Cite: Sandoval Contreras, T., Íñiguez Moreno, M., Garrido Sánchez, L., Ragazzo Sánchez, J. A., Narváez Zapata, J. A., & Calderón Santoyo, M. (2022). Effect of temperature on the interaction between *Rhizopus stolonifer* and *Colletotrichum* sp., postharvest pathogens of jackfruit (*Artocarpus heterophyllus* Lam.). *Nova Scientia*, *14*(28), 1-15.
doi.org/10.21640/ns.v14i28.2966

Effect of temperature on the interaction between *Rhizopus stolonifer* and *Colletotrichum* sp., postharvest pathogens of jackfruit (*Artocarpus heterophyllus* Lam.)

Efecto de la temperatura en la interacción entre *Rhizopus stolonifer* y *Colletotrichum* sp., patógenos de postcosecha en la yaca (*Artocarpus heterophyllus* Lam.)

Teresa Sandoval Contreras [1] - Maricarmen Íñiguez Moreno [1] - Luis Garrido Sánchez [2] - Juan Arturo Ragazzo Sánchez [1] - José Alberto Narváez Zapata [3] - Montserrat Calderón Santoyo [1] ✉

[1] Tecnológico Nacional de México. Instituto Tecnológico de Tepic
[2] Instituto Tecnológico y de Estudios Superiores de Occidente
[3] Instituto Politécnico Nacional. Centro de Biotecnología Genómica
✉ Correspondence author: mcalderon@tepic.tecnm.mx

Reception: 29-07-2021 / Acceptation: 20-10-2021

Abstract

Jackfruit (*Artocarpus heterophyllus* Lam.) is a climacteric fruit whit a high commercial value, but susceptible to decay. The most important pathogens for jackfruit *R. stolonifer* and *C. gloeosporioides* may interact in different ways. The objectives of this study were to examine the interaction between three fungal species isolated from jackfruit and describe their behavior and interactions as a function of temperature. Three pathogen isolates from jackfruit rots, cultivated individually or paired at 4 cm in a natural medium were used. The growth rate was evaluated at 13, 25, and 35 °C. The Baranyi-Roberts model was used to obtain the radial growth rate. Differences between the growth rate of each fungus are the reference to determine the type of interaction. The effect of temperature of the unpaired isolates was studied by Rosso-Robinson's model. The experiment was validated by infecting fresh jackfruit whit a mix of spores of all isolates. *Rhizopus stolonifer* is capable of rapidly colonizing the Petri dish thus reducing the space for the other fungi. The growth rates of unpaired and paired fungal isolates were statistically different showing that interactions between them exist. *Rhizopus* intermingling the others at 13 and 25 °C whereas, at the same temperatures, Colletotrichum AhCx-02 dominates AhCx-03. In contrast, dominance patterns of the *Colletotrichum* AhCx-03 strain were higher at 35 °C.

Keywords: fungal competence; dominance; growth model; postharvest pathogens; *Rhizopus stolonifer; Colletotrichum gloeosporioides*; crops; strains; temperature; jackfruit; interaction

Resumen

La yaca (*Artocarpus heterophyllus* Lam.) es un cultivo climatérico con alto valor comercial y muy susceptible al deterioro en postcosecha. *Rhizopus stolonifer* y *Colletotrichum gloeosporioides* son patógenos de postcosecha en la yaca que pueden interactuar de diversas maneras. El objetivo de esta investigación fue examinar la interacción entre tres especies de hongos patógenos aislados de la yaca y establecer su comportamiento e interacción en función de la temperatura. Se utilizaron cepas de hongos patógenos de yaca cultivados en un medio natural. Se estudió el crecimiento de los hongos solos y pareados a 4 cm de distancia a 13, 25 y 35 °C. Con el modelo de Baranyi-Roberts se obtuvieron datos de velocidad radial y se analizaron estadísticamente para determinar el tipo de interacción de acuerdo con sus diferencias. El efecto de la temperatura en cada hongo sin competencia se obtuvo con el modelo de Rosso-Robinson. Finalmente, el experimento se validó infectando yaca fresca con una mezcla de los aislados y se observó

su comportamiento. *Rizhopus stolonifer* mostró un rápido crecimiento, contrario a las dos cepas de *Colletotrichum* sp. utilizadas (AhCx-02 y AhCx-03). Las velocidades de crecimiento mostraron diferencias significativas tanto en los hongos cultivados solos como en los pareados, demostrando que existe interacción entre ellos. *Rhizopus* se entremezcla con los otros hongos, mientras que la cepa AhCx-02 de *Colletotrichum* domina a la cepa AhCx-03, tanto a 13 como a 25 °C. A temperatura de 35 °C la cepa AhCx-03 parece ser la dominante.

Palabras clave: competencia; dominancia; modelo de crecimiento; hongos patógenos postcosecha; *Rhizopus stolonifer*; *Colletotrichum gloeosporioides*; cultivos; cepas; temperatura; yaca; interacción

1. Introduction

Recently, there is interest in jackfruit culture because of its unique organoleptic characteristics and nutritive properties. Nayarit is the main producer region in Mexico and exports most of the production (Medina-Tiznado *et al.*, 2018). It has a high success at regional, national and international level due to its production yield, high commercial value (Luna-Esquivel *et al.*, 2013), and a great commercial demand in many countries (Ragazzo-Sánchez *et al.*, 2011). Jackfruit research represent an opportunity for studies because the information about growing methods or pest and rot management is scarce (Luna-Esquivel *et al.*, 2013).

Jackfruit (*Artocarpus heterophyllus* Lam.) is a climacteric fruit with a high respiration rate, and during the ripening, there are many changes in their composition as a change in acidity and sugar content. The main problem in the quality of jackfruit is the over-ripening, malformation of fruit or pedunculi and rots that cause rapid decay of fruit due to high temperatures and relative humidity, conditions highly encountered in their production area (Ragazzo-Sánchez *et al.*, 2011; Luna-Esquivel *et al.*, 2013). Concerning postharvest disease of jackfruit, *Rhizopus artocarpi* and other species of *Rhizopu*s have been reported as a cause of rots (Ghosh *et al.*, 2015; García-Estrada *et al.*, 2019), as well as *Lasiodiplodia theobromae* (Medina-Tiznado *et al.*, 2018), *Aspergillus niger* (Ragazzo-Sánchez *et al.*, 2011) and *Colletotrichum gloeosporioides* (Bhunjun *et al.*, 2019).

