Article

Physiological response and quality of mango *cv* Ataulfo infected with *Colletotrichum* spp.

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Abstract

The objective of this work was to evaluate the postharvest behavior of the fruit of mango cv. Ataulfo in a state of physiological maturity in response to infection induced by *Colletotrichum siamense* and *Colletotrichum asianum*. Mangoes were inoculated with *C. siamense* and *C. asianum* and stored for 10 days at 28 °C and RH of 85-90%. The virulence of *Colletotrichum* spp. was evaluated in the fruits. And its effect on the variables of physical, chemical quality and respiration. At 10 days of storage, *C. siamense* showed greater virulence than *C. asianum* in mango fruits, with some differences in internal color between inoculated fruits and controls. The fruits inoculated with *C. asianum* had greater respiratory activity and lower texture with respect to the control. The physical and chemical quality variables in the fruits showed significant differences with respect to storage time, but not due to the presence of the fungus. The infection of the mangoes caused by the two species of *Colletotrichum* manifested as necrotic spots in the infected area of the fruits, affecting the appearance and visual quality.

Keywords: *Colletotrichum asianum*, *Colletotrichum siamense*, postharvest, virulence.

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Introduction

Mango (*Mangifera indica* L.) is one of the most important tropical fruits in the world, due to its characteristic sweet taste, aroma, creamy texture and nutritional properties; in addition, Mexico ranks first in exports worldwide (Altendorf, 2019). The main mango producers in the country are Sinaloa (19.04%), Guerrero (18.70%), Nayarit (15.42%) and Chiapas (12.85%), where mango cv. Ataulfo is one of the most important cultivars nationwide with more than 31% of the cultivated area (63 971 ha) and is one of the most exported to the US and Canada (Ariza-Flores *et al*., 2018; SIAP, 2021).

During the maturation process of the mango fruit, biochemical reactions occur, such as the loss of firmness caused by enzymes that degrade complex polysaccharides of the cell wall and the increase in the content of sugars in the pulp (Tharanathan *et al*., 2006; Litz, 2009). These changes make it easier for the fruits to be more susceptible to the attack of phytopathogens or to the development of fungi present in a latent state, such as *Colletotrichum* spp., which reduces pre- and postharvest quality (Tovar-Pedraza *et al*., 2020; Fuentes-Aragón *et al*., 2020).

Most of the postharvest losses of the mango fruit are generated by anthracnose caused by the fungus *Colletotrichum gloeosporioides*, with losses between 30 and 60% of production, which also affects leaves and inflorescences (Benítez-Camilo *et al*., 2003; Carrillo-Fasio *et al*., 2005); Huerta-Palacios *et al*., 2009). Under inadequate conditions of agronomic management of mango crop, anthracnose can be present at any stage of fruit development. In tender fruits, the fungus can prevail in its latent form, manifesting itself after its physiological maturity with irregular dark brown or black sunken lesions on the peel, as well as small dark circular lesions on the pedicel and peduncle (Carrillo-Fasio *et al*., 2005; Tovar-Pedraza *et al*., 2020; Fuentes-Aragón *et al*., 2020).

In Mexico, at least seven cryptic species belonging to the *C. gloeosporioides* complex (*C. siamense*, *C. asianum*, *C. tropicale*, *C. alienum* , *C. fructicola*, *C. chrysophilum* and *C. queenslandicum*) have been identified based on multilocus phylogenetic analyses as the species causing the symptoms of anthracnose in mango tissues. However, *C. siamense* and *C. asianum* are the most common and widely distributed species (Tovar-Pedraza *et al*., 2020; Fuentes-Aragón *et al*., 2020; Mora-Aguilera *et al*., 2021). The above raises new questions about the physiological response of the fruit to these fungal species.

It has been reported that the pathogenicity of *Colletotrichum* species in fruits may vary due to the effect of environmental conditions and host, Sharma *et al*. (2013). So, it is important to know the physiological response and postharvest quality of mango fruits exposed to different species of *Colletotrichum*. The objective of this work was to determine the virulence of *C. siamense* and *C. asianum* in mango fruits cv. Ataulfo, in addition to estimating its effect on the parameters of quality and respiratory activity during storage.

