Original Article

CONTROL OF *Phytophthora palmivora* ON POSTHARVEST PAPAYA WITH *Trichoderma asperellum*, *T. virens*, *T. harzianum* AND *T. longibrachiatum*

CONTROLE DE Phytophthora palmivora *EM MAMÃO NA PÓS-COLHEITA POR* Trichoderma asperellum, T. virens, T. harzianum E T. longibrachiatum

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ABSTRACT: Papaya (*Carica papaya*) is one of the most cultivated and consumed tropical fruit worldwide. Its production might be limited by preharvest and postharvest diseases. The fruit rot caused by *Phytophthora palmivora* is one of the most important postharvest diseases of papaya in Brazil. The control of these diseases is usually made with fungicide applications. Therefore, studies concerning biocontrol of postharvest diseases might generate data that may reduce the environmental impacts caused by pesticides. Thus, the biological control by *Trichoderma* in postharvest diseases is an alternative to the use of fungicides for the postharvest control of *P. palmivora* in the papaya fruit. Four antagonists [*T. asperellum* (SF04), *T. virens* (255C1), *T. harzianum* (THP) and *T. longibrachiatum* (4088)] were tested, as follow: 1) *Trichoderma* spp. applied 1 hour after inoculation of *P. palmivora* and; 2) *Trichoderma* spp. applied 24 hours after inoculation of *P. palmivora*; 3) *Trichoderma* spp. applied 1 hour before inoculation of *P. palmivora*, and 4) *Trichoderma* spp. applied 24 hours before inoculation of *P. palmivora*. All *Trichoderma* significantly (P≤0,05) reduced the incidence and severity of disease. The 4088 (*T. longibrachiatum*) isolate was the best controller agent of *P. palmivora* in postharvest.

KEYWORDS: Biocontrol. Antagonism. Bioprotectors. Fruit Pathology

INTRODUCTION

Papaya (*Carica papaya*) has several phytosanitary problems such as pests, fungal and bacterial diseases mainly in post-harvest. Among the fungal diseases feature the root rot and fruit that has caused 60% loss of production (SILVA, 2001). Fruit decays account for significant levels of postharvest losses. It is estimated that about 20% of the harvested fruits are decayed by pathogens during postharvest handling (EL-GHAOUTH et al., 2001; DROBY, 2009).

The causal agent of papaya stem, root and fruit rot is *Phytophthora palmivora* (Butler) Butler. This disease occurs in almost all producing regions of Brazil, and a reason for it the absence of resistant commercial papaya cultivars (KADER, 2002). The control of *P. palmivora* is basically done with fungicide and one of those is a combination of metalaxyl and mancozeb. Fungicide application may leave toxic residues on fruits and may select pathogen isolates with resistance to it. In addition, fungicides when not applied properly, might cause environmental contamination and damage to human health (ROBERTS; KUCHAREK, 2005).

The presence of agricultural products in pesticide residues and the accumulation of these substances in the environment have stimulated research of alternative methods of disease control in postharvest, especially by public agencies concerned with health issues and trade relations (OLIVEIRA et al., 2006). Therefore, the biological control applicability is emphasized and studied for the management of various diseases even if informally. Due to the lack of studies related to postharvest especially in producing regions, such as the Southern Bahia one of the leading producers and exporters of papaya whose research and biological control application are still incipient. In addition, the biocontrol has emerged as viable technology in postharvest fruit being favored by specific targets and the fruit storage in controlled conditions, which favors the antagonist establishment (JANISIEWICZ; KORSTEN, 2002).

Trichoderma is well documented as a biological control agent (BCA), with potential use in fruit diseases caused by *Phytophthora* spp. (ALEXANDER; STEWART, 2001). *Trichoderma* species are potential antagonists of various pathogenic fungi and have several action mechanisms, the most effective being: the metabolites production and enzymes with antifungal properties, hyperparasitism and nutrients competition (HARMAN et al., 2000). As an

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additional advantage, these microorganisms are referred to as non-toxic to man and animals and as symbiotic plants associated with avirulent (MERTZ et al., 2009).

