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Control of anthracnose in papaya using UV-C irradiation and coatings containing *Lacticaseibacillus paracasei* TEP8

Manejo de antracnosis en papaya aplicando irradiación UV-C y recubrimientos adicionados con *Lacticaseibacillus* paracasei TEP8

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ABSTRACT

Fungal diseases are a major cause of postharvest losses in papaya, encouraging the search for alternatives to synthetic fungicides. This study evaluated UV-C irradiation combined with edible coatings containing the antifungal lactic acid bacterium *Lacticaseibacillus paracasei* TEP8 against *Colletotrichum gloeosporioides* in Maradol papaya. In the first assay, fruits at two ripening degrees were subjected to combinations of coating matrix (starch, carboxymethylcellulose, or uncoated) and UV-C dose (0, 0.97, 2, or 2.88 kJ m^{-2}), plus fungicide controls. Disease incidence and severity were monitored for 10 days. In a second assay, wounded degree-3 fruits were used to evaluate spore germination and lesion diameter according to the order of coating application. Factorial analysis showed that incidence was significantly affected by coating matrix, UV-C dose, ripening degree, and their interactions, whereas severity was affected by coating matrix, ripening degree, and their three-way interaction, but not by the main effect of UV-C dose. The lowest final incidence (80%) occurred in degree-1 fruits treated with 2 kJ m^{-2} UV-C followed by starch-TEP8 coating, while 0.97 kJ m^{-2} plus starch-TEP8 produced one of the lowest final severity values. Applying the coating after inoculation yielded the lowest final spore germination (52.5%), although lesion diameter did not differ from the inoculated control. Overall, treatment performance depended on the interaction among fruit maturity, coating matrix, and UV-C. Because coating-only controls without TEP8 were absent, the observed effects cannot be attributed exclusively to the bacterium. Further studies should verify TEP8 viability, fruit quality, and treatment effectiveness under postharvest storage conditions before application.

Keywords: biological control; disease incidence; disease severity; food preservation; ripening; spore germination

RESUMEN

Las enfermedades fúngicas constituyen una causa importante de pérdidas poscosecha en papaya, lo que ha impulsado la búsqueda de alternativas a los fungicidas sintéticos. Este estudio evaluó la irradiación UV-C combinada con recubrimientos comestibles que contenían la bacteria ácido láctica antifúngica *Lacticaseibacillus paracasei* TEP8 contra *Colletotrichum gloeosporioides* en papaya Maradol. En el primer ensayo, frutos con dos grados de madurez fueron sometidos a combinaciones de matriz de recubrimiento (almidón, carboximetilcelulosa o sin recubrimiento) y dosis de UV-C (0; 0,97; 2 o 2,88 kJ m^{-2}), además de controles con fungicida. La incidencia y severidad se monitorearon durante 10 días. En un segundo ensayo, frutos lesionados con grado de madurez 3 se utilizaron para evaluar la germinación de esporas y el diámetro de lesión según el orden de aplicación del recubrimiento. El análisis factorial mostró que la incidencia fue afectada significativamente por la matriz de recubrimiento, la dosis de UV-C, el grado de madurez y sus interacciones, mientras que la severidad fue afectada por la matriz, el grado de madurez y la interacción triple, pero no por el efecto principal de la dosis de UV-C. La menor incidencia final (80 %) se observó en frutos con grado de madurez 1 tratados con 2 kJ m^{-2} de UV-C y posteriormente con el recubrimiento almidón-TEP8, mientras que 0,97 kJ m^{-2} + almidón-TEP8 produjo uno de los menores valores finales de severidad. La aplicación del recubrimiento después de la inoculación produjo la menor germinación final de esporas (52,5 %), aunque el diámetro de lesión no difirió del control inoculado. En conjunto, la respuesta dependió de la interacción entre madurez, matriz de recubrimiento y UV-C. Debido a la ausencia de controles con recubrimientos sin TEP8, los efectos observados no pueden atribuirse exclusivamente a la bacteria. Estudios posteriores deberán verificar la viabilidad de TEP8, la calidad del fruto y la efectividad del tratamiento bajo condiciones de almacenamiento poscosecha antes de su aplicación.

Palabras clave: control biológico; germinación de esporas; incidencia de la enfermedad; maduración; preservación de alimentos; severidad de la enfermedad

INTRODUCTION

Papaya (*Carica papaya* L.), whose global production reached 14.23 million tons in 2023, is currently cultivated in 70 countries, mainly in India, the Dominican Republic, Indonesia, Mexico, Brazil, and Nigeria. In Mexico, approximately 20,334 hectares are cultivated, with an annual production of 1,148,545 tons (FAO, 2025), ranking the country fourth worldwide in papaya production. The Maradol variety is the most widely cultivated and consumed in Mexico and is also marketed internationally (Secretaría de Agricultura y Desarrollo Rural [SADER], 2018). However, its cultivation, harvesting, transportation, and storage face challenges in reducing losses. In the fruit sector, it is estimated that, worldwide, between 25% and 50% of production is lost after harvest because of accelerated ripening processes, insect infestation, and microbial attacks (Jat *et al*., 2024), with fungi being largely responsible for these losses.

Anthracnose is considered the main post-harvest disease of papaya fruits (Pérez-Leyva *et al*., 2025) and is caused by species of the genus *Colletotrichum*. Among the species identified as causal agents of anthracnose in fruits from different countries (Mexico, Brazil, and Trinidad and Tobago) are *C. gloeosporioides* ss*., C. truncatum* (synonym of *C. capsici*), *C. magnum, C. fructicola, and C. brevisporum* (Ruiz-Campos *et al*., 2022), with *C. gloeosporioides* being the main species involved in anthracnose (Gao *et al*., 2025). Therefore, various strategies have been designed to reduce the presence of phytopathogenic fungi, aiming to extend shelf life and maintain fruit quality (Fernandes *et al*., 2024). To control postharvest diseases, chemically synthesized fungicides such as benzimidazoles, mancozeb, and chlorothalonil are used due to their easy applicability, effectiveness, and cost (Islam *et al*., 2024). However, their extensive application can lead to negative consequences such as the development of fungicide resistance, toxicity to human health, and negative impacts on the environment (Ahmad *et al*., 2024). In addition, a decrease in the effectiveness of some of these pesticides has been reported (Salvador-Figueroa *et al*., 2017).

Consequently, safer but equally effective alternatives are being sought. Strategies that minimize or eliminate fruit contamination by toxic substances and maintain postharvest quality are needed. These include irradiation (UV-C, gamma radiation), heat treatments, inorganic salts, natural compounds (extracts and essential oils), and edible films or coatings with added microorganisms, natural antagonists, or microbial agents (Meneses-Espinosa *et al*., 2024). UV-C irradiation is a non-thermal postharvest technology that can reduce microbial decay; however, its effect on microorganisms depends largely on the dose used (Frisón *et al*., 2021). The amount of radiation received can also alter aspects of fruit physiology (Dassamiour *et al*., 2022). In papaya, Cia *et al*. (2007) reported inhibition of anthracnose development with UV-C, while Sesma-Morales *et al*. (2020) showed that UV-C dose influences physicochemical and sensory characteristics. More recent work continues to support UV-C as a component of integrated postharvest strategies, including combinations with edible coatings (Abdipour *et al*., 2020; Lee *et al*., 2024; Meneses-Espinosa *et al*., 2024).