Materials and methods

Plant material

Fruits of mango *cv* Ataulfo with physiological maturity harvested from a commercial orchard located in Los Mochis, Sinaloa, Mexico, were used. The fruits were selected according to color, uniform size and without visual damage; they were washed with 1% sodium hypochlorite for 2 min, rinsed with distilled water, sprayed with 70% alcohol and left to dry (Tovar-Pedraza *et al*., 2020).

Inoculation of mango fruits

In duplicate, the fruits were divided into 3 batches; batches 1 and 2 were inoculated with strains of *C. siamense* (UACH 334) and *C. asianum* (UACH 310) (Tovar-Pedraza *et al*., 2020) and the third was used as the control without inoculation. Previously, the isolates of *Colletotrichum* spp. were left 10 days growing in potato dextrose agar culture medium (PDA, BD Bioxon, Mexico) at 28 °C. Discs of 5 mm diameter of agar with the fungus were cut and placed in an induced wound of 1 mm in diameter by 1 mm deep on the surface of the epicarp of the fruits according to Álvarez *et al*. (2020). Subsequently, all fruits were stored at 28 °C at 85-90% relative humidity (RH).

Virulence analysis

The virulence test in the inoculated mangoes was measured with a vernier, the diameter of the lesions was considered perpendicularly and the values in each of the fruits were averaged at 0, 2, 4, 6, 8 and 10 days of storage. The results were expressed in millimeters (mm).

Color

The external and internal color of the fruits was determined with a Konica model CM-700 spectrophotometer (Minolta Inc., Japan) and the color coordinates L^*, a^*, b^*, as well as chromaticity (C) and hue angle (h) were calculated with the onColor QC version 5 program (Siller-Cepeda *et al*., 1994).

Respiration

Respiration was measured in three fruits per treatment at 1, 2, 4, 6, 8 and 10 days after inoculation (DAI), individual fruits were placed in a closed system (3.8 L) for one hour, the sample of the accumulated gas was taken, and the production of CO_2 was evaluated. The analysis was determined in an Agilent Technologies model 7820 gas chromatograph adapted with a thermal conductivity detector for CO_2 analysis, equipped with a HayeSep Q column of 1.83 m x 3.175 mm. Helium was used as a carrier gas with a flow of 25 ml min^{-1}. As standard, CO_2 at 0.52% in a mixture with nitrogen (Praxir, Mexico) was used. The results were expressed in ml CO_2 kg^{-1} h^{-1}.

Quality parameters

For the quality of the fruits, weight loss, firmness, pH, titratable acidity and total soluble solids at 0, 2, 4, 6, 8 and 10 DAI were evaluated. The percentage of weight loss was evaluated with the difference of initial and final weight of the mango fruits (Muy-Rangel *et al*., 2004). The firmness reported in Newtons (N) was determined as the effort to penetrate the pulp of the fruit using a DFGS-100 model penetrometer (Chatillon Digital Force, USA). With a cylindrical punch of 8 mm in diameter (Bourne, 1980). For total soluble solids (TSS) an RM-40 model refractometer (Mettler Toledo, Mexico) was used, the results were expressed in total soluble solids (Brix) (AOAC, 1998). Titratable acidity (TA) reported in (%) of citric acid and pH were determined in a model T-50 automatic titrator (Mettler Toledo, Mexico) (AOAC, 1998).

Statistical analysis

For the test of virulence in the mango fruits with the two species of *Colletotrichum* (*C. siamense* and *C. asianum*), a repeated measures design was used. The response variable was lesion diameter. For the analysis of firmness, pH, titratable acidity, total soluble solids and internal color, a completely randomized design with factorial arrangement A x B was used. Factor A was the treatments: mango-*C. siamense*, mango-*C. asianum* and the control, and factor B was the sampling days (0, 2, 4, 6, 8 and 10 DAI). For the variables of weight loss, external color and respiration in the fruits, a design of repeated measures over time was used. The differences of the evaluated parameters were determined by an Anova with significance of 5% and a Tukey test in the NCSS statistical package, 2020.