Fruit surface is normally colonized by a mixture of microorganisms as epiphytes. Normally, most of these microorganisms are not pathogenic, but they role in fruit health, quality and disease resistance, before and after harvest is largely unknown (Droby & Wisniewski, 2018). Regarding postharvest disease, the impact of interactions of the pathogenic fungi is little known. Interspecific interactions can occur between fungi including mutual intermingling, mutual inhibition, and dominance by one species over another. The main types of interaction between filamentous fungi are competition for space, consequently, competition for resources in the substratum. However, biotic and abiotic factors affect the spatial competition such as temperature, pH, humidity, type of substratum, species identity, etcetera (Kolesidis *et al.*, 2019). It might give them an advantage to outcompete other fungal colonizers. Understanding their interactions and their dominance will result in a prediction of their growth with the aim of avoiding damage and loss of quality in storage or transport conditions of jackfruit. The objectives of this study were to *in vitro* examine the effect of temperature on the growth of three fungal species isolated from jackfruit epicarp and their interaction.

2. Methods, techniques, and instruments

Fungal strains

Rhizopus stolonifer AhRs-01 isolated from jackfruit (*Artocarpus heterophyllus* Lam.) held in the Laboratory of Food Microbiology of the Technological Institute of Tepic, Nayarit Mexico, and two native molds

isolated directly from different rots of jackfruit were included in this research. Macro and microscopical characterization of the two isolates were carried out according to Barnett & Hunter (1998) and Carrillo (2003).

Medium

Jackfruit pericarp agar (AJ) was specifically designed for this study, in which composition contains 0.6 % of pectin (Xu *et al.*, 2018). The AJ medium contains 28 g of finely ground jackfruit pericarp and 15 g of agar per 1,000 mL of distilled water at pH 5.5 ± 0.2. The medium was autoclaved and poured into 90-mm sterile petri dishes. The water activity (*wa*) was measured at the beginning and at the end of the experiments (AquaLab Pre Water Activity Meter, Decagon Devices, Pullman, WA), and must remain stable during the experiment and near 1.

Inoculation

All the strains were cultured on PDA at 25 °C. From the margin of a 5-day old growing colony, a disc of 5 mm diameter agar made with a core borer was taken for all strains, except for one faster growing-strain, which a one-day old colony was taken. A disc of the paired isolate was taken and transferred to the AJ plate and placed 4.0 cm apart from the other in the dish. Petri dishes of AJ medium were inoculated centrally with agar the disc of each isolate as a control. They were incubated at 13, 25, and 35 °C. Once the growth started, radial measurements were periodically made of each colony, one on a line joining the two inoculation points, the other at the opposite side of the colony. All isolates were cultures alone at the same temperature conditions as a control.

The radius of the colony was plotted versus time to calculate the growth rate. Nonlinear regression was applied to estimate the maximum growth rate (μ_{max}, mm/day), by fitting the experimental data to the Baranyi & Roberts model (Baranyi & Roberts, 1994) (equations 1 and 2).

$$R = R_0 + \mu_{max} A - \frac{1}{\mu} \ln\{1 + \frac{[\exp(\mu_{max} A) - 1]}{\exp(R_{max} - R_0)}\} \quad (1)$$

$$A = t + \left(\frac{1}{\mu_{max}}\right) \ln[\exp(-\mu_{max} t) + \exp(-\mu_{max} \lambda) - \exp(-\mu_{max} t - \mu_{max} \lambda)] \quad (2)$$

Where: R is the colony radius (mm); R_0 is the colony radius at the time $t = 0$, R_{max} is the maximum colony radius in Petri dishes, and A is an integral variable running from 0 to t, in function of the curvature of the plot. The curve fitting procedure used Marquardt's algorithm of the StatGraphics Centurion XV.II (Maryland, USA) with 95 % of confidence.

Effect of temperature

The effect of temperature on μ_{max} for each unpaired fungus was estimated using the cardinal model with inflexion (CMI) developed by Rosso *et al*. (1993) (equation 3). Cardinal temperatures (T_{min}, T_{max}, and T_{opt}) values were estimated using this model. Temperatures in this work were chosen to simulate the optimum and the extremes conditions that may surround the jackfruit supply chain.

$$\mu_{max} = \frac{\mu_{opt}(T - T_{max})(T - T_{min})^2}{(T_{opt} - T_{\min})\{(T_{opt} - T_{min})(T - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T)\}} \quad (3)$$

Where μ_{max} is the growth rate (mm/d); μ_{opt} is the growth rate at optimal temperature; T is the temperature (° C); and T_{min}, T_{max}, and T_{opt} are cardinal temperatures. Marquardt's algorithm was used for model fitting with Statgraphics Centurion XV.II (Maryland, USA) with 95 % of confidence.

Types of interaction and ID calculation

Fungal paired interaction was observed as a function of the growth rate of each fungus alone or paired to another. Data were statistically analyzed by the HSD test. When significant differences existed in the growth rate of fungus in the line face of the other fungi, and when it grew at the opposite side and/or alone, implied that fungus was affected by the other. Then, a numerical score assigned based on the type of interaction according to Magan & Lacey method: [1] when mutual intermingling exist; [2] mutual antagonism on contact or with free space between fungus colonies < 2 mm; [3] mutual antagonism at a distance; dominance on contact [4 for the dominant species, 0 for the inhibited species] and dominance at a distance [5 for the dominant species, 0 for the inhibited species] (Magan & Lacey, 1985; Sempere & Santamarina, 2010).

Microscopic observation

A microscopic study of the fungi interactions according to Li *et al*. (2013). was performed. Brief, glass microscope slides with PDA were placed into sterile Petri dishes whit a humidified medical tissue. Discs (5 mm) were cut from the edge of each colony as previously described. One disc of each isolate was transferred to one end of each agar-covered slide and one of the other isolates to the other end, then the two discs were 2 cm apart. Slides containing the isolate growing alone served as controls. Slides into the Petri dishes were incubated at 25 °C in the dark until an interaction zone appeared. Interactions between each paired isolate were observed under a microscope Motic BA310 and results were photographed.

Validation

An experiment was performed on jackfruit to compare the growth of mold on the surface of the fruit and validate the predictions of the models. Jackfruit was disinfected with sodium hypochlorite (1 % v/v) for 2 min and rinsed with sterile water (Arias & Toledo, 2000), then, fruit was inoculated by triplicate with either single or mixed isolates of the fungal species into an artificial wound (3 mm diameter by 3mm deep) with 10 µl of suspensions adjusted to 10^{6} spores/ml (Iñiguez-Moreno *et al.*, 2020). The fruit was incubated at 25 °C at a RH > 85 %. The diameter of infection and lesion was daily measured. The radius of each infection against time was plotted and the Baranyi & Roberts model was used to calculate the growth rate. Comparison between the predicted growth rate and the observed growth rate was assessed by using the accuracy, A_f and the bias B_f factors (Ross, 1996).

$$B_f = 10^{[\Sigma \log (\frac{tv_{predicted}}{tv_{observed}})/n]} \quad (2)$$

$$A_f = 10^{[\Sigma |\log (\frac{tv_{predicted}}{tv_{observed}})|/n]} \quad (3)$$

The fungal genera could be differentiated from each other on the jackfruit peel based on their color and morphology, thus a microscopical observation of rots was performed (Steel *et al.*, 2011).