Lactic acid bacteria (LAB) are one of the most effective sources of antifungal and antibacterial substances used in food preservation. Antifungal compounds produced by LAB can include fatty and organic acids, protein compounds, cyclic dipeptides, phenolic compounds, and H_2O_2 (Cirat *et al*., 2024). LAB with antifungal capacity have been incorporated into alginate (Li *et al*., 2024) and starch edible coatings (ECs) (Marín *et al*., 2019), with LAB maintaining their viability within the ECs. *Lacticaseibacillus paracasei* TEP8 has previously shown antifungal activity against *C. gloeosporioides* (Barrios-Roblero

et al., 2019), providing a biological basis for its incorporation into coating matrices. Recently, *L. paracasei* TEP8 was incorporated into starch and carboxymethylcellulose ECs, achieving *in vitro* inhibition of up to 90% of the development of *C. gloeosporioides* (Cadena de Aquino, 2025).

The reports mentioned above, along with others in the literature, have already established that UV-C radiation can inhibit postharvest fungal development and that coatings (either alone or enriched with lactic acid bacteria) can provide complementary protection (Abdipour *et al*., 2020; Lee *et al*., 2024; Razali *et al*., 2021). However, knowledge gaps remain regarding whether combining UV-C with a starch- or carboxymethylcellulose-based coating containing *L. paracasei* TEP8 achieves a consistent reduction in anthracnose development in inoculated Maradol papayas, and whether the timing of coating application relative to inoculation influences spore germination and lesion formation.

To contribute to bridging this gap, it was hypothesized that UV-C exposure at doses that do not visibly damage the fruit, followed by application of a starch- or carboxymethylcellulose-based coating containing *L. paracasei* TEP8, would delay disease development relative to the corresponding uncoated or untreated conditions, whereas application of the coating at or after inoculation would reduce spore germination and lesion development. Accordingly, the specific aims were: (1) to characterize the effects of ripening degree, coating matrix, and UV-C dose on disease incidence and severity during storage, and (2) to evaluate the effect of coating application order on spore germination and lesion diameter in wounded degree-3 papaya fruits.

MATERIALS AND METHODS

Microorganisms

The phytopathogenic fungus *Colletotrichum gloeosporioides* Penz (isolated from diseased Maradol papaya fruits) and the antifungal lactic acid bacterium (LAB) *Lacticaseibacillus paracasei* TEP8 (Barrios-Roblero *et al*., 2019) were used. Both were provided by the strain collection of the Instituto de Biociencias of the Universidad Autónoma de Chiapas (IBC-UNACH), Chiapas, Mexico. The fungus *C. gloeosporioides* was isolated and characterized by Vázquez-Ovando *et al*. (2018), who also fulfilled Koch's postulates using Maradol papaya fruits. To date, the fungus has not been molecularly characterized. *L. paracasei* TEP8 was previously isolated from tepache (a fermented pineapple beverage) and molecularly characterized by Barrios-Roblero *et al*. (2019), with GenBank accession number MF632299.1.

L. paracasei TEP8 was reactivated by continuous subculturing on MRS agar at pH 6.5 for 48 h. Gram stain and a catalase test were used as purity checks. Cultures were then prepared in 10 mL of MRS broth and incubated at 150 rpm for 48 h in a Luzern shaker (H2-300).

To reactivate the phytopathogenic fungus, mycelium fragments were reseeded on Petri dishes containing Potato Dextrose Agar (PDA) at pH 7 and grown at 30 °C for 8 days until the mycelium covered the entire area of the plate.

Spore suspension

PDA culture medium was prepared at pH 7, and 15 mL was poured into Roux culture bottles. After solidification, approximately 5-mm mycelial fragments were transferred and incubated until sporulation was observed (Vázquez-Ovando *et al*., 2018). After 15 days or when sporulation was observed, the mycelium was washed with sterile Ringer's solution, and spores were recovered using glass beads. Spore concentration

was determined microscopically (40×) using a Neubauer chamber and adjusted to 1 × 10^6 spores mL^{-1}.

Papaya fruits

The study comprised two stages. In the first stage, 260 *C. papaya* L. var. Maradol fruits with an average weight of 1.75 kg were obtained from Agromod S. A. de C. V. (14° 46' 09.07'' N; 92° 13' 40.74'' W). The plants were cultivated using the producing company's traditional agronomic management practices for papaya (information not provided). The fruits were supplied immediately after harvest, and no prior antifungal treatment was applied. Sixty-five fruits were classified as physiological maturity degree 1 (green) and 65 as degree 3 (changing to orange). In stage 2, 50 fruits of the same source, also at degree 3, were used. Fruits were washed with potable water and neutral soap, immersed in 130 ppm sodium hypochlorite for 5 min, and dried with absorbent paper towels.

Stage 1 assay

The experiment comprised a 2 × 3 × 4 treatment structure: ripening degree (1 and 3), EC biopolymer matrix (starch, carboxymethylcellulose, or uncoated), and UV-C dose (0, 0.97, 2, and 2.88 kJ m^{-2}), yielding 24 treatment combinations. Two additional Mancozeb® 80 WP treatments, one for each ripening degree, were included, for a total of 26 treatment conditions. The starch and CMC ECs were supplemented with *L. paracasei* TEP8; therefore, the design did not include coating-only treatments without LAB. The code and composition of each treatment are shown in Table 1.

Table 1. *Treatments evaluated to reduce the incidence of the Colletotrichum gloeosporioides in Maradol papaya fruits at ripening degrees 1 and 3*

Code	Ripening degree	Coating	UVC dose (kJ m^{-2})
A-1	1	Starch	0.00
UVC1-A-1	1	Starch	0.97
UVC2-A-1	1	Starch	2.00
UVC3-A-1	1	Starch	2.88
UVC1-1	1	Uncoated	0.97
UVC2-1	1	Uncoated	2.00
UVC3-1	1	Uncoated	2.88
C-1	1	Carboxymethylcellulose	0.00
UVC1-C-1	1	Carboxymethylcellulose	0.97
UVC2-C-1	1	Carboxymethylcellulose	2.00
UVC3-C-1	1	Carboxymethylcellulose	2.88
Control-1	1	Uncoated	0.00
A-3	3	Starch	0.00
UVC1-A-3	3	Starch	0.97
UVC2-A-3	3	Starch	2.00
UVC3-A-3	3	Starch	2.88
UVC1-3	3	Uncoated	0.97
UVC2-3	3	Uncoated	2.00
UVC3-3	3	Uncoated	2.88
C-3	3	Carboxymethylcellulose	0.00
UVC1-C-3	3	Carboxymethylcellulose	0.97
UVC2-C-3	3	Carboxymethylcellulose	2.00
UVC3-C-3	3	Carboxymethylcellulose	2.88
Control-3	3	Uncoated	0.00

Preparation of edible coatings solutions

For starch coatings, a 1.5% (w/v) solution of cassava starch in distilled water was prepared, stirred until completely dissolved, and heated at 80 °C for 10 min. Then, 30% freshly extracted *Aloe vera gel* and 1.5% glycerol were added. The mixture was stirred until homogenized, and then ultrasound was applied (40 Hz, frequency 60%, activity 50 s, inactivity 5 s) for 15 min (Cadena de Aquino, 2025).