Results and discussion

Virulence analysis

The isolate of *C. siamense* showed a cottony growth, circular in shape, white and slightly gray, while *C. asianum* showed a cottony and irregular mycelial growth, of white-gray coloration; both characteristics were similar to that reported by Tovar-Pedraza *et al*. (2020), which supports the reproducibility of the fungus. The symptoms of the disease were characterized by black, irregular and sunken lesions, as well as gray mycelial growth and production of asexual fruiting bodies (acervuli) of the fungus in the cuticle of the fruit.

The species *C. asianum* and *C. siamense* have been most frequently associated with anthracnose with high pathogenicity in different mango cultivars and distribution worldwide (Lima *et al*., 2013; Sharma *et al*., 2013; Pardo-De la Hoz *et al*., 2016; Liu *et al*., 2017; Fuentes-Aragón *et al*., 2020; Tovar-Pedraza *et al*., 2020; Mora-Aguilera *et al*., 2021). In the virulence analysis, a larger lesion size was observed in the fruits inoculated with *C. siamense* with maximum values of 33 mm at 10 days of storage (Figure 1), similar to those reported by Li *et al*. (2019), for mango fruits inoculated with *C. gloeosporioides* stored at eight days at 25 °C at a RH of 85-90%.

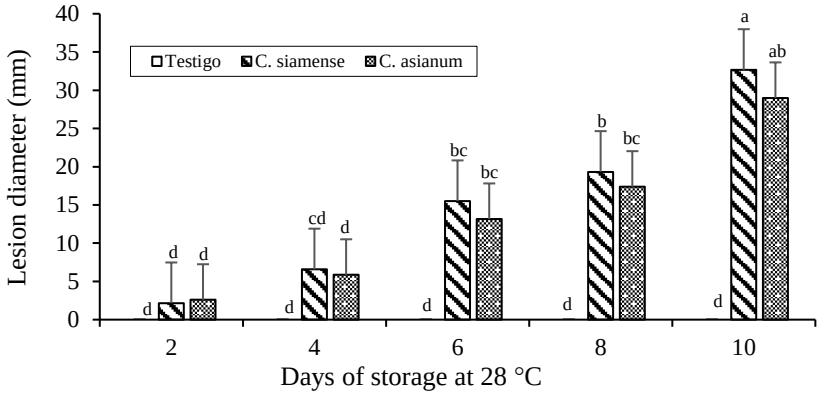

Figure 1. Lesion diameter in fruits of mango *cv* Ataulfo inoculated with *Colletotrichum siamense* and *C. asianum*, stored for 10 days at 28 °C with 85-90% RH. The bars indicate standard deviation. Same letters in superscripts are statistically equal (Tukey $p > 0.05$).

Tovar-Pedraza *et al*. (2020) observed in Manila mango a shorter propagation time of *Colletotrichum* spp. (5 DAI) against 8 DAI. The Ataulfo mango has greater amounts of phenols, flavonoids and beta-carotenes both in pulp and in peel (Manthey and Perkins-Veazie, 2009; Sulaiman and Ooi, 2012), which could act as components of inhibition of the growth of the fungus. In addition, in the peel of immature mango fruits are some antifungal compounds such as chitinases, gallotannins and resorcinols (5-12-cisheptadecenyl resorcinol and 5-pentadecenyl resorcinol), which decrease with maturation and are involved in resistance to pathogens such as *Alternaria alternata* and *C. gloeosporioides* (Droby *et al*., 1987; Karunanayake *et al*., 2011; Sinniah *et al*., 2012).

The concentration of these antifungal compounds can vary between mango cultivars, which generates a differential resistance against *Colletotrichum* infection, but this response can also be different in the same cultivar against different species of *Colletotrichum* (Karunanayake *et al*., 2011; Sinniah *et al*., 2012; Fuentes-Aragón *et al*., 2020).