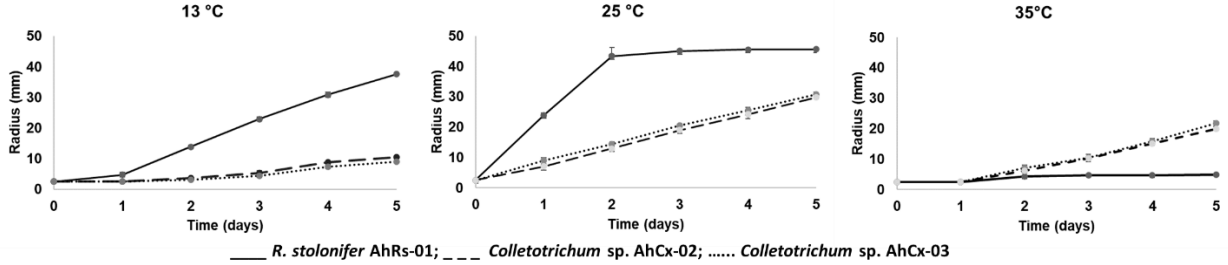
3. Results and discussion

3.1 Fungal isolates

The two isolates taken from different rot lesions from jackfruits were morphologically different at the naked eye. The microscopic structural characters were recorded with a digital camera (data not shown). Both isolates were identified following standard literature (Barnett & Hunter, 1998; Carrillo, 2003) as *Colletotrichum* sp., named AhCx-02 and AhCx-03.

The experiments were performed at three different temperatures and the *wa* remains stable during the incubation time at all conditions (*aw* = 0.97 ± 0.03). The data of each isolate individually cultured on AJ medium at 13, 25, and 35 °C were plotted against time to individually obtain the growth curves (figure 1).

Figure 1. *Radii of growth versus time describing the growth of «R. stolonifer» and «Colletotrichum» sp. strains AhCx.02 and AhX-03 isolated from jackfruit rots.*
Figura 1. *Radios de crecimiento contra el tiempo que describen el crecimiento de las cepas «R. stolonifer» and «Colletotrichum» sp. AhCx.02 y AhX-03 aisladas de pudriciones de yaca.*



Growth of the unpaired isolates followed a biphasic model at extremes temperatures. *Rhizopus stolonifer* showed a linear growth at 35 °C, then a linear regression by Excel was used to evaluate μ_{max} (table 1) with a determination coefficient $R^2 > 0.82$. For all the other strains and conditions, the Baranyi & Roberts model was used to estimate μ_{max} ($R^2 > 0.95$) (data not shown). All sets of data were statistically examined.

Table 1. *Radial growth rate (μ_{max}) estimated by linear regression or the Baranyi-Roberts model for «Rhizopus» AhRs-01 and «Colletotrichum» AhC-02 and AhC-03 isolates from jackfruit epicarp.*
Tabla 1. *Velocidad radial (μ_{max}) estimada por regression lineal con el modelo de Baranyi-Roberts para «Rhizopus» AhRs-01 y «Colletotrichum» AhCx-02 and AhCx-03 respectivamente, aislados del epicarpo de yaca.*

	***μ_{max} (mm/d) at 13 °C**	***μ_{max} (mm/d) at 25 °C**	***μ_{max} (mm/d) at 35 °C**
AhRs-01[a]	8 ± 0.1	18.9 ± 2.3	0.6 ± 0.1
AhCx-02 [b]	1.9 ± 0.05	5.9 ± 0.1	4.4 ± 0.1
AhCx-03[b]	1.8 ± 0.05	6.1 ± 0.1	3.4 ± 0.1

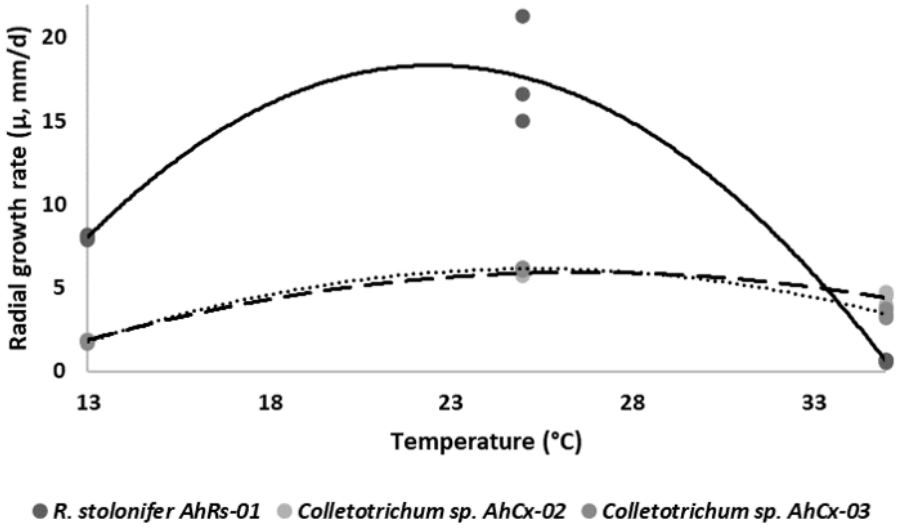
Note: * Values are means ± standard errors. Different letters indicate significant differences ($P < 0.05$).
Nota: * Los valores son las medias ± el error estándar. Letras diferentes indican diferencias significativas ($P < 0.05$).

In general, growth rates increased with temperatures near to de optimum and decreased at extreme conditions. Significant differences were observed among the unpaired isolates. *Colletotrichum* AhCx-02 and 03 formed a homogeneous group ($P > 0.05$) with a very similar value in μ_{max} at the same temperature.

On the other hand, *R. stolonifer* grew faster than the others at 13 and 25 °C occupying all the area in the Petri dish in a few days ($P < 0.05$). However, at 35 °C this isolate had poor growth.

By the CMI model (equation 3), the effect of temperature on μ_{max} was determined and the cardinal values were estimated. The fitted curves showing the influence of temperature on μ_{max} are presented in figure 2.

