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Survival of *Acidovorax citrulli* in infected melon tissues and in different edafoclimatic conditions

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Abstract- The survival of *Acidovorax citrulli Aac1*^{Rif} was accessed in infected melon tissues (fruits and leaves) incorporated to the soil at 0, 5, 10 and 15 cm depth, in seven different types of soil, at temperatures 10, 15, 20, 25, 30 and 35 ºC and moisture field capacity of 50 and 100% in the absence of the host plant. *Aac1*^{Rif} was detected in melon tissues at 0, 5 and 10 cm until 21 days and at 15 cm until 14 days. The highest and lowest relative extinction rate of the population (RERP) for *Aac1*^{Rif} occurred respectively in fruit tissues and leaf tissues at depths of 0 and 5 cm. *Aac1*^{Rif} survived in seven types of soil only for three days. The lowest RERP occurred at 10 or 15 ºC and the highest at 30 or 35 ºC. Greater concentrations of Na^+, silt, and greater populations of actinomycetes and *Trichoderma* were correlated with highest RERP of the *Aac1*^{Rif} in the soil. There was significant difference between RERP at 100% and 50% of field capacity. The soil was not considered potential primary source of *A. citrulli* inoculum. Infected melon fruits and leaves in soil were considered as such sources, at least for 21 days.
Index terms: *Cucumis melo,* bacterial fruit blotch, populational density, ecology, management

Sobrevivência de *Acidovorax citrulli* em tecidos infectados de melão e em diferentes condições edafoclimáticas

Resumo - A sobrevivência de *Acidovorax citrulli Aac1*^{Rif} foi estudada em tecidos infectados de frutos e folhas de melão incorporadas ao solo a 0; 5; 10 e 15 cm de profundidade, em sete diferentes tipos de solo, às temperaturas de 10; 15; 20; 25; 30 e 35 ºC e umidade na capacidade de campo de 50 e 100%, na ausência da planta hospedeira. *Aac1*^{Rif} foi detectado em frutos de melão e tecidos foliares a 0; 5 e 10 cm até 21 dias, e a 15 cm até 14 dias. A taxa de extinção relativa mais alta e mais baixa da população (TERP) para *Aac1*^{Rif} ocorreu, respectivamente, nos tecidos de frutos e nos tecidos foliares, às profundidades de 0 e 5 cm. Para a maioria dos solos, a TERP mais baixa ocorreu a 10 ou 15 ºC, e a mais alta, a 30 ou 35 ºC. Maiores concentrações de Na^+, silte e maiores populações de actinomicetos e *Trichoderma* foram correlacionadas com maior TERP de *Aac1*^{Rif} no solo. Houve diferença significativa entre a TERP em 100% e 50% da capacidade do campo. O solo não foi considerado fonte primária potencial de inóculo de *A. citrulli*. Os frutos e folhas de melão infectados no solo foram considerados como fontes de inóculo, pelo menos por 21 dias.
Termos para indexação: *Cucumis melo*, mancha aquosa, densidade populacional, ecologia, manejo

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Introduction

Bacterial fruit blotch (BFB) is one of the most destructive diseases in melon (*Cucumis melo* L.) and watermelon (*Citrullus lanatus* (Thunb.) Matsum. & Nakai) on producing regions throughout the world (BURDMAN; WALCOTT, 2012). BFB is caused by *Acidovorax citrulli* (Schaad et al.) Schaad et al. (Schaad et al., 2008) that appears in the EPPO (European and Mediterranean Plant Protection Organization) Alert List (CABI, 2015). In Brazil, the most significant economic impact of BFB is on melon crops (CARVALHO et al., 2013) mainly cultivated and exported in Ceará and Rio Grande do Norte. In these states, BFB crop losses are estimated at 40-50%, but could reach 100% (EPPO, 2013).