Mycelial growth and disease symptoms in mangoes inoculated with *Colletotrichum* spp. showed significant differences at day 10 of storage with respect to the control; but not between species (Figure 1). However, Figure 2 subjectively shows that *C. siamense* caused larger irregular black sunken lesions compared to those produced by *C. asianum*. So, to deduce the effect of the biotic stress caused by *Colletotrichum* spp. in mango, it is important to analyze the physiological changes and the defense response at the biochemical and molecular level in the fruits.

Color

The variables of the external and internal color of the fruits (Figure 2) were not statistically different between mangoes, but during the storage time ($p < 0.05$). For the external color at day zero, the fruits were less luminous, of lower saturation and with values of 90 °h, which correspond to mangoes in physiological maturity of yellow-green color; variables that changed significantly in the first four days of storage. Subsequently, the fruits showed a trend of change with final values of L= 62, greater color saturation= 60 and a hue= 65 °h that corresponds to fruits in maturity of consumption, of yellow-orange color, according to the color circle of Minolta (1994).

C. siamense

	Color (day 0)	
	Exter	Inter
L	63.9_{a}	71.7_{AD}
C	39.7_{d}	62_{AB}
°h	90.6_{a}	74.2_{AB}

	Color (day 4)	
	Exter	Inter
L	65_{a}	62.3_{BCD}
C	59.5_{b}	59.7_{B}
°h	71.5_{b}	67.2_{BC}

	Color (day 8)	
	Exter	Inter
L	63.7_{a}	60.1_{BC}
C	61.3_{a}	56.5_{B}
°h	66.1_{c}	61.6_{D}

	Color (day 10)	
	Exter	Inter
L	62.6_{ab}	60.5_{BC}
C	59.9_{b}	60.9_{B}
°h	64.6_{d}	62.7_{D}

C. asianum

	Color (day 0)	
	Exter	Inter
L	56.2_{c}	72_{AD}
C	39.3_{d}	60.3_{B}
°h	90.4_{a}	73.5_{AB}

	Color (day 4)	
	Exter	Inter
L	62.6_{ab}	61.6_{BC}
C	56.5_{c}	58.8_{B}
°h	74.2_{b}	64.4_{CD}

	Color (day 8)	
	Exter	Inter
L	62.8_{ab}	61.6_{BC}
C	62_{a}	62_{AB}
°h	66.2_{c}	63.1_{CD}

	Color (day 10)	
	Exter	Inter
L	62.3_{b}	60.6_{BC}
C	61.3_{a}	63.1_{AB}
°h	64.8_{d}	61.5_{D}

Control

	Color (day 0)	
	Exter	Inter
L	56.3_{c}	76.4_{A}
C	40.1_{d}	76.7_{A}
°h	91.5_{a}	76.1_{AB}

	Color (day 4)	
	Exter	Inter
L	61.9_{b}	65.4_{BCD}
C	55.2_{c}	60.3_{B}
°h	75.3_{b}	67.9_{BC}

	Color (day 8)	
	Exter	Inter
L	62.5_{ab}	61.3_{BC}
C	60.8_{a}	63.2_{AB}
°h	66.1_{c}	64.2_{CD}

	Color (day 10)	
	Exter	Inter
L	61.5_{b}	60.6_{BC}
C	60.3_{b}	63.4_{AB}
°h	64.9_{d}	62.9_{CD}

Figure 2. Effect of anthracnose symptoms induced by *Colletotrichum siamense* and *C. asianum* on the appearance of mango *cv* Ataulfo and data on external (exter) and internal (inter) color in luminosity (L); chromaticity (C); and hue angle (°h) stored 10 days at 28 °C with 85-90% RH. Values with equal letters by response variable between external color (lowercase) and internal color (uppercase) are statistically equal (Tukey $p > 0.05$).

