Research Institute in Camagüey and Santiago de Cuba, where the conidial suspension was prepared at the aforementioned concentration.

Three and a half months after inoculation, the stalks were cut and split longitudinally. For disease symptom evaluation, the following criteria were considered: coloration; length and width of the lesion; presence, abundance, and characteristics of white spots produced by infection; number of infected internodes; and green, yellow, or dead terminal bud. Cultivar reaction was determined using a scale that considered the aforementioned criteria (Table 1) (17).

Disease severity in the cultivars was determined based on the reaction to *C. falcatum* of each inoculated plant and was calculated using the following mathematical expression (30):

Table 1. Scale for evaluating resistance to red rot of sugarcane

Degree	Categories	Description
1	Highly Resistant (HR)	Intense or dark red coloration occupying the entire inoculated internode but not extending to adjacent internodes. Absence of transverse white bands. Green terminal buds.
2	Resistant (R)	Elongated intense or dark red coloration occupying the entire inoculated internode and extending to adjacent internodes as wide bands. Absence of transverse white bands. Green terminal buds.
3	Moderately Resistant (MR)	Intense or dark red coloration occupying the entire inoculated internode and extending to adjacent internodes. Presence of small transverse white bands. Green terminal buds.
4	Susceptible (S)	Intense or dark red coloration occupying several internodes. Presence of abundant and prominent transverse white bands. Green terminal buds.
5	Highly Susceptible (HS)	Intense or dark red coloration occupying several internodes. Presence of abundant and prominent transverse white bands. Hollow internodes with mycelium production. Yellow or dead terminal buds.

$$SE = \left(\frac{\sum n * v}{i * N}\right) * 100$$

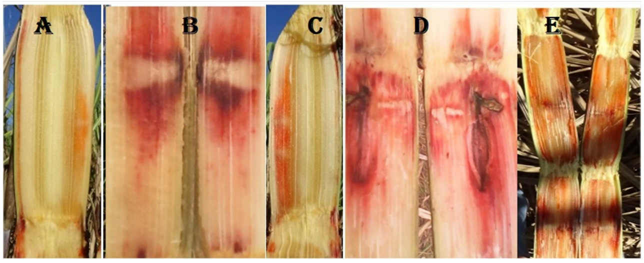
Where:
SE: disease severity
n: number of stalks infected for each disease rating
v: rating on the scale
i: highest rating on the scale
N: total number of samples

The meteorological variables: rainfall, minimum, average, and maximum temperatures, as well as relative humidity for the periods between fungal inoculation and disease severity evaluation, were collected and processed at the Güira de Melena Station of the National Institute of Meteorology (INSMET) (Table 2). This station is located 5 km from the site where the experiment was established, in the municipality of Güira de Melena, Artemisa province.

Statistical analysis was performed using a single-classification ANOVA and Tukey's test ($p < 0.05$) to compare disease severity percentages among cultivars and years. A simple linear regression analysis was also conducted to relate disease severity percentages to rainfall, maximum, minimum, and mean temperatures, and relative humidity ($p < 0.01$). All analyses were carried out using InfoStat statistical software, version 2015.

RESULTS AND DISCUSSION

The injected conidial suspension of *C. falcatum* was sufficiently virulent to produce disease in the inoculated sugarcane stalks (Figure 1). The cultivars categorized as resistance patterns to red rot achieved the infection levels proposed to evaluate their resistance and stability against this disease (29), thus confirming them as pattern cultivars for each category of the scale.