Seeds represent the most important source of primary inoculum for BFB outbreaks. This confirms what has been observed in field, that expanded leaves and stems are the main inoculum sources for melon blossoms and fruit (ALVES et al., 2010). Volunteer cucurbit seedlings, noncucurbit and cucurbit weeds, and infected plant debris are also potential inoculum sources; however, these are not important in all environments (BURDMAN; WALCOTT, 2012). Developing better management practices depends on having knowledge of saprophytic dynamics of *A. citrulli*, but few studies were conducted on survival of the bacteria in crop residues and soil. *A. citrulli* survives in the soil for a few weeks during the summer in the absence of watermelon plants (ISAKEIT, 1999). However, Silva et al. (2006) detected the decline of *A. citrulli* populations over a 60-day period, from more than 10^7 cells mL^{-1} to 10 cells and to 100 cells, in rhizosphere soil and melon roots, respectively. In crop residues, the bacterium has been recovered from fragments of watermelon rinds buried at 20 to 30 cm for 10 months (LATIN et al., 1995).

The bacterial survival in soil is influenced by abiotic factors such as temperature, moisture and pH (JANSE, 2005) and depends on their ability to resist a lack of nutrients and water, and exposure to heavy metals (GREY; STECK, 2001; HASHIMOTO et al., 2006). None study were found about the influence of chemical and physical characteristics of the soil on the survival of *A. citrulli*.

Seed disinfestation treatments, seed health testing and chemical control in the field are limited in their ability to reduce the yield losses associated with BFB. In addition, to date, there are no reliable sources of BFB resistance (BURDMAN; WALCOTT, 2012). A better comprehension of the ecology of *A. citrulli* will contribute toward the development of BFB new management strategies.

This study aimed to analyze the survival of the bacterium (i) in fruit and leaf tissues incorporated in the soil at different depths, and (ii) in different types of soil of the northeast region of Brazil in the absence of the host plant under the influence of different temperatures and moisture levels.

Material and Methods

Bacterial strain

The *A. citrulli* spontaneous rifampicin-resistant mutant (*Aac1*^{Rif}) used in this study was obtained from the Culture Collection Rosa Mariano of the Laboratory of Plant Bacteriology, Federal Rural University of Pernambuco, and was originally isolated from melon plant exhibiting typical BFB symptoms. The strain *Aac1* (syn. AAC201-21) belongs to the Group I of *A. citrulli* sensu Walcott et al., which includes strains isolated mainly from non-watermelon plants (WALCOTT et al., 2004).

In previous studies, *A. citrulli Aac1*^{Rif} showed growth in liquid culture medium and pathogenicity similar to the rifampicin-sensitive parental strain (SILVA et al., 2006). In all survival studies, *A. citrulli Aac1*^{Rif} was cultivated on nutrient yeast dextrose agar medium (PUSEY; WILSON, 1984) amended with rifampicin at 1 mg mL^{-1} (NYDA^{Rif}); suspensions of mutant were prepared in distilled water and the optical density was adjusted in a spectrophotometer model 500 M (Analyser) to A_{560} = 0.25, which corresponds to 3.4 x 10^7 colony forming units (CFU) mL^{-1}.

Survival of *A. citrulli* in infected melon tissues buried in the soil at different depths

To produce infected melon tissues, fruits and leaves of yellow hybrid AF4945 were inoculated with the *A. citrulli Aac1*^{Rif} utilizing the sub-epidermal injection method (SOMODI et al., 1991) and atomization until run-off, respectively. Ten days after inoculation, symptomatic fruits and leaves were collected separately and fragmented into pieces of approximately 1 cm. Ten grams of tissue were individually placed in plastic mesh bags with openings of 2 x 2 mm and dimensions of 8 x 5 cm and incorporated to the soil [pH in water = 5.1; P^{+}= 6 mg $(dm^3)^{-1}$; Na^{+} = 0.31 mg $(dm^{-3})^{-1}$; K^{+} = 0.03 mg $(dm^{-3})^{-1}$; Ca^{2+} + Mg^{2+} = 1.45 mg $(dm^{-3})^{-1}$; Ca^{2+} = 0.9 mg $(dm^{-3})^{-1}$; Al^{3+} = 0.25 mg $(dm^{-3})^{-1}$; potential acidity (H + Al) = 5.2 mg $(dm^{-3})^{-1}$; organic C. = 11.52 mg $(dm^{-3})^{-1}$; organic matter. = 19.86 mg $(dm^{-3})^{-1}$]. The bags were buried side by side at four depths [0 (surface), 5, 10 and 15 cm] in plastic columns of 20 cm height x 10 cm diameter, containing 2 kg of soil, and kept inside a greenhouse where the average air and soil temperature, and the relative humidity were monitored. The plastic columns were periodically wetted to maintain the soil moisture close to the field capacity (90%).