In several fruits such as apple, strawberry, citrus and banana the *Trichoderma*, has been used to effectively control postharvest diseases (PRATELLA; MARI, 1993; PAJMON et al., 1995; BATTA, 2004; NALLATHAMBI et al., 2009). Therefore, the potential in the postharvest diseases' management using biocontrol are relevant to diverse cultures in postharvest, subject to environmental and agribusinesses (JANISIEWICZ; KORSTEN, 2002). Although the Bahia south and Espírito Santo northern are responsible for most of Brazilian production and export of papaya, few studies refer to the biocontrol application in the postharvest fruit and this has compromised the commercial competitiveness. Therefore, the aim of this study was to evaluate the efficacy of *Trichoderma* spp. for the biocontrol of *P. palmivora* in postharvest papaya.

MATERIAL AND METHODS

The isolate 356 *P. palmivora* was obtained from the Collection of *Phytophthora* 'Arnaldo Medeiros' at the section of Plant Pathology in the Research Center of Cocoa Crop (CEPLAC), Bahia, Brazil, and, where the experiments were conducted.

Phytophthora palmivora (356) was transferred to Petri dishes containing selective medium PARPH (KANNWISCHER; MITCHELL, 1978), grown for five days in the dark (25 ±2ºC), and, then transferred to carrot agar medium (CA). The dishes were kept in incubator (BOD - Biochemistry Oxygen Demand) under continuous light (25 ± 2 ºC), for nine days. Then the pathogen was inoculated into healthy fruit for observation of the *P. palmivora* characteristic symptoms and again isolated following Koch's postulates. Finally, the cultures were maintained in test tubes containing culture medium CA and preserved for further studies. The four isolates of *Trichoderma* spp. used in this study were provided by the Collection of fungi antagonists at the Biocontrol Laboratory of CEPLAC. These antagonists were selected as effective biocontrol agents in other studies: SF04 (*T. asperellum*) (TOCAFUNDO et al., 2010), 255C1 (*T. virens*) e THP (*T. harzianum*) (TAVARES, 2009); and, 4088 (*T. longibrachiatum*) (OLIVEIRA et al., 2009). *Trichoderma* spp. isolate were transferred to Petri dishes with medium Potato Dextrose Agar (PDA) and maintained for 10 days at 25 ± 2 ºC with 12h light.

Preparation of *P. palmivora* and *Trichoderma* spp. suspensions

Suspensions of *P. palmivora* were obtained from 20 dishes containing the pathogen zoosporangia. To each plate was added 8 mL of sterile distilled water (SDW). For liberation of zoospores the dishes were subjected to thermal shock [5 (± 2 ºC) / 20 min followed by 25 (± 2 ºC) / 25 min]. Afterwards, the zoospores concentration was determined and standardized in 5 x 10^5 zoospores mL^{-1} suspension using Neubauer chamber, adding 2 drops of fixative solution FAA (formaldehyde, alcohol and acetic acid) for standstill of zoospores.

For *Trichoderma* spp. suspension was obtained from 20 dishes containing the antagonist candidate. Eight mL of SDW were added on the colonies surface and conidia were removed by friction with a brush. This suspension was filtered through sterile double gauze and conidial concentration (10^8 mL^{-1}) was determined and adjusted in hemocytometer.

Evaluation of *Trichoderma* spp. for biocontrol of postharvest papaya fruit rot

The experiments were performed in the 'Phytophthora' Laboratory, Plant Pathology Section of the Crop Research Center Cocoa (CEPLAC). Two experiments were conducted identically, one in March / 2010 and another in April / 2010. The second one was performed 30 d after the first (Experiment 1). For the evaluation of potential biocontrol were used fruit variety Sunrise Solo, maturation stage 2 (RITZINGER; SOUZA, 2000), from Farm 'Alegria', located at Vera Cruz City, Bahia, Brazil.

The fruits were washed with mild soap and water and dried at room temperature. The *Trichoderma* were then applied as follows: *Trichoderma* spp. applied 1 h after inoculation of *P. palmivora*; *Trichoderma* spp. applied 24h after inoculation of *P. palmivora*; *Trichoderma* spp. applied 1h before inoculation of *P. palmivora*; *Trichoderma* spp. applied 24h before inoculation of *P. palmivora*. Two *P. palmivora* inoculation methods were also tested: a) $5x10^5$ de zoospores mL^{-1} (20 µL), applied over three 2-mm injured holes at the equatorial region of fruit, and; b) zoospores suspension spray. Each *Trichoderma* isolate was sprayed as conidial suspension (10^8 conidia mL^{-1}) in both types of pathogen inoculation. For comparison purposes with the *Trichoderma* spp., a fungicide (mancozeb + metalaxyl) treatment was applied on fruits. In addition, a group of fruits was pathogen inoculated and treated with SDW. All treatments

were submitted to incubation in humidity chamber for 72h at 25 ± 2 ºC.