Infection degrees caused by *C. falcatum* on sugarcane stalks. A, degree 1; B, degree 2; C, degree 3; D, degree 4; E, degree 5

Figure 1. Red rot symptoms on stalks of the highly susceptible cultivar 'C137-81'

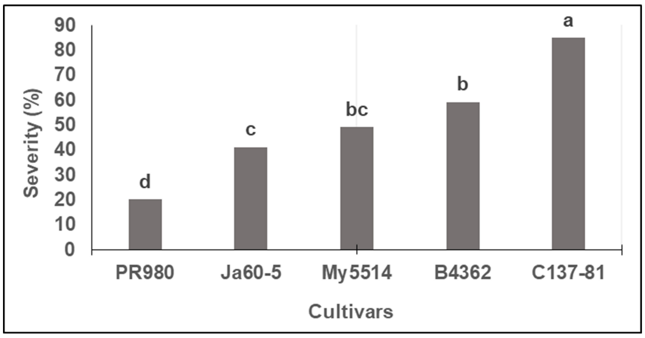
Table 2. Meteorological data recorded at the Güira de Melena Station

Years	Months	Temperatures (°C)			Relative humidity (%)	Rainfalls (mm)
		Minimum	Maximum	Means		
2015	November	21.8	30.9	25.5	80	83
2015	December	21.4	29.4	24.7	84	54
2016	January	18.0	26.2	21.6	81	192
2016	February	16.2	26.5	21.0	75	22
2016	November	17.4	29.3	23.2	74	0
2016	December	19.5	29.9	24.5	81	12
2017	January	16.7	27.5	22.0	78	6.5
2017	February	19.0	29.4	23.6	77	21
2017	November	20.0	29.6	24.3	81	169
2017	December	18.1	27.9	22.5	81	24
2018	January	17.4	27.9	22.1	75	94
2018	February	17.3	28.3	17.6	74	22
2018	November	17.5	30.3	23.5	76	31
2018	December	17.3	28.4	29.9	79	52
2019	January	16.9	26.9	21.6	80	10
2019	February	18.0	29.0	22.3	79	14
2019	November	19.2	28.1	23.6	79	43
2019	December	20.6	27.8	24.6	82	12
2020	January	17.3	28.5	22.5	73	0
2020	February	16.7	27.8	22.5	75	39
2020	November	20.4	30.2	25.1	80	55
2020	December	18.6	28.9	23.3	80	0
2021	January	16.8	25.8	21.9	77	31
2021	February	18.4	26.9	22.6	76	5

Source: Prepared by the National Institute of Meteorology (INSMET), Güira de Melena Station

For statistical analysis, a single-classification analysis of variance (ANOVA) and Tukey's mean comparison test ($p < 0.05$) were used to compare disease severity percentages among the evaluated cultivars and across years. Additionally, a simple linear regression analysis was performed to relate disease severity percentages to rainfall, maximum, minimum, and mean temperatures, as well as relative humidity ($p < 0.01$). All analyses were conducted using the statistical software package InfoStat, version 2015.

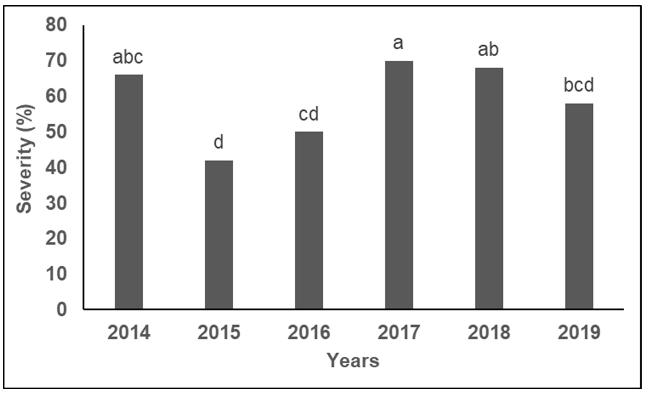
Significant differences ($p \leq 0.05$) were observed in disease severity percentages for each cultivar categorized as a resistance pattern to red rot (Figure 2). Severity ranged from 20 to 100 %. For 'PR980', it was 20 %; for 'Ja60-5', it ranged from 34 to 52 %; for 'My5514', from 38 to 58 %; for 'B4362', from 42 to 70 %; and for 'C137-81', from 70 to 100 %.