For carboxymethylcellulose (CMC) coatings, a 1.5% (w/v) low-molecular-weight carboxymethylcellulose solution in distilled water was prepared, and 1.5% (v/v) glycerol was added. The mixture was stirred for 10 min until homogenized. Subsequently, 30% freshly extracted *Aloe vera* gel was added, and ultrasound (40 Hz, frequency 60%, activity 50 s, inactivity 5 s) was applied for 15 min (Cadena de Aquino, 2025).

L. paracasei TEP8 incorporation was performed following the procedure used in previous work with *L. paracasei* TEP6 (Barragán-Menéndez *et al*., 2020). The required volume of 48-h MRS culture was centrifuged at 5,880 × *g* for 20 min to achieve a concentration of 9.02 log CFU g^{-1} of EC (Barragán-Menéndez *et al*., 2020). The pellet was washed twice with 1 mL of saline solution and incorporated into the starch and CMC solutions. Viable LAB counts were not independently verified in the EC at application or during storage in this experiment.

Papaya fruit inoculation and treatments

Before treatment application, all 130 degree 1 fruits were inoculated by spraying with 500 µL of a *C. gloeosporioides* spore suspension (1×10^6 spores mL^{-1}), following the procedure described by Pérez-Leyva *et al*. (2025). Fruits were held for 24 h at 28 ± 3 °C in an area with 70% RH. After 24 h, fruits were assigned to the 26 treatment conditions (Table 1), with five fruits per treatment in each experimental replicate. The complete experiment was independently performed twice simultaneously, resulting in 130 fruits per replicate and a total of 260 fruits evaluated in Stage 1.

Fruits that received UV-C irradiation treatment were placed on an aluminum platform 30 cm away from the UV light source (unfiltered germicidal lamps, Sankyo Denki Model G15T8, 15 W) previously stabilized for 30 min. UV-C intensity was measured using a photoradiometer (Delta Ohm LP9021 UVC, Padova, Italy) and was measured as 0.97 kJ m^2 after an exposure time of 3 min. Depending on the dose applied to each batch, the fruits were exposed for 0 (0 kJ m^{-2}), 3 (0.97 kJ m^{-2}), 6 (2 kJ m^{-2}), and 9 (2.88 kJ m^{-2}) min (Pinheiro *et al*., 2016). The fruits were rotated three times (depending on the total exposure time) to achieve greater exposure of the fruits' total surface area. In treatments where UV-C was combined with EC, UV-C was applied before coating application.

For the application of EC, 5 mL of the corresponding starch or CMC solution containing *L. paracasei* TEP8 was applied to the fruit surface with a polyurethane brush.

Storage and monitoring

Fruits were stored together in a common storage area at 30 ± 3 °C and 70% RH. Incidence (%) and severity (%) of the disease were recorded every 24 h for 10 days. Incidence was calculated as the number of diseased fruits divided by the total number of fruits in the treatment × 100. Disease severity was estimated as the area of visible damage caused by the fungus divided by the total area of the fruit × 100, adapting the procedure suggested by Pérez-Leyva *et al*. (2025). The areas were estimated from photographic images and analyzed using ImageJ software (Schneider *et al*., 2012).

Stage 2. EC–pathogen–fruit interaction

To verify the interaction between *C. gloeosporioides* and the EC (or EC-forming solution)

applied to the fruits, the second phase was carried out. Fifty degree-3 fruits were divided among five conditions (10 fruits per condition) shown in Table 2. Four circular wounds, 6 mm in diameter and 10 mm deep, were made on each fruit using a sterile drill bit (Hernández-Montiel *et al*., 2018), before or after application of the starch EC solution containing *L. paracasei* TEP8 and inoculation with 10 µL of spore suspension (Hernández-Montiel *et al*., 2018), according to Table 2. Fruits were stored for 17 days at 30 ± 3 °C and 70% RH in the same common storage environment. Spore germination (%) and lesion diameter (mm) were recorded every 24 h (Quiróz-López *et al*., 2021; Sandoval-Niebles *et al*., 2022).

Table 2. *Treatments evaluated to verify the inhibitory effect using Maradol papaya fruits with a degree of ripeness changing from green to orange*

Treatments	Order of application
T-1	4 lesions + inoculation of 10 µL of the spore suspension
T-2	4 lesions + inoculation of 10 µL of spore suspension + 50 µL of coating solution with LAB
T-3	4 lesions + 50 µL of LAB coating solution
T-4	Application of LAB coatings to the entire fruit + 4 lesions + inoculation of 10 µL of the spore suspension
T-5	Application of LAB coatings to the entire fruit + 4 lesions