Experimental design, disease assessment and statistical analysis

The experiments were conducted in a completely randomized design. The 24 experimental treatments, that were composed by a combination of six *Trichoderma* isolates with four types of *Trichoderma* application. All treatments had 10 replications of one fruit for disease severity evaluation and three replications of six fruits each, for disease incidence enumeration. The severity was measured for six days after fruit removal from humidity chamber, by determining injured considering the diameter (mm) of the lesion in two diametrically opposite directions, using the following formula: $S = (\pi \times D1 \times D2)/4$. Where: S = colony area; D1 = diameter1; D2 = diameter2. The data obtained in the experiments underwent hypothesis t Student test ($P \leq 0.05$) and then analysis of variance, the average cluster made by the Scott-Knott test ($P \leq 0.05$) [SISVAR 5.3 (FERREIRA, 2011)].

RESULTS AND DISCUSSION

When comparing the two experiments, there was no statistical difference by Student's t test ($P \leq 0.05$), between them. Therefore, it was possible to use combined experiment data analysis. Once the pathogen was inoculated 1h or 24 h before the antagonists, it was observed that the isolated 225C1 (*T. virens*) induced a disease lesion area reduction, compared to the control without any treatment (Table 1). This area reduction on disease severity corresponds to around 49 to 61% of control (Table 2). In addition, it was observed that 225C1 was better than the fungicide treatment, in reduction of the diseased area when the pathogen was applied 24 h before *Trichoderma* (Tables 1 and 2). The most efficient treatment was the fungicide when the pathogen was applied 1 h before *Trichoderma* (Tables 1 and 2).

For treatments where the biocontrol agent was applied 1h and 24h before the pathogen, it was observed that isolate 4088 (*T. longibrachiatum*) caused a decrease on lesion area (Table 1). The isolate 4088 when applied 1h before the pathogen, showed a disease control of over 74% (Table 2). The isolates 225C1, SF04 and THP also significantly reduced disease severity and increased disease control, compared to the untreated control (Tables 1 and 2).

Table 1. Effect of *Trichoderma* on the area of the lesion caused by *Phytophthora palmivora* in post-harvest, six days after incubation.

	Area of the lesion of papaya-fruit-rot (mm^2)			
	P. palmivora before *Trichoderma*		*Trichoderma* before *P. palmivora*	
Treatment[(1)]	1h	24h	1h	24h
T. longibrachiatum	5760.0[(2)] cC[(3)]	6627.3 bD	2170.1 bB	925.4 aA
T. virens	4108.0 bA	4270.0 aA	4136.4 cA	3586.9 bA
T. asperellum	5498.1 cB	6157.8 bB	4066.7 cA	3114.6 bA
T. harzianum	5131.5 cB	5713.1 bB	3446.6 cA	3733.3 bA
Fungicide	0.0 aA	6741.3 bB	471.2 aA	473.1 aA
Check control	7714.3 dA	10158.5 cB	8088.3 dA	7929.8 cA
CV%	28.3	21.0	20.4	22.5

[(1)] *Trichoderma*: *T. longibrachiatum* 4088; *T. virens* 225C1; *T. asperellum* SF04 -; *T. harzianum* THP -; Fungicide – mancozeb + metalaxyl; Check control - sterilized destiled water; [(2)] Area of mycelial growth of *P. palmivora* in mm ^{2}for 20 repetitions ; [(3)] Values followed by the same lowercase letter in each column and the same capital letter on a line, do not differ statistically, according to the test of Scott-Knott (P≤0,05).