Figure 2. *Radial growth rate (μ_{max}, mm/day) versus temperature (T, °C) for «R. stolonifer» (continuous line), «Colletotrichum» ps. AhCx-02 (hatched line) and «Colletotrichum» ps AhCx-03 (dotted line) isolated from jackfruit. Points are observed data, and lines indicate the fit of the data to the cardinal model with inflexion (equation 3).*
Figura 2. *Velocidad radial (μ_{max}, mm/day) versus temperatura (T, °C) para «R. stolonifer» (línea continua), «Colletotrichum» ps. AhCx-02 (línea rayada) y Colletotrichum ps. AhCx-03 (línea punteada) aislados de la yaca. Los puntos gruesos indican los datos observados y las líneas indican el ajuste de los datos al modelo cardinal con inflexión (ecuación. 3).*



Coefficient estimations are in table 2 for all strains. The calculated optimal μ_{max} was higher for the strain AhRs-01, value three times higher than the other strains. The theoretical T_{max} was different depending on the isolate, varying between 35 – 43 °C and the theoretical T_{opt} was 22 to 27 °C. All parameters obtained are in the 95 % confidence interval.

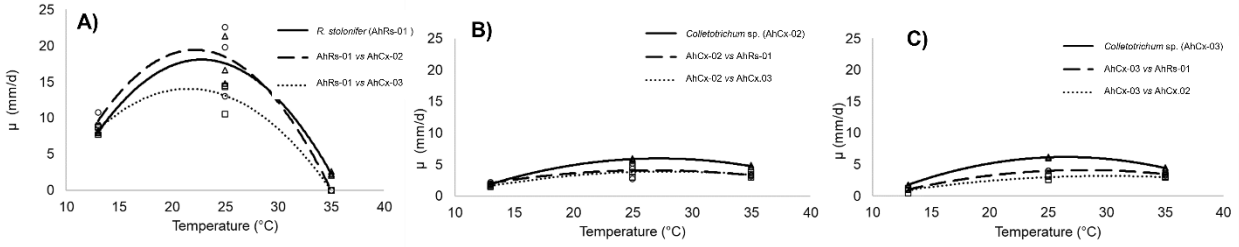
Table 2. *Estimated cardinal coefficients for the Rosso* et al. *(1993) model fitted to growth rates for three unpaired fungal isolates from jackfruit on AJ medium*.*
Tabla 2. *Coeficientes cardinales estimados de la ecuación de Rosso* et al. *(1993) ajustado a las velocidades de crecimiento de tres aislados de yaca, no pareados, en medio de cultivo AJ*.*

Isolate	μ_{opt}	T_{opt}	T_{max}	T_{min}	R^2	***RMSE***
R. stolonifer AhRs-01	18.20 ± 1.2	22.76 ± 1E-7	35.20 ± 0.4	8.06 ± 2.4	0.95	4.3
Colletotrichum sp. AhCx-02	5.90 ± 0.1	27.00 ± 1E-8	43.00 ± 1.1	10.50 ± 3.7	0.98	0.1
Colletotrichum sp. AhCx-03	6.14 ± 0.1	25.34 ± 1E-8	39.90 ± 0.3	11.13 ± 0.9	0.99	0.03

Note: * Values are means ± standard deviations.
Nota: * Los valores son las medias ± la desviación estándar.

The plug of the mycelium of each species inoculated on Petri dishes in all experiments expands outwards in a radially symmetric way that competition was determined by observing the colony peripheries. Thus, a one-dimensional mathematical model sufficient to investigate such competition corresponding to the growth along the line connecting the centers of the inoculation sites, compared with the opposite side. The dual growth of each strain was affected by the temperature and the paired species. The effect of temperature on growth for each fungus alone or paired to the other species was showed in figure 3. Initially, the growth of the paired colonies was not affected. Later, the interaction significantly influenced their development depending on the temperature.

Figure 3. *Effect of temperature on growth of A): «R. stolonifer», B): «Colletotrichum» sp. AhCx-02, and C) Colletotrichum sp. AhCx.03 in competence to the two other species. The different lines indicate the difference in growth rate as a function of temperature when fungus grows alone or paired.*
Figura 3. *Efecto de la temperatura en el crecimiento de A): «R. stolonifer», B): «Colletotrichum» sp. AhCx-02, y C) «Colletotrichum» sp. AhCx.03 en competencia con las otras especies. Las diferentes líneas en cada gráfico indican la diferencia en la velocidad de crecimiento en función de la temperatura cuando el hongo crece solo o pareado a otro.*



At extremes temperatures, the interaction was no significant for all paired isolates while near the optimal conditions their interactions were more clearly observed.

Three distinct types of interactions have been observed: I) intermingling, when fungi overlap and both fungal colonies coexist such as pairwise AhRs-01 *versus* AhCx-02 at 13 and 25 °C; II) mutual antagonism with a significant reduction in the growth rate, even if they overlap like strains AhCx-02 *versus* AhCx-03 at 35 °C, and III): dominance on contact (strain AhCx-02 *versus* AhCx-03 at 13 °C) (table 3).

The isolates AhCx-02 and AhCx-03 cultured unpaired, their growth rate appeared to be similar ($P > 0.05$), but based on their interactions, they are not ($P < 0.05$).

Table 3. *Index of dominance I_D* given to different species when growing a paired with another species.*
Tabla 3. *Índice de dominancia I_D* obtenido para las diferentes especies cuando crecen pareadas a otra.*