Means with different letters are significantly different according to Tukey's mean comparison test ($p < 0.05$)

Figure 2. Mean red rot severity percentages for each cultivar evaluated over the six study years (2014-2019)

The average severity percentages of *C. falcatum* in the cultivars used as resistance patterns showed significant differences across the study years ($p \leq 0.05$) (Figure 3). The highest values were reached in 2014, 2015, and 2018, with a marked decrease in 2015 and 2016.



Different letters differ significantly according to Tukey's mean comparison test ($p < 0.05$)

Figure 3. Average severity percentages of red rot for each year of the study, across the cultivars used as resistance patterns to this disease

Mean red rot severity percentages were poorly correlated with rainfall (Figure 4), minimum (Figure 5), mean (Figure 6), and maximum temperatures (Figure 7), as well as relative humidity (Figure 8) during the 2014-2019 evaluation period. This may be attributed to the narrow range of variation in meteorological variables during the months between fungal inoculation and symptom assessment. These findings confirm that this time of year is suitable for *C. falcatum* inoculation in sugarcane stalks to evaluate their response to the disease.

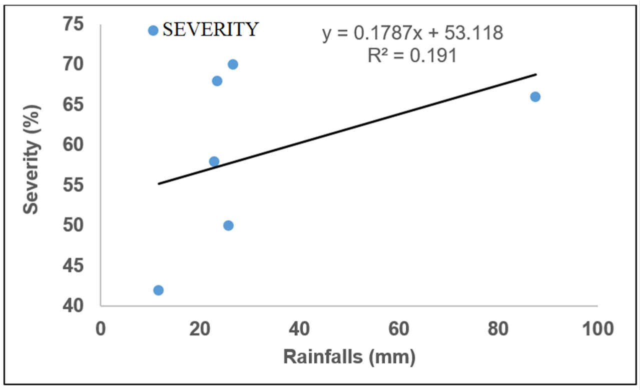


Figure 4. Relationship between mean red rot severity in resistance pattern cultivars to *C. falcatum* and rainfall, based on simple linear regression analysis ($p < 0.01$)

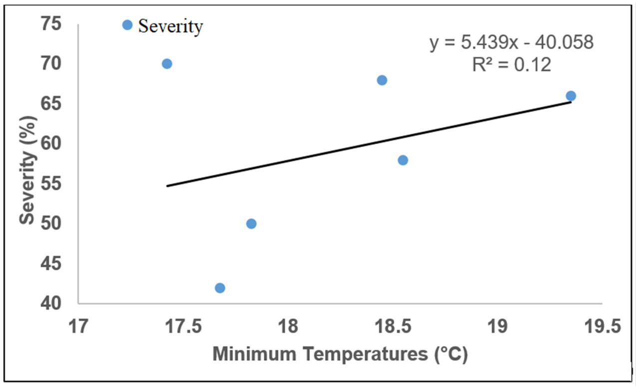


Figure 5. Relationship between mean red rot severity in resistance pattern cultivars to *C. falcatum* and minimum temperatures, based on simple linear regression analysis ($p < 0.01$)

Statistically significant differences were observed in the average severity percentages of *C. falcatum* reached in the cultivars used as resistance patterns to red rot across the evaluated periods, even though these experiments were weakly influenced by the values of the meteorological variables. However, studies conducted in commercial sugarcane plantations demonstrated the influence of minimum temperatures between 25.8 and 26.4 °C, relative humidity in the range of 70 to 90 %, and monthly rainfall exceeding 30 mm on the increased incidence of this disease (31).