Data analysis

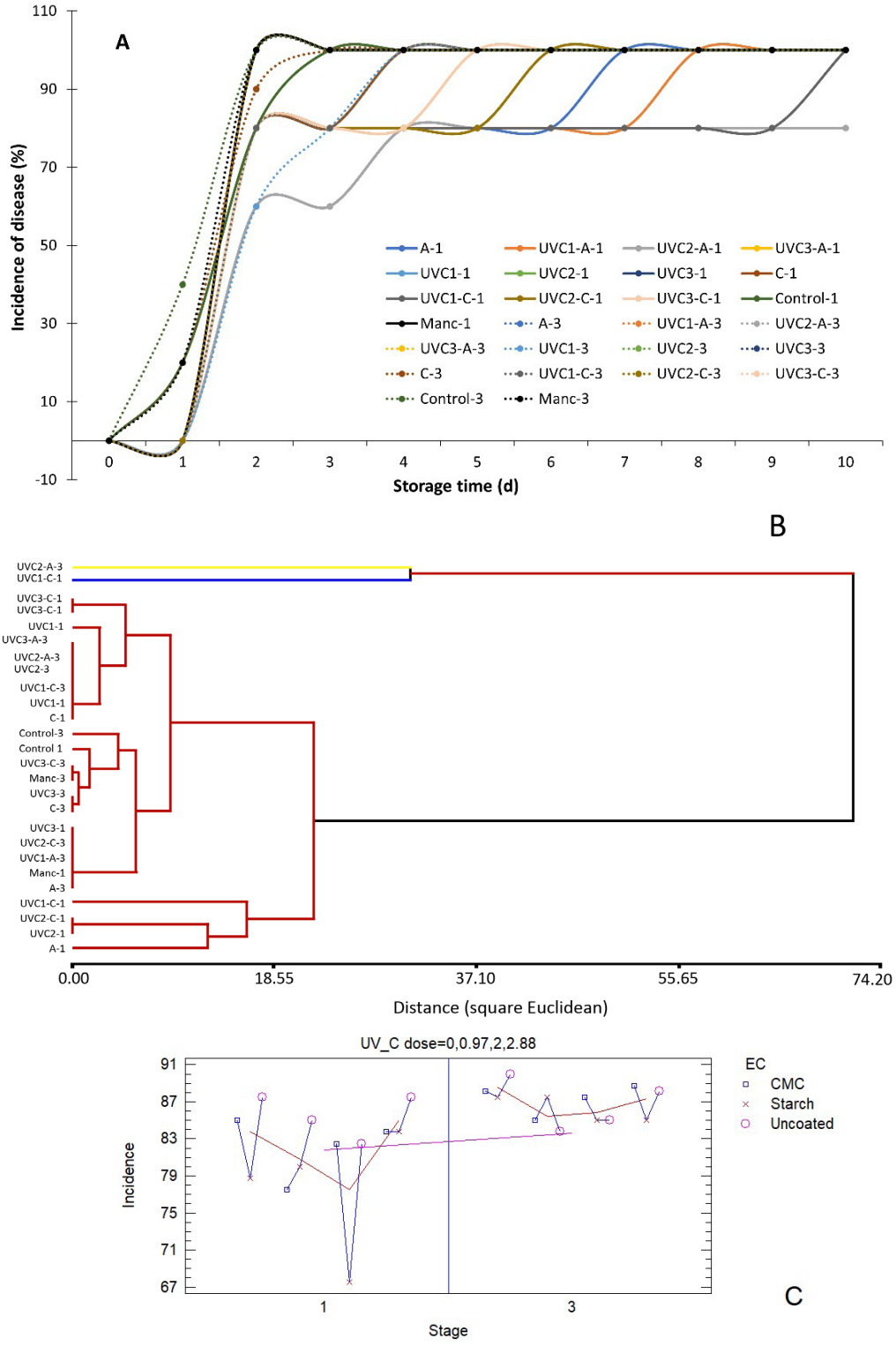
The data are presented as measures of central tendency and standard deviation as a function of storage time. Data on incidence and severity (%) were transformed using the arcosin(√n) to meet the assumptions of the analysis of variance. The average values were analyzed using factorial analysis of variance to estimate the significance of the main effects and two-way and three-way interactions using Statgraphics XV Centurion; differences were assessed using the Bonferroni test. The remaining data were taken as continuous variables and subjected to one-way or repeated-measures ANOVA, with subsequent comparison of means by the Bonferroni test ($\alpha = 0.05$). To group common treatments (combinations of factors), incidence data were analyzed by a multivariate statistical method (cluster analysis with standardized data), using the InfoStat software version 2020 (Di Rienzo *et al*., 2020). Severity data were transformed using the logistic transformation formula $Ln=y\ (1-y)^{-1}$ and subsequently plotted against sampling time. The slope value was estimated from the linear equation and considered the specific rate of severity, expressed in d^{-1} (Mckay *et al*., 2014).

RESULTS

Incidence of disease

Figure 1A shows the average incidence values in papaya fruits at ripening degrees 1 and 3 treated with different strategies against *C. gloeosporioides*. The values and trends of some treatments during storage were identical; therefore overlapping lines are observed. Infection was detected from day 1 of storage in some treatments, while all treatments showed some incidence value on day 2. Except for treatments UVC1-C-3, UVC2-3, UVC2-A-3, UVC3-A-3, and UVC1-3, all other fruits at ripening degree 3 were contaminated (100% incidence) by day 3. Similarly, fruits treated with Mancozeb® 80 WP (both ripening degrees) and untreated degree (Control-3) showed 100% incidence at 48 h after inoculation. On the other hand, treatments A-1, UVC2-A-1 and UVC1-3 showed the lowest incidence value (60%) at 48 h. On day 8 of storage, almost all treatments

showed 100% incidence, the exceptions were fruits from treatments UVC2-A-1 and UVC1-C-1 whose incidence remained at 80%. However, the incidence in fruits from treatment UVC1-C-1 reached 100% on day 10.



Note. (A) Average values. (B) Dendrogram from cluster analysis with standardized data using squared Euclidean distance and the average linkage method. (C) Multi-vari chart.

Figure 1. *Incidence of disease caused by Colletotrichum gloeosporioides in Maradol papaya fruits with ripening degrees 1 and 3 during 10 days of storage*

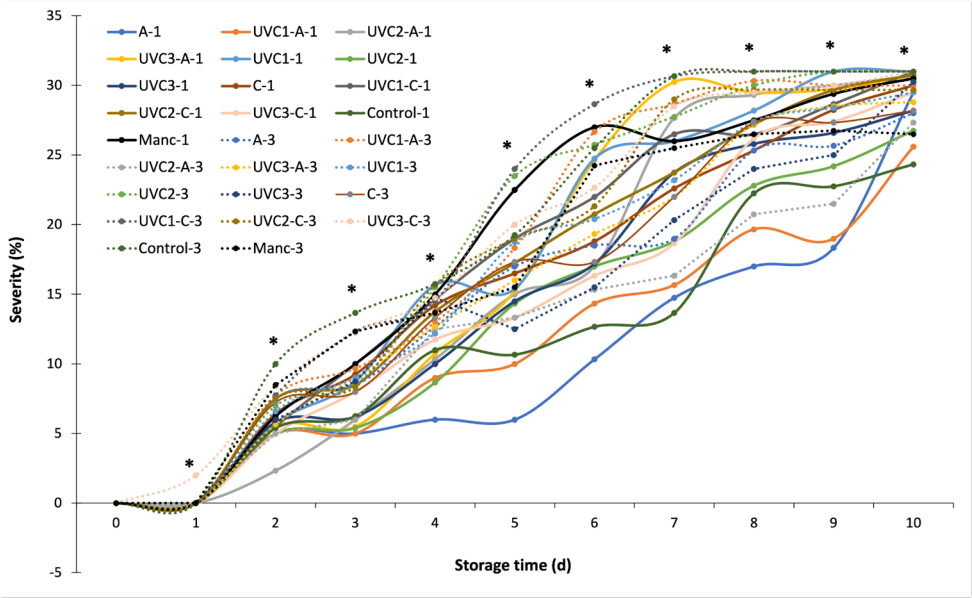
On the other hand, fruits treated with UVC2-A-1 maintained an incidence of 80% until the end of storage. For this reason, these two treatments (blue and yellow, respectively) are separated from the rest of the treatments (red) in the dendrogram derived from the cluster analysis of incidence values (Figure 1B). The treatments that showed the highest incidence values since the beginning of storage were all grouped in red (Figure 1B), and although some subgroups were formed, these did not reach the sufficient distance to belong to another set. Completely similar treatments were also observed in this set (distance = 0). Analysis of variance of the transformed mean values revealed significance for all individual factors, as well as for the two-way and three-way interactions (Table 3). Figure 1C shows the graphical representation of these interactions.