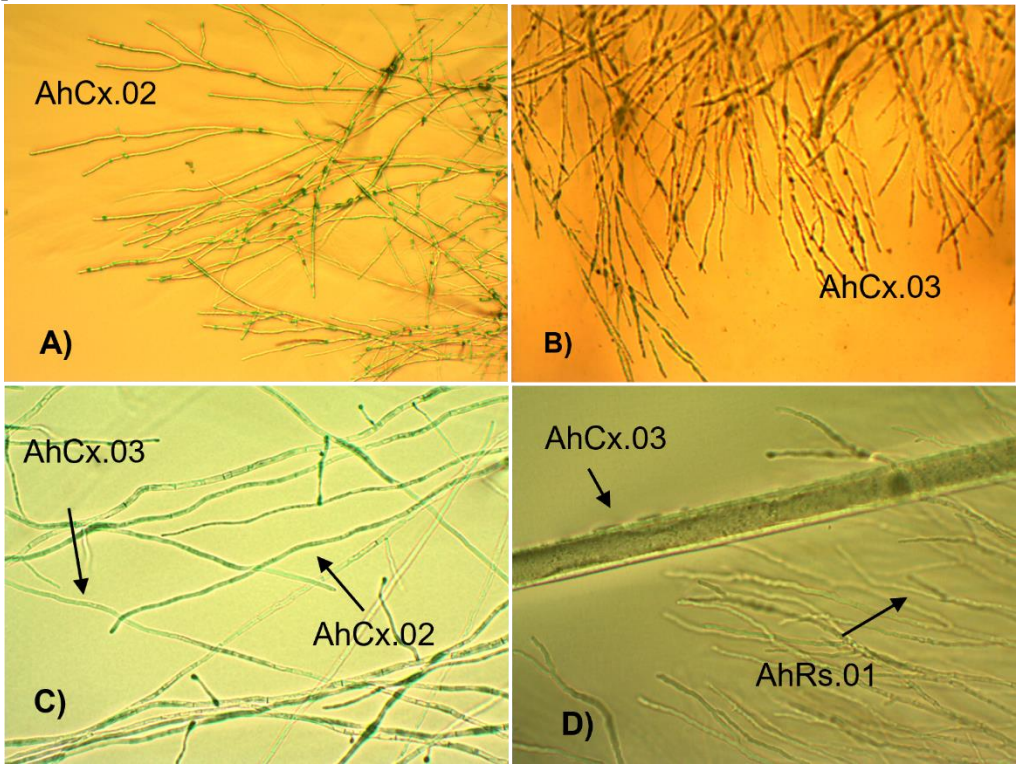
Temperature	Isolate *vs*/	AhRs-01	AhCx-02	AhCx-03	I_D
13 °C	*R. stolonifer* AhRs-01	_	1	1	2
	Colletotrichum sp. AhCx-02	1	_	4	5
	Colletotrichum sp. AhCx-03	1	0	_	1
25 °C	*R. stolonifer* AhRs-01	_	1	0	2
	Colletotrichum sp. AhCx-02	1	_	4	5
	Colletotrichum sp. AhCx-03	4	0	_	4
35 °C	*R. stolonifer* AhRs-01	_	0	0	0
	Colletotrichum sp. AhCx-02	1	_	2	3
	Colletotrichum sp. AhCx-03	1	2	_	3

Note: * I_D refers to the sum of scores at a certain temperature for each strain based on the interaction scores for each species. Types of interaction: a) Mutual intermingling [1]; b) mutual antagonism on contact [2]; c) mutual antagonism at a distance [3]; d) dominance on contact [4 for the dominant species, 0 for the inhibited species], and d) dominance at a distance [5 for the dominant species, 0 for the inhibited species].
Nota: * I_D refiere a la suma de los puntajes a cierta temperatura para cada aislado basado en la puntuación para cada especie. Tipos de interacciones: a) entremezclado mutuo [1]; b) antagonismo mutuo al contacto [2]; c) antagonismo mutuo a distancia [3]; d) dominancia al contacto [4 para la especie dominante, 0 para la especie inhibida], y d) dominancia a distancia [5 para la especie dominante, 0 para la especie inhibida].

The type of interactions was evaluated based on their growth rate at different conditions are shown in table 3. At 13 °C the growth of *R. stolonifer* has been enhanced in contact with *Colletotrichum* AhCx-02 ($P < 0.05$), but not near *Colletotrichum* AhCx-03, while AhCx-02 has affected the strain AhCx-03 diminishing their growth rate ($P < 0.05$). Near the optimal conditions, the strain AhCx-02 enhances the growth rate of *R. stolonifer* with no significant differences while AhC-03 diminishes their growth rate ($P < 0.05$). On the other hand, the isolate AhCx-03 was affected by AhCx-02 also diminishing their growth rate ($P < 0.05$). Moreover, at 35 °C, the temperature appears to inhibit the growth of strain AhRs-01, and the strains AhCx-02 and AhCx-03 showed mutual antagonism. According to de Index of Dominance (I_D) obtained, the strain AhCx-02 was more competitive at low temperature and optimal conditions than the others, while at 35°C, AhCx-02 and AhCx-03 coexist.

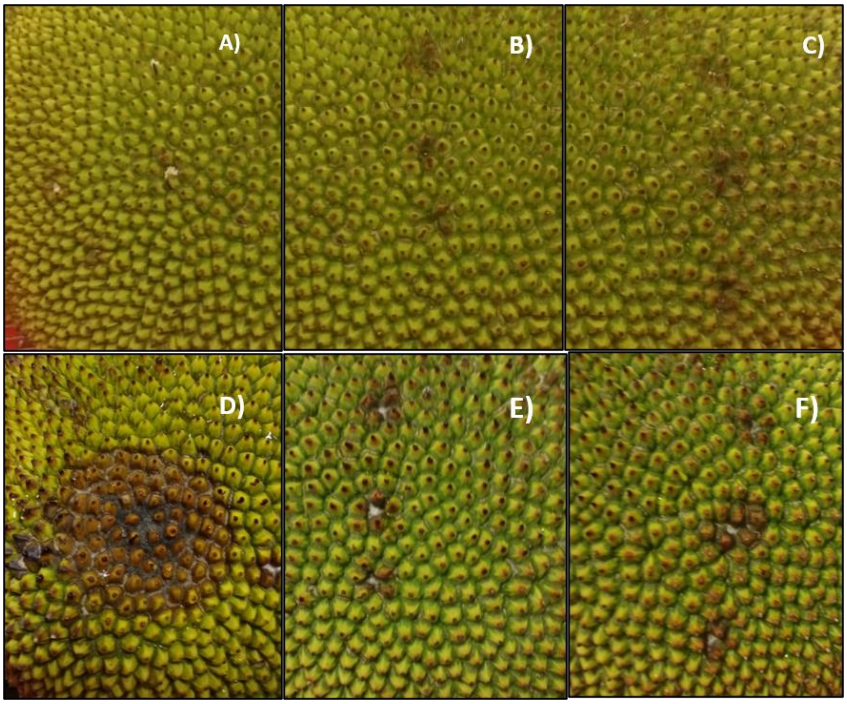
Microscopically, when a *Colletotrichum* sp. grew unpaired, the hyphae freely develop (figure 4 A and B) as well as they are paired (figure 4 C). The thin hyphae of *R. stolonifer* grows invading the space and intermingling with the *Colletotrichum* sp. (figure 4 D). When the strain AhCx-03 was paired to AhCx-02 at 13 °C, only growth rate slows down ($P < 0.05$) with no apparent changes in the hyphae.

Figure 4. *Micrographs (40 X) of A): «Colletotrichum» sp. AhCx-02; B): «Colletotrichum» sp. AhCx-03 individually cultured; C) «Colletotrichum» sp. AhCx-02 and AhCx-03 paired with each other, and D) «R. stolonifer» paired to Colletotrichum AhCx-03.*
Figura 4. *Micrografías (40 X) de A): «Colletotrichum» sp. AhCx-02, B): «Colletotrichum» sp. AhCx-03 cultivados individualmente; C) «Colletotrichum» sp. AhCx-02 y AhCx-03 pareados entre sí, y D) R. stolonifer pareado a «Colletotrichum» AhCx-03.*



To validate the models, individual and mixed infections were done at room temperature on jackfruit. Individual infections were as expected (figure 5). All infections start the day after being inoculated with a 100 % of incidence, being *R. stolonifer* the faster-growing fungus. On the fourth day after the inoculation, *R. stolonifer* showed the fruit surface covered by white fungal mycelia and black spores while the *Colletotrichum* species shows a white mycelium.