The color of the mango pulp showed a change from light yellow (h= 74) to yellow to slightly orange (h= 63). The luminosity and hue values showed on average a linear change trend of -2.4 units for every two days of storage, while the color saturation was polynomial of order 2 with an increase during the maturation of the fruits. Huerta-Palacios *et al*. (2009) reported darkening, rotting and development of acervuli between the pulp and the surface of the peel in areas damaged by *Colletotrichum*.

Similarly, a change in the color of the mango peel is reported due to the effect of the fungus, due to the presence of hyphae that can penetrate the epidermal cells and degrade the cell walls through hydrolytic enzymes, causing the death of the host tissue (Sandoval-Chávez *et al*., 2015). The change in mango color during maturation occurs due to the degradation of chlorophyll and the accumulation of carotenoids and phenolic pigments (Tharanathan *et al*., 2006). Mango cultivars such as Ataulfo and Manila Super lose their green color and yellow and orange hues appear on fruits in maturity of consumption, which is mainly provided by carotenoids (Ortiz *et al*., 2002; Singh *et al*., 2013), as observed in the fruits of this experiment.

Respiration

The mango fruits presented their maximum respiratory activity at two days of storage, with no significant difference between the inoculated fruits (153.6 for *C. siamense* and 168.7 ml CO_2 kg^{-1} h^{-1} for *C. asianum*), but with the control fruits (132.2 ml CO_2 kg^{-1} h^{-1}) (Figure 3). The increase in respiratory activity within a few days of storage can be related to fruits of advanced maturity at the beginning of the experiment, which is corroborated by values of lower acidity and higher total soluble solids at the cut; which coincides with what is cited by Osuna-García *et al*. (2002).

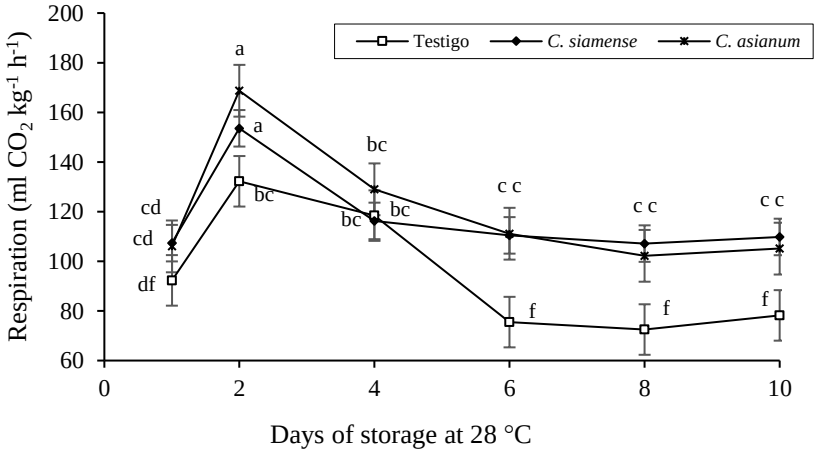

Figure 3. Respiratory behavior in fruits of mango cv. Ataulfo inoculated with *Colletotrichum siamense* and *C. asianum*, stored for 10 days at 28 °C with 85-90% RH. The bars indicate standard error (n= 3). Same letters by treatment and day are statistically equal (Tukey, $p> 0.05$).

For their part, Cienfuegos *et al*. (2004) reported for fruits of mango *cv* Kent at physiological maturity maximum respiratory activity at six days of storage at 27 °C (62.7 ml CO_2 kg^{-1} h^{-1}), while Dautt-Castro *et al*. (2019) observed it at 13 days at 20 °C (62.29 ml CO_2 kg^{-1} h^{-1}) for mango *cv* Kent. Similar to this work, Palafox-Carlos *et al*. (2012) reported a respiratory activity of 122.2 ml CO_2 kg^{-1} h^{-1} in mango *cv* Ataulfo, which related it to a stage of advanced maturity.