Treatment with isolated 4088 inoculated 24 h before the pathogen, was statistically as effective as the fungicide (Table 1), with a control of around 88% (Table 2) and statistically different from the others. Once the *Trichoderma* spp. require period of conidial humectation in order to germinate and penetrate into the plant pathogenic fungi (BATTA, 2004a; 2004b). The beneficial action of *T. virens* for pre-treatment of cotton seedlings has been reported. It was demonstrated that the induction of plant defense system and the suppression of pathogen germination by antagonistic compounds produced by germinating cotton seedlings were the dominant biocontrol mechanisms (HOWELL; PUCKHABER, 2005).

Although *Trichoderma* is well reported as a biocontrol agent (BCA) against various species of *Phytophthora* (AMORIM; ITAMAR, 1999; COSTA et al., 2000; ALEXANDER; STEWART, 2001; 2) the specific effect on *P. palmivora* in papaya has not been studied and the results obtained so far are few significant (BUENO; SILVA, 2001; DIANESE et al., 2012; TOCAFUNDO et al., 2010). The control effectiveness of the isolates 225C1 (*T. virens*), SF04 (*T. asperellum*) and 4088 (*T. longibrachiatum*), indicated that these isolates are important for management of the papaya fruit rot (*P. palmivora*).

Table 2. Percentage of control of fruit rot of papaya caused by *Phytophthora palmivora* on the post-harvest 6 days after inoculation.

	Control (%) of *Phytophthora palmivora* under treatment with *Trichoderma* spp.			
	Pathogen before the biocontroler		Biocontroler before the pathogen	
Isolate[(1)]	1h	24h	1h	24h
4088	28.61[(2)] bA[(3)]	39.77bA	74.63 cB	88.64 cC
225C1	49.09 cA	61.19 cA	51.62 bA	56.01 bA
SF04	36.42 bA	43.99 bA	52.44 bB	61.79 bB
THP	36.42 bA	48.08 bB	59.69 bB	54.20 bB
Fungicide	100.00 dB	38.64 bA	94.48 dB	94.18 cB
Check control	0.00 aA	0.00 aA	0.00 aA	0.00 aA
CV%	12.34	13.47	10.45	9.56

[(1)]4088 - *Trichoderma longibrachiatum*; 225C1 - *T. virens*; SF04 - *T. asperellum*; THP - *T. harzianum*; Fungicide - mancozeb+metalaxyl; check control - Sterile distilled water; [(2)] Percentage of disease control (%) in relation to check control. [(3)] Values followed by the same lowercase letter in each column and the same capital letter on a line, do not differ statistically, according to the test of Scott-Knott (P≤0,05).

Regarding the incidence of *P. palmivora* in papaya fruit, where the pathogen was inoculated 1h before the BCA and evaluated the 3d following the removal of humid chamber, the isolates 4088, 225C1 and THP induced a delay on disease (Figure 1). Even as evaluated six days after removal of the humid chamber, the isolate 4088 and 225C1 obtained 50% disease control, statistical differing from the other isolates and check control.

In the treatment where the pathogen was inoculated 24 h before antagonists none of isolates has demonstrated the ability to control or slow the growth of *P. palmivora* on papaya. Therefore, 24 h of pathogen incubating in fruit was enough for its establishment and consequently reduce the effectiveness of the BCA. The BCA 4088 and 225C1 when inoculated 1 h before the pathogen and evaluated 6 d after incubation, resulted in a significant disease reduction. However, when BCA were applied 24 h before the pathogen resulted in 100% control. The 225C1 (*T. longibrachiatum*) and 4088 (*T. virens*) isolates reduced the incidence of disease to under 30% and improved the disease control to over 60%. (Figure 1). This implies that it is necessary an establishment period of the antagonist before having contact with the pathogen, to enable effective disease control. Rapid colonization of fruit wound by the antagonist is critical for decay control, and manipulations leading to improved colonization enhance biocontrol (MERCIER; WILSON, 1994). Thus, microbial antagonists should have the ability to grow more rapidly than the pathogen. Similarly, it should have the ability to survive even under conditions that are unfavorable to the pathogen (DROBY et al., 1992).