Figure 5. *Infections caused by «R. stolonifer» (A and D); «Colletotrichum» sp. AhCx-02 (B and E) and «Colletotrichum» sp. AhCx.03 (C and F) on the second- and fourth-day following inoculation on jackfruit, respectively.*
Figura 5. *Infecciones causadas por «R. stolonifer» (A y D); «Colletotrichum» sp. AhCx-02 (B y E) y «Colletotrichum» sp. AhCx.03 (C y F) en el segundo y cuarto día después de la inoculación sobre yaca, respectivamente.*



The radial growth rate of each isolate individually showed a different behavior comparing when they were mixed. *Rhizopus stolonifer* showed a halo of injury three times higher than the infection area. The other isolates grew slower than in the AJ medium (table 4).

Table 4. *Radial growth rate of «R. stolonifer»; «Colletotrichum» sp. AhCx-01 and «Colletotrichum» sp. AhCx.03 on jackfruit at 25 °C, when grew individually and mixed, and their bias and accuracy factors comparing data* in vitro *and* in vivo.
Tabla 4. *Velocidad radial de crecimiento de «R. stolonifer»; «Colletotrichum» sp. AhCx-01 y «Colletotrichum» sp. AhCx.03 en yaca fresca de forma individual y en mezcla, y sus factores de sesgo y de precisión que compara los datos* in vitro *e* in vivo.

Mold	***μ (mm/d)**	**R^2**	B_f	A_f
R. stolonifer	19.18 ± 0.3	> 0.95	2.2	2.2
Colletotrichum sp.	0.67 ± 0.2	> 0.88	2.5	2.5
Colletotrichum sp.	0.70 ± 0.2	> 0.85	2.6	2.6
Fungal mix	36.26 ± 3.5	> 0.98	#0.72	#1.4

Note: *Values are means ± standard deviations. # The B_f and the A_f was calculated comparing μ of the mixed infection on jackfruit with μ of *R. stolonifer* in AJ medium at 25 °C.
Nota: *Los valores son las medias ± la desviación estándar. #El B_f y A_f fueron calculados comparando μ de la infección mixta en yaca con μ de *R. stolonifer* en el medio AJ a 25 °C.

The bias and the accuracy factors (B_f and the A_f) were calculated for each isolate at room temperature to compare the predicted model in AJ medium. The values were > 2.2 indicating no correlations.
Otherwise, during the mixed infection with all spores, the symptoms were observed at the second day after the inoculation and the severity was greater showing a halo of injury twice the size of the initial infection. On the third day, the halo of injury was more than three times higher than the infection, and, on subsequent days, intermingles with the infection. The presence of *R. stolonifer* in the infection was evident (figure 6) from the beginning. At the fifth day, the infection showed a cottony appearance around the edges, indicating the coexistence of *Colletotrichum* sp.

Figure 6. *Infection on jackfruit with of «R. stolonifer» and «Colletotrichum» sp. AhCx-02 and AhCx-03 strains at the fourth day after inoculation.*
Figura 6. *Infección de yaca fresca con «R. stolonifer» y «Colletotrichum» sp. Cepas AhCx-02 y AhCx-03 al cuarto día después de la inoculación.*

The B_f and the A_f for the mixed infection were calculated comparing with the faster-growing fungus *Rhizopus* AhR-01 *in vitro*. The values were B_f: 0.72 and A_f: 1.4.

3.2 Discussion

To obtain a good quality jackfruit, it must be ripened on the tree. After harvesting, the fruit ripens in 3 – 7 days (Elevitch, Craig & Manner, 2012). Due to their short shelf life, rot diseases are an important problem resulting in huge losses due to deterioration (Amusa, Kehinde & Ashaye, 2002). The fungal isolates used in this research belong to the main one that causing rots on jackfruit in all producers zones (Nelson, 2005; Bhunjun *et al.*, 2019). The environmental factors affect the growth of fungal disease and may vary on each other. Moreover, these factors influence the outcomes of spatial competition between fungal species. *Rhizopus* is a fast-growing fungus as it was observed in this work. *Rhizopus stolonifer* has been reported

with the highest mycelial growth rate (μ_{opt} = 94.32 mm/d), five times faster than the results obtained in this study. To our knowledge, there are only a few reports about the growth rate for *Rhizopus* species, and no jackfruit varieties have been reported to have resistance to the Rhizopus rot (Nelson, 2005).

On the other hand, our results showed that the two isolates of *Colletotrichum* used in this work had a similar behavior if they grow individually, even if their macroscopic appearance was different, reason for these strains to be chosen. The cultures are often highly divergent within a species. According to Weir *et al*. (2012), the differences are probably a reflection of different storage histories, especially with repeated sub-culturing, resulting in staling of the cultures, changes in the appearance and color of the mycelium, and variable in growth rate (Weir, Johnston & Damm, 2012). In this case, their growth rate was similar to those obtained for *C. gloeosporioides* isolated from papaya fruit at 25 °C (Sandoval-Contreras *et al.*, 2020). Optimal temperatures for growth of *C. gloeosporioides* from avocado have been reported in a wide range: 25 to 35 °C (Judet-Correia *et al.*, 2010) while for *C. gloeosporioides* from papaya at 27 – 31 °C (Sandoval-Contreras *et al.*, 2020). No reports have been done for growth at 5 °C and no germination at 37 °C (Pitt & Hocking, 2009).

Regarding models of *in vitro* unpaired isolates, the minimum temperature estimated for all strains was near to the temperature for storage. The temperatures recommended for storage depend on the type of fruit and the variety, normally between 7 and 13 °C (Arias & Toledo, 2000). *Rhizopus stolonifer* has been reported to grow from 5 °C up to 30 °C or 35 – 37 °C, being their optimal growth at 25 °C, but at 37 °C usually colonies do not growth. According to Bautista-Baños *et al*. (2008), *Rhizopus* rot progression is temperature related with a maximal fungal growth at 27 °C, temperature higher than the theoretical optimal temperature obtained in this study (Bautista-Baños *et al.*, 2008).