Table 3. *Analysis of variance (Type III sum of squares) for anthracnose incidence in papaya fruit at two ripening degrees (degree) treated with edible coatings (EC) and UV-C radiation*

Source	Sum of squares	Degrees of freedom	Mean square	F	P value
EC (A)	0.0259703	2	0.0129852	24.41	0.0000
UV-C (B)	0.0323299	3	0.0107766	20.26	0.0000
Degree (C)	0.0533717	1	0.0533717	100.35	0.0000
A B	0.0213379	6	0.00355632	6.69	0.0003
A C	0.0179976	2	0.00899881	16.92	0.0000
B C	0.00767181	3	0.00255727	4.81	0.0092
A B × C	0.0141391	6	0.00235651	4.43	0.0038
Residual	0.0127645	24	0.000531853		
Total (corrected)	0.185583	47			

Disease severity

The severity of damage caused by the fungus over the 10 days of storage is shown in Figure 2. Among the six treatments that showed incidence on day 1 (Figure 1A), visible damage was recorded only in fruits from treatment UVC3-C-3 (2%). By 48 h, signs of disease were evident in all treatments, with the highest severity (10.4%) observed in untreated fruits at ripening degree 3 (Control 3), in contrast to treatment UVC2-A-1, which had the lowest value (2.33%). Excluding day 0, significant differences (p<0.05) were found among treatments on every subsequent day. Fruits from the Control 3 treatment exhibited the highest severity values throughout nearly the entire storage period, whereas the opposite pattern was observed in untreated fruits at ripening degree 1: despite their high incidence (Figures 1A and 1B), severity in these fruits remained lower than in most other treatments. By the end of storage, the treatments with the highest severity values (31%) were UVC1-1, UVC1-C-3, UVC3-C-3 and Control 3, while the lowest values were recorded in Control 1 (24.33%) and UVC1-A-1 (25.66%). The evaluation of factor effects revealed significant effects of the coating type and fruit maturity stage (Table 4), but not of the UV-C dose. Furthermore, no significance effects were observed for the two-way interactions, whereas the three-way interaction was significant.



Note. Storage times marked with an asterisk at the top indicate significant differences (P<0.05) between treatments.

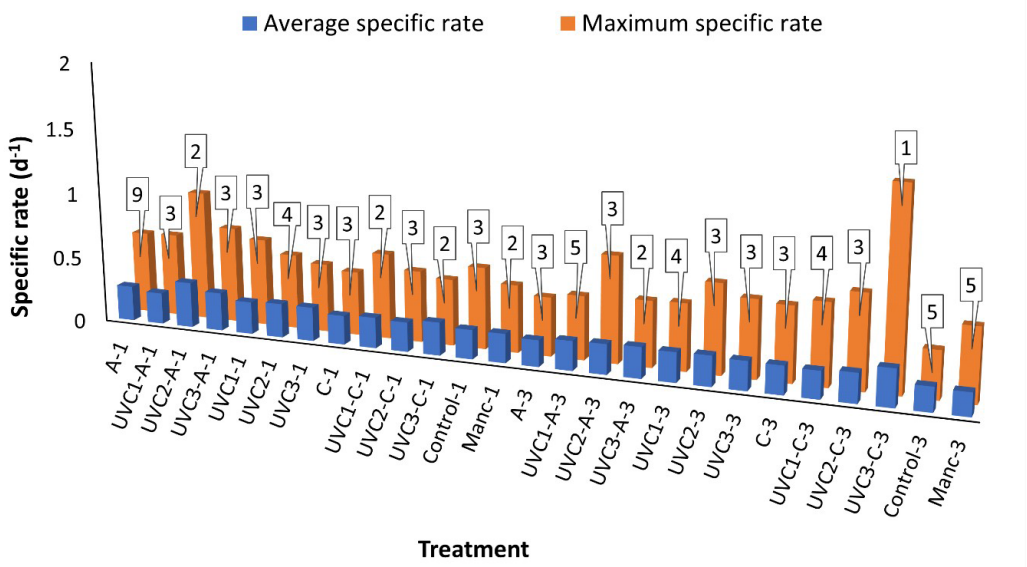
Figure 2. *Severity of the disease caused by Colletotrichum gloeosporioides on papaya var. Maradol fruits with ripening degree 1 and 3 during 10 days of storage*

Table 4. *Analysis of variance (Type III sum of squares) for disease severity caused by Colletotrichum gloeosporioides in papaya fruits at two ripening degrees treated with edible coatings (EC) and UV-C radiation*

Source	Sum of squares	Degrees of freedom	Mean square	F	P value
EC (A)	0.0122478	2	0.00612389	5.77	0.0043
UV-C (B)	0.00616588	3	0.00205529	1.94	0.1288
Degree (C)	0.0158077	1	0.0158077	14.89	0.0002
A B	0.0116074	6	0.00193457	1.82	0.1026
A C	0.0012738	2	0.000636902	0.60	0.5508
B C	0.00265617	3	0.000885391	0.83	0.4783
A B × C	0.0224671	6	0.00374452	3.53	0.0034
Residual	0.101886	96	0.00106131		
Total (corrected)	0.174112	119			