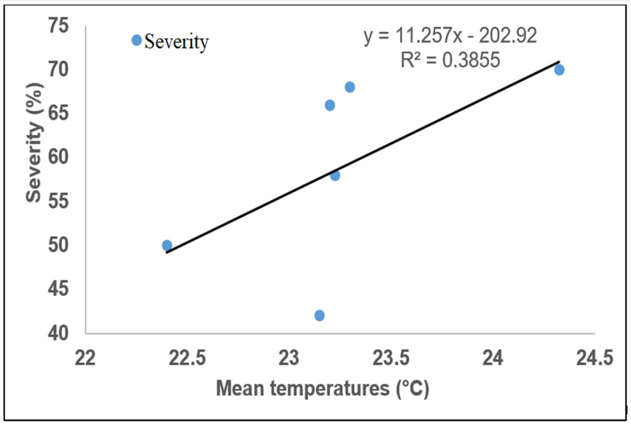


Figure 6. Relationship between mean red rot severity in resistance pattern cultivars to *C. falcatum* and mean temperatures, based on simple linear regression analysis ($p < 0.01$)

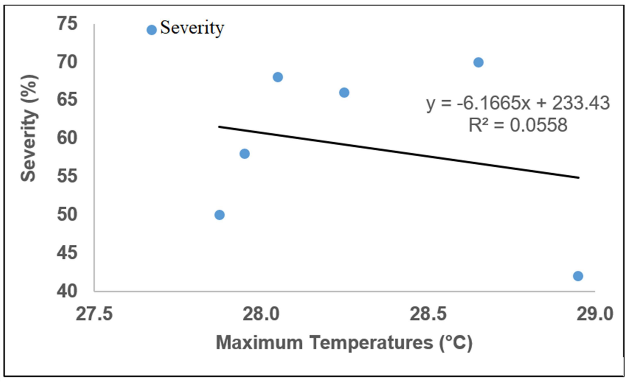


Figure 7. Relationship between mean red rot severity in resistance pattern cultivars to *C. falcatum* and maximum temperatures, based on simple linear regression analysis ($p < 0.01$)

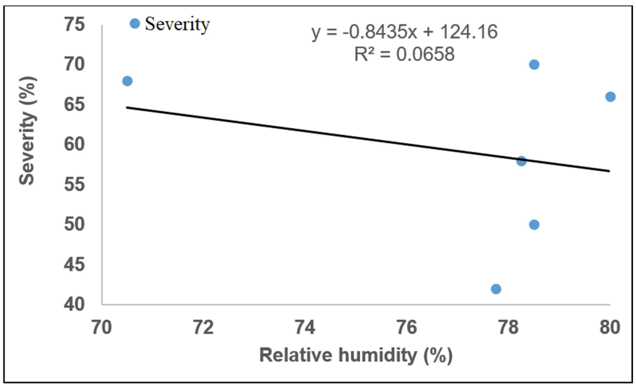


Figure 8. Relationship between average red rot severity in cultivars used as resistance patterns to *C. falcatum* and relative humidity, according to simple linear regression analysis ($p < 0.01$)

Indeed, rainfall and relative humidity are factors that favor the conditions for red rot development, and the highest incidence percentages of this disease were detected in regions where these factors annually reached the highest values, 4387 mm and 85 %, respectively (32). The vital role of meteorological variables, particularly rainfall and temperatures, in the development of *C. falcatum* is also reported, as infection severity increases with temperatures between 23 and 24 °C (minimum), 34 and 37 °C (maximum), and a high rainfall regime (33,34).

Rainfall enhances the dissemination and infection of the fungus in plants, while the most favorable temperatures for disease development are between 29 and 31 °C, with relative humidity values above 85 % (35). In this regard, it has been noted that the minimum temperature for *C. falcatum* infection to occur is 15 °C, while the optimum temperature is 30 °C (36).

CONCLUSIONS

- Red rot severity in cultivars used as resistance patterns to *C. falcatum* showed significant differences ($p \leq 0.05$), with a wide contrast between the highly resistant and the highly susceptible cultivars.
- The years in which the cultivars used as resistance patterns to *C. falcatum* reached the highest average red rot severity percentages were 2014, 2015, and 2018.
- The average red rot severity percentages in the cultivars are poorly related to minimum, mean, and maximum temperature values, as well as to rainfall and relative humidity during the evaluated period from 2014 to 2019.

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