In the current study, the impact of temperature on the growth rate of all isolated was evident. *Rhizopus* had a high growth rate compared to the other isolates. At the low temperature used in the experiments, all strains showed a certain growth rate, which increases as the temperature increases, then decreases as the temperature continues to increase. Similar results were obtained for *C. gloeosporioides* isolate from papaya fruit (Sandoval-Contreras *et al.*, 2020). Comparative studies showed that *Rhizopus* had higher growth rates compared to other genera, taking a few days in became visible at the naked eye. Ochoa-Velasco et al. (2018) reports μ_{max} = 0.31 (1/h) (~7.44 mm/d) for *R. stolonifer* at 28 °C. The mycelial growth rate for *R. stolonifer* on pears has been estimated at 3.93 mm/h (94.32 mm/d) (Sardella, Gatt & Valdramidis, 2018). On the other hand, similar results were found for *C. gloeosporioides* isolated from papaya. The maximal radial growth rate μ_{max} was 3.6 mm/d (Teixeira *et al.*, 2007) and between 2.3 and 4.3 mm/d (Sandoval-Contreras *et al.*, 2020), both at 25 °C. High frequency of saprophytic fungi exists on fruit surface, but some of them are pathogens. Wind, rain, and insects dislodge and spread the fungal spores and expose the fruit to postharvest infections. Such conditions favor an increase in fruit mycobiota diversity increasing the risk or severity of rots. For this reason it was important to investigate the interaction of postharvest pathogenic molds (Lorenzini *et al.*, 2013).

The main type of interaction between filamentous fungi is competition for space and nutrients. At first, resource exploitation and direct harmful replacement after physical contact exist, or antagonism at a distance if a volatile substance or diffusible chemical is produced. This ability to capture unoccupied space might be inhibited or enhanced by the presence of adjacent or distant mycelia. (Kolesidis *et al.*, 2019). In the case of *R. stolonifer*, it belongs to the class of the Zygomycetes, with aseptate hyphae or very few septa that enhance rapid translocation and absorption of nutrients, thus allow rapidity of growth to the fungus (Pitt & Hocking, 2009; Sardella, Gatt & Valdramidis, 2018). In our study, *R. stolonifer* covers the plate in a few days, paired or unpaired, just as it did *in vivo* due to their fast growth. The presence of *Colletotrichum*

affects its growth by a slight stimulation or by inhibiting it depending on the isolate, however, they coexist even in extreme conditions.

Regarding I_D index, *Colletotrichum* isolate AhCx-02 shows more competitiveness face to the other *Colletotrichum* AhCx-03 isolate, but at high temperature, the interaction was not observed. The temperature is one of the main abiotic elements that influence the outcomes of spatial competition (Kolesidis *et al.*, 2019) such as we observed in this study. Seasonal temperatures predispose the incidence of a fungal specie responsible for rot infection. Research about the interaction between *C. acutatum*, *Botrytis cinerea*, and *Greeneria uvicola* on berries, *B. cinerea* was reduced when co-inoculated with *C. acutatum* at 20 or 27 °C, but with *G. uvicola* was only reduced at 27 °C. They concluded that *B. cinerea* was the predominant pathogen at 20 °C, whereas at 27 °C predominates *C. acutatum* (Steel *et al.*, 2011). It can be inferred that depending on the ambient temperature, a high virulent strain may appear and dominates over other species causing rots in fruits. Both isolates of *Colletotrichum* coexist with *Rhizopus* at 13 and 25 °C but no at 35 °C. Although *Colletotrichum* AhCx-03 inhibits the growth of *Rhizopus*, it continues its growth a little slower and they end up coexisting. To our knowledge, the mixed infection or interaction of *Rhizopus* and *Colletotrichum* on jackfruit have not been addressed. Stimulation of enzymatic activity in mixed infections of *Pleurotus ostreatus* and *Ceriporiopsis subvermispora* have been reported in the degradation of aspen wood (Chi, Hatakka & Maijala, 2007). In the same way, it may be a mixed fungal infection that enhances the enzymatic degradation of fruit. Spores of pathogens can cause a primary seasonally infection, but a subsequent infection caused by another pathogen produces a mixed infection increasing deterioration (Agrios, 2005). High temperatures did no favor *Rhizopus* growth because, in these conditions, *Colletotrichum* species were the dominant ones. At low temperatures or near optimal conditions, both *Rhizopus* and *Colletotrichum* coexist.

4. Conclusions

In conclusion, temperature influences competition between the studied species, being *Colletotrichum* sp. the most competitive fungus. On the other hand, *R. stolonifer* and *Colletotrichum* sp. appear to be more harmful to jackfruit when they coexist on jackfruit surface rather than when either is present alone. Understanding the interactions of epiphytic communities may contribute to the development of new postharvest control systems. Hierarchical or intransitive competition has yet to be studied in further detail; nevertheless, these findings may have relevance in the knowledge of postharvest treatment of jackfruit.

5. Supplementary information

No.

6. Acknowledgements

To Mexican National Council of Science and Technology (CONACyT) for the postdoctoral scholarship.

Authors information

Teresa Sandoval Contreras [1]
Maricarmen Íñiguez Moreno [1] 0000-0001-9538-6935
Luis Garrido Sánchez [2] 0000-0001-7034-4341
Juan Arturo Ragazzo Sánchez [1] 0000-0002-2298-3306
José Alberto Narváez Zapata [3]
Montserrat Calderón Santoyo [1] 0000-0002-8744-1815

Contribution of the authors in the development of the work

The authors declare that they contributed equally.

Interest conflict

The authors declare that there is no conflict of interest.

References

Agrios, G. N. (2005). *Plant Pathology*. 5th. Amsterdam: Elsevier.

Amusa, N. A., Kehinde, I. A. & Ashaye, O. A. (2002). Bio-deterioration of breadfruit (*Artocarpus Communis*) in storage and its effects on the nutrient composition. *African Journal of Biotechnology*, 1(December), 57–60. 10.5897/AJB2002.000-010.

Arias Velázquez, C. J., & Toledo Hevia, J. (2000). *Manual de manejo postcosecha de frutos tropicales (papaya, piña, plátano, cítricos), Técnicas mejoradas de postcosecha, procesamiento y comercialización de frutas*. ONU-FAO.

Baranyi, J., & Roberts, T. A. (1994). A dynamic approach to predicting bacterial growth in food. *International journal of food microbiology*, *23*(3–4), 277–294. 10.1016/0168-1605(94)90157-0.

Barnett, H. L., & Hunter, B. B. (1998) *Illustrated Genera of Imperfect Fungi.* APS, St. Paul, Minnesota.