Specific rate of severity

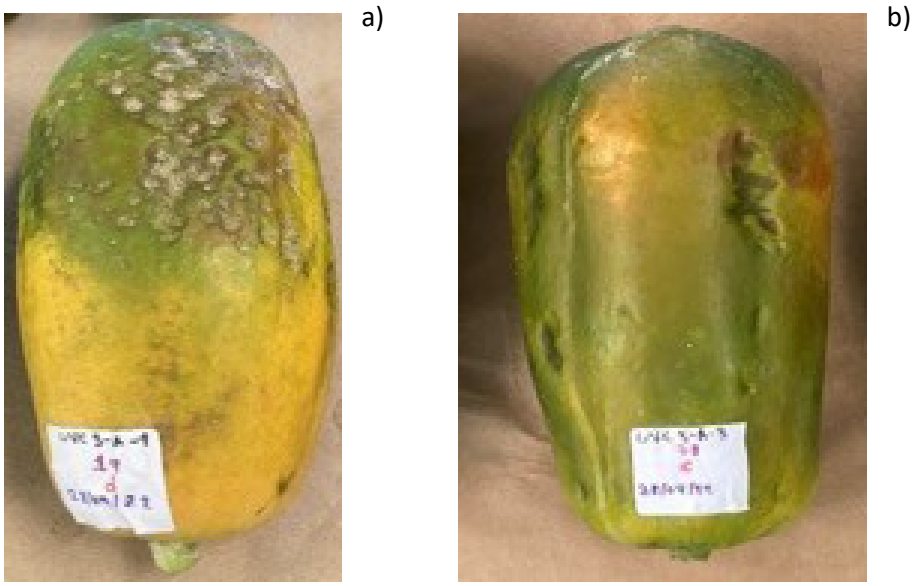
Figure 3 shows the specific rate of the severity caused by *C. gloeosporioides* in fruits. In general, the fruits from treatments with the lowest severity (Figure 2) corresponded to those with the lowest average specific rate (UVC1-A-1, A-1). Fruits from treatment UVC3-C-3 presented the highest maximum specific rate of all the treatments, which occurred from day 1 of storage (box above the bar, Figure 3), unlike the other treatments, in which the maximum specific rate occurred after day 2 and, in one treatment, as late as day 9.



Note. The numbers in boxes above the bars indicate the day with the highest specific rate value.

Figure 3. *Specific rate of severity caused by Colletotrichum gloeosporioides on Maradol papaya fruits treated with different strategies*

Although quality and texture parameters were not evaluated, fruits treated with the highest dose and at the green-yellow ripening showed signs of damage immediately after treatment application. The fruit epidermis appeared slightly dehydrated (rough) and had an opaque coloration that is not characteristic of papaya fruits during the postharvest period (Figure 4).

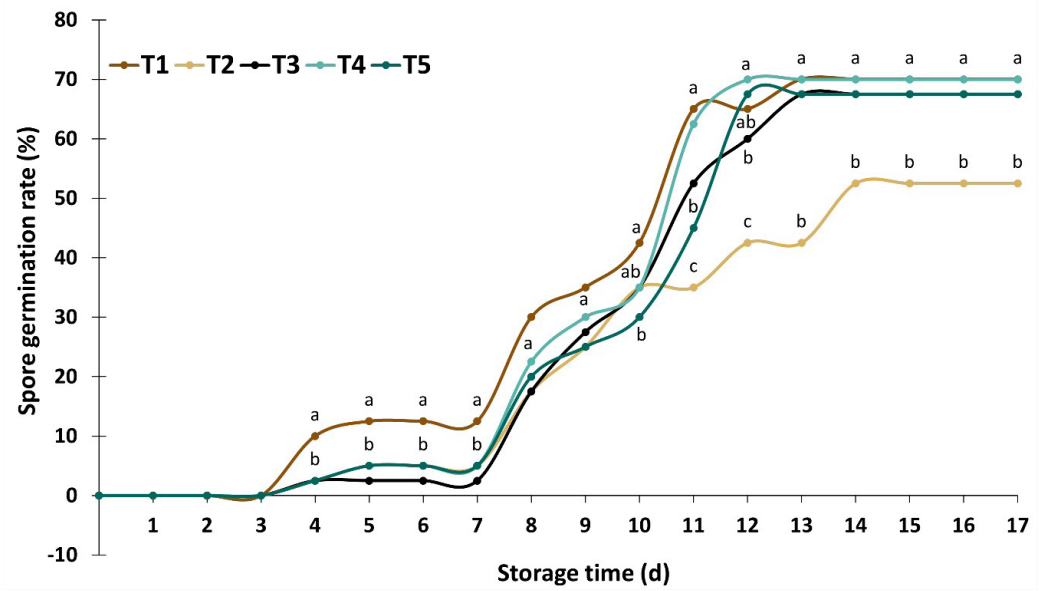


Note. A) ripeness level 1 (physiological ripeness) after 5 days of storage, B) ripeness level 3 (changing from green to yellow) after 2 days of storage.

Figure 4. *'Maradol' Papaya fruits irradiated with 2.88 kJ m-2 of UV-C at different ripening degrees*

Fungus-EC-fruit interaction

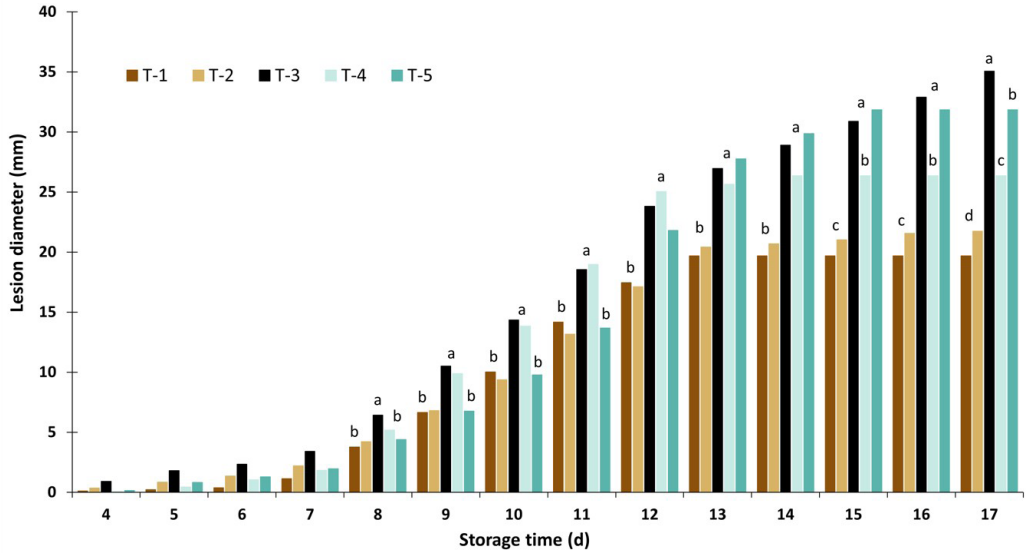
Figure 5 shows the results of the spore germination (%) inoculated directly into papaya fruits lesions. During the first 3 days, no evidence of spore germination was observed. From day 4 of storage, the highest value (10%) was recorded in T-1, while the remaining treatments had a value of 2.5%. On the eighth day, a pronounced increase was observed in all treatments; this increase continued until the 13th day of storage, especially for treatments T1 and T3-T5. After day 13, no further increase in spore germination was observed. Treatment T2 (lesions + inoculation with spore suspension + coating solution with LAB) showed the lowest germination rate from day 11 until the end of the study (52.5%), while the value for the other treatments was above 67.5%.



Note. Means with different letters for each sampling day are significantly different (repeated measures ANOVA and Bonferroni test, α = 0.05).