In general, the respiratory activity of mango fruits depends on the variety, the state of maturity and storage temperature, with a climacteric behavior and a decreasing respiratory activity until the senescence of the fruits (Martínez-González *et al*., 2017; Palafox-Carlos *et al*., 2012), which accelerates in the presence of phytopathogens, as observed in this study. The production of ethylene

and CO_2 during climacteric maturation triggers the transduction of signals for the activation of several transcription factors that in turn activate the expression of genes that encode enzymes that catalyze changes in maturation such as color, flavor, texture, aroma, among others (Grierson, 2013; Martínez-González *et al*., 2017; Dautt-Castro *et al*., 2019).

Firmness

The loss of firmness was similar between the inoculated and control fruits during storage, 85% of loss occurred in the first four days, without significant changes during the subsequent days of storage, with average values of 7 N (Figure 4). It is important to mention that the fungus affected a localized area of the fruit (Figure 2) and the values of the texture of the fruit were not taken exactly in the damaged area. Quintero *et al*. (2013); Dautt-Castro *et al*. (2019) reported a loss of firmness below 20 N at 10 days in mango fruits from different cultivars and different storage conditions.

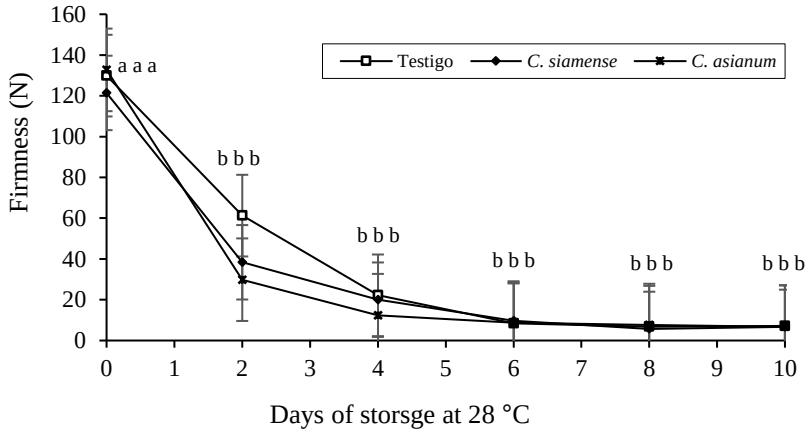

Figure 4. Firmness in fruits of mango *cv* Ataulfo inoculated with *Colletotrichum siamense* and *C. asianum*, stored for 10 days at 28 °C with 85-90% RH. The bars indicate standard error (n= 5). Same letters by treatment and day are statistically equal (Tukey $p > 0.05$).

For their part, Carrillo-Fasio *et al*. (2005) observed a firmness of 20 N at 12 days in mango *cv* Kent with anthracnose. However, Kader (2008) pointed out that the firmness of the mango for consumption should be between 13 and 26 N, which occurred at four days for the mangoes in this study, possibly due to the state of maturity and the storage temperature. Naturally, the change in the firmness of the mango is due to the rupture of the cell walls, caused by the enzymatic degradation of polysaccharides such as celluloses, hemicelluloses and pectins and the generation of low molecular weight sugars soluble in water and quantified as soluble solids (Islas-Osuna *et al*., 2010; Quintero *et al*., 2013; Singh *et al*., 2013).

The inoculated mangoes of this study subjectively showed a greater rupture of the peel, possibly induced by the hydrolytic enzymes of the pathogen, compared to the control fruits (Figure 2). It has been suggested that a firmness of less than 20 N in mango fruits can cause mechanical damage during handling and storage, in addition to being considered of poor quality for commercialization (Quintero *et al*., 2013).

Weight loss

All mango fruits showed on average 1% weight loss per day, with an increasing linear behavior, dependent on storage days and without significant difference between inoculated mangoes and controls. The weight loss (WL) of the fruits is expressed: inoculated with *C. siamense* (%WL= 1.159∗day-0.01, R^2= 0.9998), *C. asianum* (%WL= 1.047∗day-0.05, R^2= 0.99) and the control (%WL= 1.039∗day-0.09, R^2= 0.9983), with significant difference between the storage time due to the effect of physiological activity.