Bautista-Baños, S. *et al.* (2008). Rhizopus stolonifer-Tomato interaction. In E. A. Barca, & C. Clément. (Eds.). *Plant-Microbe Interactions* (pp. 269-289). Kerala, India: Rerearch Signpost

Bhunjun, C. S. *et al.* (2019). Multigene phylogenetic characterisation of *Colletotrichum artocarpicola* sp. Nov. From *Artocarpus heterophyllus* in northern Thailand. *Phytotaxa*, *418*(3), 273-286. 10.11646/phytotaxa.418.3.3.

Carrillo, L. (2003). *Los hongos de los alimentos y forrajes*. Universidad Nacional de Salta.

Chi, Y., Hatakka, A., & Maijala, P. (2007). Can co-culturing of two white-rot fungi increase lignin degradation and the production of lignin-degrading enzymes? *International Biodeterioration and Biodegradation*, *59*(1), 32-39. 10.1016/j.ibiod.2006.06.025.

Droby, S., & Wisniewski, M. (2018). The fruit microbiome: A new frontier for postharvest biocontrol and postharvest biology. *Postharvest Biology and Technology*, *140*(January), 107-112. 10.1016/j.postharvbio.2018.03.004.

Elevitch, Craig R., & Manner, H. I. (2012). *Artocarpus heterophyllus* Lamarck. *Edible Medicinal And Non-Medicinal Plants*, *3*(April), 318-336. 10.1007/978-94-007-2534-8.

García-Estrada, R. S. *et al.* (2019). First Report of *Rhizopus stolonifer* causing fruit rot in Jackfruit (*Artocarpus heterophyllus*) in Mexico. *Plant Disease*, *103*(11), 2957–2957. https://doi.org/10.1094/PDIS-02-19-0395-PDN.

Ghosh, R. *et al.* (2015). Biological control of fruit-rot of jackfruit by rhizobacteria and food grade lactic acid bacteria. *Biological control*, 83, 29-36. 10.1016/j.biocontrol.2014.12.020.

Íñiguez-Moreno, M. *et al.* (2020). Sodium alginate coatings added with *Meyerozyma caribbica*: Postharvest biocontrol of *Colletotrichum gloeosporioides* in avocado (*Persea americana* Mill. cv Hass). *Postharvest Biology and Technology*, *163*(January), p. 111123. 10.1016/j.postharvbio.2020.111123.

Judet-Correia, D. *et al.* (2010). Validation of a predictive model for the growth of *Botrytis cinerea* and Penicillium expansum on grape berries. *International Journal of Food Microbiology*, *142*(1-2), 106–113. 10.1016/j.ijfoodmicro.2010.06.009.

Kolesidis, D. A. *et al.* (2019). Predicting fungal community dynamics driven by competition for space.

Fungal Ecology, 41, 13-22. 10.1016/j.funeco.2019.04.003.

Li, Y. *et al*. (2013). The inhibitory effect of *Epicoccum nigrum* strain XF1 against *Phytophthora infestans*. *Biological Control*, *67*(3), 462-468. 10.1016/j.biocontrol.2013.09.007.

Lorenzini, M. *et al.* (2013). Postharvest grape infection of *Botrytis cinerea* and its interactions with other moulds under withering conditions to produce noble-rotten grapes. *Journal of Applied Microbiology*, *114*(3), 762-770. 10.1111/jam.12075.

Luna-Esquivel, G. *et al.* (2013). La yaca (*Artocarpus heterophyllus* Lam.) un fruto de exportación. *Agro Productividad*, 65-70.

Magan, N., & Lacey, J. (1985). Interactions between field, and storage fungi on wheat grain. *Transactions of the British Mycological Society*, *85*(1), 29-37. 10.1016/s0007-1536(85)80153-4.

Medina-Tiznado, M. A. *et al*. (2018). Lasiodiplodia theobromae agente causal de la pudrición blanda de frutos de *Artocarpus heterophyllus* Lam. en Nayarit, México. *Revista Brasileira de Frutidultura*, *40*(5), 1–5. http://dx.doi.org /10.1590/0100-29452018018.

Nelson, S. (2005). Rhizopus Rot of Jackfruit. *Plant disease*, p. PD-29.

Ochoa-Velasco, C. E. *et al.* (2018). Growth modeling to control (in vitro) *Fusarium verticillioides* and *Rhizopus stolonifer* with thymol and carvacrol. *Revista Argentina de Microbiologia*, *50*(1), 70-74. 10.1016/j.ram.2016.11.010.

Pitt, J. I., & Hocking, A. D. (2009) *Fungi and Food Spoilage*. Springer.

Ragazzo-Sánchez, J. A. *et al.* (2011). Molecular identification of the fungus causing postharvest rot in jackfruit. *Revista Mexicana de Micología*, 34, 9-15.

Ross, T. (1996). Indices for performance evaluation of predictive models in food microbiology. *Journal of Appilied Bacteriology*, 81, 501-508.

Rosso, L., Lobry, J. R., & Flandrois, J. P. (1993). An unexpected correlation between cardinal temperatures of microbial growth highlighted by a new model. *Journal of Theoretical Biology*, *162*(4), 447–463. 10.1006/jtbi.1993.1099.

Sandoval-Contreras, T. *et al.* (2020). A predictive model for the effect of the environmental conditions on the postharvest development of *Colletotrichum gloeosporioides* strains isolated from papaya (Carica papaya L.) Accepted article. *Journal of Food Protection*, *80*(9), 1495-1504. 10.1017/CBO9781107415324.004.

Sardella, D., Gatt, R., & Valdramidis, V. P. (2018). Modelling the growth of pear postharvest fungal isolates at different temperatures. *Food Microbiology*, *76*(April), 450-456. 10.1016/j.fm.2018.07.010.

Sempere, F., & Santamarina, M. P. (2010). Study of the interactions between *Penicillium oxalicum* currie & thom and *Alternaria alternata* (Fr.) keissler. *Brazilian Journal of Microbiology*, *41*(3). 10.1590/S1517-83822010005000003.

Steel, C. C. *et al.* (2011). Effect of temperature on *Botrytis cinerea*, *Colletotrichum acutatum* and *Greeneria uvicola* mixed fungal infection of *Vitis vinifera* grape berries. *Vitis*, *50*(2), 69-71.

Teixeira, J. A. *et al.* (2007). Papaya (*Carica papaya* L.) Biology and Biotechnology. *Tree and Forestry Science and Biotechnology*, *1*(1), 47-73. 10.1136/bmj.282.6264.598.

Weir, B. S., Johnston, P. R., & Damm, U. (2012). *The Colletotrichum gloeosporioides species complex*. *Stud Mycol*, 73, 115–180. 10.3114/sim0011.

Xu, S. *et al.* (2018). Ultrasonic-microwave assisted extraction, characterization and biological activity of pectin from jackfruit peel. *LWT - Food Science and Technology*, 90, 577-582. 10.1016/j.lwt.2018.01.007.