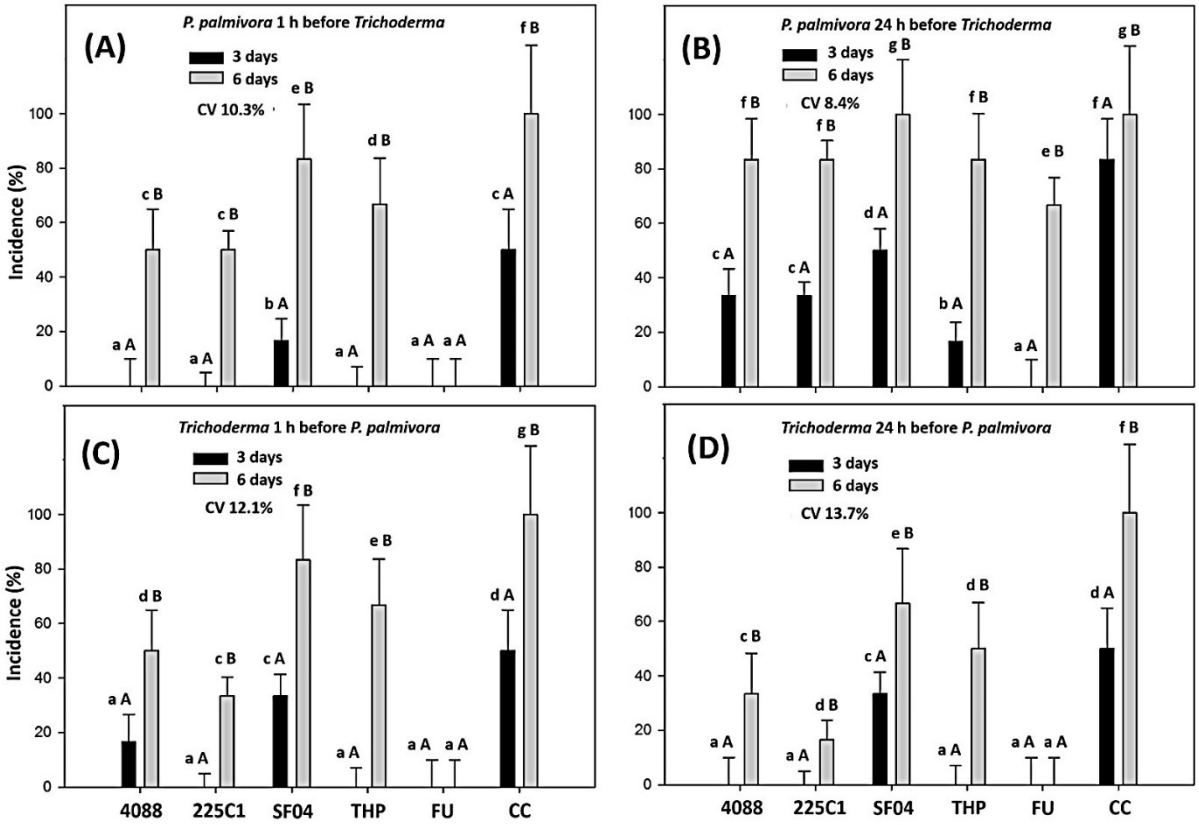

Figure 1. Incidence of *P. palmivora* causing rot of papaya fruit on post-harvest, under the control of *Trichoderma* spp. applied by spraying, 3 and 6 days after incubation. (A) Inoculation of *P. palmivora* 1 hour after inoculation of *Trichoderma* spp.; (B Inoculation of *P. palmivora* 24 hours after inoculation of *Trichoderma* spp.; (C) Inoculation of *Trichoderma* spp. 1 hour after inoculation of *P. palmivora*.; (D) Inoculation of *Trichoderma* spp. 24 hours after inoculation of *P. palmivora*. [(1)]4088 - *Trichoderma longibrachiatum*; 225C1 - *T. virens*; SF04 - *T. asperellum*; THP - *T. harzianum*; Fungicide - mancozeb+metalaxyl; Check control - Sterile distilled water Values followed by the same lowercase letter in each period (3 or 6) and the same capital letter in the same treatment, did not differ statistically (Scott-Knott Test, P≤0,05).