For mango *cv* Ataulfo stored for 12 days at 20 °C a weight loss of 9% is reported and for the same conditions, values between 2.5 and 5.4% for mangoes *cv* Haden, Manila Rosa, Tommy Atkins and, Kent (Siller-Cepeda *et al*., 2009), where WL is clearly related to variety. On the other hand, Carrillo-Fasio *et al*. (2005) found a weight loss of 4.6% in mango *cv* Kent stored nine days at 20 °C with the presence of *C. gloeosporioides*. The greatest weight loss in the mango fruits in this study may be due to the higher storage temperature used (28 °C).

pH, titratable acidity and total soluble solids (TSS)

During the maturation process of the fruits, the pH and soluble solids increased, and the acidity decreased significantly during the storage time, while the inoculation of the fruits with the two species of *Colletotrichum* had no significant effect compared to the control. At day zero and 10 days at 28 °C, the mangoes had values of pH from 2.9 to 5, acidity from 3.2 to 0.12% of citric acid and TSS from 10 to 18 °Brix, which correspond to a normal behavior of maturity of the fruit with good chemical quality.

Quintero *et al*. (2013) reported for mango a pH range from 3.3 to 5.3, acidity from 1.5 to 0.35% of citric acid and TSS from 10.5 to 20 °Brix during 10 days of storage at 25 °C. The results cited are similar to those of the mature mango *cv* Ataulfo, as the green Ataulfo mango is more acidic than most mangoes, which is reflected in pH and acidity. Siller-Cepeda *et al*. (2009) studied Haden, Manila Rosa, Tommy Atkins and Kent mangoes stored for 12 days at 20 °C and observed an initial pH from 2.9 to 4.7 and a final pH from 3.9 to 4.9. The °Brix found in these same cultivars at maximum maturation were less than the 18 °Brix found in Ataulfo mango, which indicates that mangoes *cv* Ataulfo may develop greater sweetness.

Biochemical changes that occur during fruit maturation include the degradation of polysaccharides that are reduced to low molecular weight carbohydrates, and the decrease in organic acids (Tharanathan *et al*., 2006; Siller-Cepeda *et al*., 2009; Quintero *et al*., 2013). With the presence of monosaccharides such as sucrose, fructose and glucose during storage days, TSS (°Brix) and the sweetness of mango increase (Tharanathan *et al*., 2006; Siller-Cepeda *et al*., 2009). While the decrease in acidity in diseased fruits is due to the fact that pathogens use organic acids to carry out respiratory activity (Ruiz and Guadarrama, 1992). On the contrary, in the results of this study, all the fruits showed a similar behavior in the variables of chemical quality, this could have occurred because the disease was not severe enough and was localized to cause changes in the quality of the rest of the fruit.

Pathogens break down pre-existing barriers in plants and fruits (cuticle, cell wall and preformed antifungal compounds) and immediately the recognition and activation of induced structural and/or biochemical defenses at the local or systemic level that prevent infection and pathogen from spreading begins (Sinniah *et al*., 2012). The induction of these defenses causes the accumulation of phytoalexins, the synthesis of proteins that strengthen the cell wall and proteins related to pathogenesis. Sinniah *et al*. (2012) reported transcriptional activation of defense genes in the peel of mango fruits (*cv* Karutha Colomban and Willard) in response to *C. gloeosporioides* infection. Therefore, it is important to know the role of the defenses induced at the biochemical and molecular level of mango fruits in the resistance to pathogens such as *Colletotrichum* spp.

Conclusions

Infection induced by *Colletotrichum siamense* and *C. asianum* in fruits of mango *cv* Ataulfo in a state of physiological maturity did not generate significant differences in most of the quality parameters with respect to the control, except for the internal color and respiration. *Colletotrichum siamense* presented greater virulence compared to *C. asianum* in fruits of mango *cv* Ataulfo inoculated and both species affected the visual appearance in the inoculated area with respect to the control fruits. Therefore, it is important to study the biochemical and molecular response in areas near the diseased tissue to understand the defense response of the fruits to the different species of *Colletotrichum* to generate data that help establish different control strategies.

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