Figure 5. *Spore germination rate (%) of Colletotrichum gloeosporioides in papaya fruits subjected to different treatments for 17 d after spore inoculation*

Treatment T-3 (lesions + LAB coating solution), although it was not inoculated in the lesions with the spore solution, presented the largest lesion diameter at the end of the study (Figure 6). In general, the conditions that showed the greatest lesion diameters were treatments T-3, T-4, and T5, which at the end of the study (day 17) presented values of 35.05, 26.36, and 31.85 mm, respectively. Except for days 4-7, significant differences ($P < 0.05$) between treatments were found on all other days. Treatments T1 and T2 showed no significant differences between them throughout the study, and at the end, they presented the lowest values in the size of the lesion (19.68 and 21.74 mm, respectively).



Note. Bars with different letters are significantly different for the same day (one-way ANOVA per day, Bonferroni test, $P < 0.05$).

Figure 6. *Average lesion diameter (mm) caused by Colletotrichum gloeosporioides in papaya fruits inoculated with spores, subjected to different treatments and stored for 17 d*

DISCUSSION

The study evaluated whether UV-C irradiation combined with starch- or CMC-based coatings containing *L. paracasei* TEP8 could modify anthracnose development in Maradol papaya fruits at two ripening degrees, and whether the order of coating application relative to pathogen inoculation influenced spore germination and lesion development in wounded fruit. Overall, the results indicate that disease development was strongly dependent on fruit ripening degree and coating matrix, while the contribution of UV-C depended on the particular experimental factors. Therefore, the effects observed should be interpreted as responses to specific treatment combinations rather than as independent effects of UV-C irradiation or *L. paracasei* TEP8.

Fruit ripening degree was an important determinant of anthracnose development. Fruits at ripening degree 1 generally showed lower disease severity than fruits at degree 3, and ripening degree had a significant effect on both incidence and severity (Figures 1-3). This response is consistent with the biology of *Colletotrichum* spp., which can remain quiescent in immature fruit tissues and resume development as ripening progresses. Changes associated with ripening, including increased ethylene production and modifications of host tissue susceptibility, may favor fungal colonization and symptom development (Chávez-Espinoza *et al*., 2020). The lower disease progression observed in less mature fruits therefore suggests that physiological maturity at treatment application is an important factor when considering postharvest strategies against papaya anthracnose. However, because physiological and biochemical variables related to ripening were not measured in the present study, the contribution of ethylene or other ripening-associated mechanisms cannot be directly established from our data.

The coating matrix also significantly influenced disease development. Treatments containing the starch-based coating with *L. paracasei* TEP8 generally showed lower incidence, severity, and specific severity rates than several CMC-coated or uncoated

treatments, particularly in degree-1 fruits. Although the result is not conclusive, it can be assumed that the LAB used exerts its antifungal activity more effectively when incorporated into a starch matrix. Previous studies have demonstrated that *L. paracasei* TEP8 can retain antifungal activity against *C. gloeosporioides* (Barrios-Roblero *et al*., 2019) by synthesizing some active compounds such as organic acids, carbon dioxide, ethanol, hydrogen peroxide, fatty acids, acetoin, diacetyl, cyclic dipeptides, and bacteriocins (De Simone *et al*., 2021). Likewise, Marín *et al*. (2019) reported effective control of *B. cinerea* in grapefruits when *Lactobacillus plantarum* was incorporated into starch-based coatings. Nevertheless, the better performance of some starch-based treatments in the present study cannot be attributed exclusively to *L. paracasei* TEP8. All starch and CMC coatings used in Stage 1 contained TEP8, and coating-only treatments without the bacterium were not included. Consequently, the experimental design does not allow the physical effects of the coating matrix to be separated from the biological effects of the incorporated LAB. The effect of the coatings on disease development may be because related to the fact that starch coatings generally have adequate resistance to water vapor, which may reduce dehydration but also delays gas exchange (permeability to O_2 and CO_2), and consequently, affect endogenous ethylene production. It has been reported that ethylene functions as a signal that promotes the infection process of the fungus *Colletotrichum* spp. (Dassamiour *et al*., 2022; Hernández-Guerrero *et al*., 2023; Ochoa-Velasco *et al*., 2021). The physiological properties of the fruits could have contributed to the observed disease response independently of, or in combination with, the antifungal activity of TEP8. Future experiments should therefore include equivalent coatings with and without TEP8 to quantify the specific contribution of the bacterial antagonist.

A further limitation is that viable counts of *L. paracasei* TEP8 were not independently determined in the coating immediately before application or during fruit storage. Although the inoculum concentration was established during coating preparation and previous studies have demonstrated the ability of related LAB strains to remain viable in edible matrices (Barragán-Menéndez *et al*., 2020; Li *et al*., 2024), the actual survival of TEP8 under the conditions used in this experiment remains unknown. Therefore, variation in bacterial viability among coating matrices or during storage cannot be excluded as a factor contributing to differences among treatments. Monitoring viable LAB populations on the fruit surface throughout storage would help establish a more direct relationship between bacterial survival and antifungal effectiveness.

The response to UV-C irradiation was more complex than that suggested by comparison of individual treatment means. For disease incidence, factorial analysis showed significant effects of UV-C dose, coating matrix, and ripening degree, as well as significant two-way and three-way interactions. These interactions indicate that the effect of UV-C was dependent on both coating type and fruit maturity. Thus, a single generalized effect of UV-C irradiation cannot be inferred across all experimental conditions. In particular, treatment UVC2-A-1, corresponding to degree-1 fruits irradiated at 2 kJ m^{-2} and subsequently coated with the starch–TEP8 formulation, maintained the lowest final incidence observed in Stage 1 (80%). This response identifies this combination as one of the more favorable conditions evaluated; however, it should not be interpreted as evidence that 2 kJ m^{-2} was universally the most effective UV-C dose. Nevertheless, under the conditions evaluated, it appears to be the most appropriate among the doses tested. This distinction is even more important for disease severity. The factorial analysis showed significant effects of coating matrix and ripening degree, whereas the main effect of UV-C dose was not significant. Moreover, none of the two-way interactions involving UV-C were significant, although the three-way interaction