In addition, competition for nutrient and space between the pathogen and the antagonist is considered as the major modes of action by which microbial agents control pathogens causing postharvest decay (IPPOLITO et al., 2000; JIJAKLI et al., 2001). To compete successfully with pathogen at the wound site, the microbial antagonist should be adapted to various environmental and nutritional conditions than the pathogen (BARKAI-GOLAN, 2001; EL-GHAOUTH et al., 2004). In addition, production of antibiotics (antibiosis), direct parasitism, and possibly induced resistance are other modes of action of the microbial antagonists by which they suppress the activity of postharvest pathogens on fruits and vegetables (JANISIEWICZ et al., 2000; BARKAI-GOLAN, 2001; EL-GHAOUTH et al., 2004).

Tavares (2009) reported 50% control of papaya seedlings root rot (*P. palmivora*) using *T. virens*. In addition, Tocafundo et al. (2010) used *T. asperellum* (SF04) obtained similar value of control (~57%). The *T. longibrachiatum* (4088) isolate was successfully used by Oliveira et al. (2009) observing the inhibition of mycelial growth of *Fusarium subglutinans* f.sp. *ananas*, indicating how efficient this antagonist was. Adedeji et al. (2008) demonstrated a reduction of the incidence of cocoa black pod (*P. megakarya*) of 85% when treated with a strain of *T. harzianum*. Dianese et al. (2012) worked with *Trichoderma* spp. on papaya and found significant inhibition of *P. palmivora* by some isolates. Parasitism by *Trichoderma* spp. on *P. palmivora* is one common action mechanism (HARMAN, 2000; KUBICEK et al., 2001; BENÍTEZ et al., 2004). Thus, it is evident that the mechanisms of parasitism, antibiosis, and competition for substrates are the possible modes of action of *Trichoderma* against fungal plant pathogens (VERMA et al., 2007). It is postulated that *Trichoderma* initially succeed in antagonism via

hyphal interactions, probable primal step in antagonism. Later, the BCA fungi kill the pathogenic fungi by means of toxins and consume them using a combination of lysozymes (WHIPPS; LUMSDEN, 2001).

The BCA 4088 could parasitize the pathogen as well inhibited its growth on fruit. It is known that of a BCA success as an antagonist depends of its parasitic capacity, and course of their specificity for the pathogen, requiring some characteristics which antagonists its ability to compete for nutrients, and, its production of pathogen toxic metabolites. The biological control is applied to reduce the amount of inoculum or activity of the pathogen. Biological control rarely eliminates the pathogen site, but reduce the seedlings population, and hence its ability to produce disease. The use of biological control agents, such as fungi of the genus *Trichoderma*, cannot be the only solution to control the papaya fruit rot, but based on these results, it is a viable tool that can be used in the management of this disease (SHARMA et al., 2009).

As conclusion, the data of this work showed that *T. longibrachiatum* (4088) was the best BCA of *P. palmivora* in postharvest, when it was applied 24h before the pathogen.

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RESUMO: O mamão é uma fruta muito cultivada e consumida nas regiões tropicais e subtropicais do mundo e apresenta diversos problemas fitossanitários. Sendo assim, estudos de doenças pós-colheita com biocontroladores viabilizam a diminuição de impactos causados pelo uso de fungicidas. A podridão-dos-frutos (*Phytophthora palmivora*) é uma importante doença pós-colheita em mamão no Brasil. Neste contexto, o controle biológico desta doença na pós-colheita com *Trichoderma* é uma alternativa viável ao uso de fungicidas e foi aplicado neste estudo para avaliar a eficácia de *Trichoderma* spp. para o biocontrole de *P. palmivora* em mamão na pós-colheita. Foram utilizados quatro potenciais antagonistas: *T. asperellum* (SF04), *T. virens* (255C1), *T. harzianum* (THP) e *T. longibrachiatum* (4088). E as frutas foram submetidas aos seguintes tratamentos: Inoculação de *P. palmivora* e 1 hora depois inoculação do *Trichoderma* spp.; Inoculação de *P. palmivora* e 24 horas depois inoculação do *Trichoderma* spp.; Inoculação de *Trichoderma* spp. e 1 hora depois inoculação do *P. palmivora* e; Inoculação de *Trichoderma* spp. e 24 horas depois inoculação do *P. palmivora.* Todos os isolados de *Trichoderma* reduziram significativamente tanto na incidência como na severidade da doença. O isolado 4088 (*T. longibrachiatum*) foi o melhor no controle da podridão.

PALAVRAS-CHAVE: Controle. Antagonismo. Bioprotetores. Patologia de Frutas.

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