among coating matrix, UV-C dose, and ripening degree was significant. Therefore, UV-C irradiation alone cannot be considered to have consistently reduced severity. Instead, its response depended on the complete treatment context. For example, UVC1-A-1, combining 0.97 kJ m^{-2} with the starch–TEP8 coating in degree-1 fruit, showed one of the lowest final severity values, whereas the highest UV-C dose did not consistently improve disease control. The response observed at moderate UV-C doses is nevertheless compatible with previous reports showing that UV-C can reduce postharvest fungal development. Cia *et al*. (2007), for example, reported reduced germination of *C. gloeosporioides* conidia following UV-C treatment of papaya, while other studies have shown that appropriate UV-C doses can reduce microbial deterioration in harvested plant products (Frisón *et al*., 2021; Smith *et al*., 2024). Direct microbial inactivation by UV-C is commonly associated with photochemical damage to nucleic acids, including pyrimidine dimer formation, which interferes with DNA replication and cellular functions (Zhu *et al*., 2025). However, spore viability immediately after UV-C irradiation was not measured in Stage 1 of the present study. Consequently, the observed disease responses cannot be unequivocally attributed to direct germicidal activity, and possible indirect effects mediated through the fruit should also be considered. Increasing UV-C exposure did not result in progressively greater disease suppression. In particular, the highest dose evaluated, 2.88 kJ m^{-2}, was not associated with superior anthracnose control and produced visible alterations of the fruit epidermis. Fruits exposed to this treatment showed a rougher, slightly dehydrated surface and atypical opaque coloration. Similar dose-dependent changes in papaya quality after UV-C treatment have previously been reported (Sesma-Morales *et al*., 2020). These observations emphasize the importance of optimizing UV-C exposure rather than assuming that higher doses provide greater protection. Because fruit firmness, color, weight loss, respiration, ethylene production, or other physicochemical and sensory attributes were not quantitatively evaluated in this study, the physiological consequences and commercial relevance of the visible UV-C injury cannot be determined. This represents an important limitation for recommending the treatments for postharvest application.

The Stage 2 experiment provided additional information regarding the interaction between the starch–TEP8 coating and *C. gloeosporioides* in wounded fruits. Treatment T2, in which the coating solution containing TEP8 was placed directly in the wound after pathogen inoculation, showed the lowest final spore germination value (52.5%), whereas germination in the remaining treatments exceeded 67.5%. This result suggests that direct contact between the coating formulation and recently inoculated spores may interfere with germination. Such an effect would be consistent with the previously demonstrated antifungal activity of TEP8 (Barrios-Roblero *et al*., 2019). Nevertheless, because a starch coating without TEP8 was not included, it is not possible to establish whether the lower germination resulted primarily from bacterial antagonism, properties of the coating formulation, or their combined action. Furthermore, the reduction in spore germination observed in T2 did not translate into a statistically lower final lesion diameter relative to the inoculated control T1. At day 17, T1 and T2 showed lesion diameters of 19.68 and 21.74 mm, respectively, and they were not significantly different. Thus, the results support an effect of treatment T2 on spore germination but do not demonstrate a corresponding reduction in subsequent lesion expansion. This distinction is relevant because inhibition of an early infection event does not necessarily imply effective suppression of disease once fungal colonization has been established. Interpretation of Stage 2 is also limited by the occurrence of lesions in treatments that were not deliberately inoculated with *C. gloeosporioides*. T3, which received wounds and the LAB-containing coating solution but

no intentional pathogen inoculation, ultimately developed the largest lesion diameter. Because fruits from all treatments were maintained in the same storage environment, unintended exposure to fungal propagules or cross-contamination among treatments cannot be excluded. Accordingly, lesion development in the non-inoculated conditions should not be interpreted as evidence of failure of the coating itself. Future experiments should physically separate inoculated and non-inoculated controls or use independent storage units to minimize this source of uncertainty.

The synthetic fungicide Mancozeb® did not provide effective disease suppression under the conditions evaluated. Previous work has also reported limited effectiveness of this fungicide against phytopathogenic fungi associated with papaya (Salvador-Figueroa *et al*., 2017). However, the present experiment was not designed to investigate fungicide resistance, and no sensitivity assays or molecular analyses of resistance mechanisms were conducted. Therefore, the poor response to Mancozeb® cannot be specifically attributed to fungicide resistance based on the current data.

An additional methodological limitation concerns the identity of the fungal isolate. The *C. gloeosporioides* strain used in this study was previously isolated from diseased Maradol papaya and its pathogenicity was demonstrated through Koch's postulates, but it has not been molecularly characterized. Because the *C. gloeosporioides* species complex comprises closely related taxa that can differ in pathogenic behavior, molecular identification of the isolate would strengthen future studies and facilitate comparisons of treatment responses across *Colletotrichum* species and isolates. Taken together, the results indicate that anthracnose development in Maradol papaya was determined by the interaction among fruit maturity, coating matrix, and UV-C treatment rather than by any single factor. The most favorable responses occurred predominantly in degree-1 fruits treated with starch-based formulations containing *L. paracasei* TEP8, with some combinations involving moderate UV-C doses showing delayed disease development. However, the absence of coating-only controls, the lack of measurements of TEP8 viability during storage, the absence of quantitative fruit-quality measurements, the possibility of cross-contamination in Stage 2, and the lack of molecular identification of the fungal isolate limit mechanistic interpretation and prevent attribution of the observed effects exclusively to the LAB or UV-C treatment. Future studies addressing these limitations, together with validation under commercial postharvest conditions, are required before the combined strategy can be recommended as an alternative anthracnose-control treatment.

CONCLUSIONS

Anthracnose development in Maradol papaya was influenced by fruit ripening degree, coating matrix, and UV-C treatment, with significant interactions among these factors. The most favorable responses were generally observed in degree-1 fruits treated with starch-based coatings containing *Lacticaseibacillus paracasei* TEP8. In particular, the combination of 2 kJ m^{-2} UV-C followed by the starch–TEP8 coating resulted in the lowest final disease incidence observed (80%), whereas 0.97 kJ m^{-2} UV-C combined with the same coating produced one of the lowest final severity values among treated fruits. However, UV-C dose did not have a significant main effect on disease severity, indicating that its performance depended on the specific combination of coating matrix and fruit ripening degree rather than on UV-C exposure alone. In wounded fruits, application of the starch–TEP8 coating solution after pathogen inoculation reduced final spore

germination relative to the other evaluated conditions, but did not significantly reduce final lesion diameter compared with the inoculated control. Therefore, suppression of spore germination did not necessarily result in reduced lesion expansion. The results suggest that starch-based coatings containing TEP8, particularly when applied to less mature fruits and combined with moderate UV-C doses, may represent promising components of an integrated postharvest strategy against papaya anthracnose. Nevertheless, since coating-only treatments without TEP8 were not included, viable TEP8 populations were not monitored during storage, fruit quality parameters were not quantitatively evaluated, and the fungal isolate lacked molecular identification, the individual contribution of the LAB and the commercial applicability of the treatments cannot yet be established. Further studies incorporating appropriate coating controls, monitoring bacterial viability and fruit quality, molecularly identifying the pathogen, and validating the treatments under commercial storage conditions are needed before practical recommendations can be made.

AUTHOR CONTRIBUTIONS

Conceptualization, A.V.O., and I.R.D.; Methodology, M.S.F., W.A., and R.R.Q.; Validation, I.R.D., A.V.O., and R.R.Q.; Formal Analysis, I.R.D., and A.V.O.; Investigation, I.R.D.; Resources, A.V.O., and M.S.F.; Data Curation, A.V.O.; Writing—Original Draft Preparation, I.R.D., and A.V.O.; Writing—Review & Editing, all authors.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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