The bags containing plant tissue were sampled at 7-day intervals until two successive samples failed in detected *A. citrulli Aac1*^{Rif}. Five bags per depth and type of infected tissue were collected in each sample. Following manual sample homogenization, 1 g from each bag was macerated. This macerated tissue was added to 9 mL of sterilized distilled water (SDW) in tubes, and sonicated with an Ultra Sony™ 5B (Dentsply Neytech) for 5 min

at 46 KHz. Serial dilutions were made up to 10^{-3}, and 0.1 mL of each dilution was plated on NYDA^{Rif}, with three replicates. The Petri dishes were incubated for 36 h at 30 ºC in a Biochemical Oxygen Demand incubator (B.O.D. Mod. TE-391, Tecnal).

Evaluations were carried out by counting the number of typical colonies of the *A. citrulli Aac1*^{Rif}, to determine the population in CFU g^{-1} of soil. These data were used to calculate the relative extinction rate of the population (RERP), determined by the formula RERP = - [(log Y_f – log Y_0)/(T_f – T_0)], whereY_0 is the population at the first evaluation, Y_f is the population at the last evaluation prior to reaching zero, T_f is the time (in days) of the last evaluation prior to reaching zero and T_0 is the time of the first evaluation (KOCKS, 1998).

The experimental design was completely randomized, with four treatments for leaves or fruit, representing the depths distribution (0, 5, 10, 15 cm) of the bags in the soil with five replicates for each sampling, where each replicate constituted a plastic mesh bag containing infected tissue.

Survival of *A. citrulli* in different types of soil

The survival of *A. citrulli Aac1*^{Rif} was evaluated in seven types of soil collected, from a depth of 0-20 cm, in the states of Rio Grande do Norte and Ceará (Brazil) in melon fields with the occurrence of bacterial fruit blotch epidemics (Table 1). Soil samples were air dried over 15 days.

Each soil sample (200 g) was placed in a plastic box (Gerbox® – 11 cm x 11 cm x 4 cm) and 50 mL of *A. citrulli Aac1*^{Rif} suspension was added at a concentration of 3.4 x 10^9 CFU mL^{-1} and mixed using a glass stirring rod. The boxes were incubated in B.O.D. (Bio-Oxygen Demand) at 30 °C. For the evaluation of the pathogen population counts, 1 g samples of soil were processed daily, based on the methodology described in the previous experiment. The experimental design was completely randomized, with seven treatments and four replicates, where one gerbox constituted a replicate.

The samples of the seven types of soil were analyzed for physical, chemical and microbiological characteristics. The physical and chemical analyses were performed immediately after soil sampling (EMBRAPA, 1997). These included granulometry, flocculation, residual moisture, available water, pH in water, available P (mg $(dm^3)^{-1}$), Na^+, K^+, Ca^{2+} + Mg^{2+}, changeable Ca^{2+} and Al^{3+} (cmol $(dm^3)^{-1}$), potential acidity (H+Al), organic C and organic matter (g kg^{-1}).

In the microbiological analysis, ten sub-samples of 10 g each were mixed, and from this composite sample, 0.5 g was weighed out. To this aliquot, 4.5 mL of SDW was added, homogenized, and serial dilutions were performed until reaching 10^{-3} of the original. Next, 0.1 mL of each dilution was plated on different culture media with three replicates. Potato dextrose agar medium-PDA (TUITE, 1969) with the addition of 250 ppm of tetracycline was used to isolate fungi; NYDA for isolating bacteria; King's B medium-KB (KING et al., 1954) to isolate fluorescent *Pseudomonas* spp.; Starch casein agar modified medium-MSCA (VARGAS GIL et al., 2009) to isolate actinomycetes; and Malt extract agar medium-MEA (MARTIN, 1950) for the selective isolation of *Trichoderma* spp. In the *Bacillus* spp. isolation, the dilutions were heated in a water bath (Evlab®) at 80 °C for 20 minutes, with subsequent plating on PDA medium. For fungal isolation, plates were incubated at 25 ± 2 ºC under alternating light (12 h of 15-watt light/12 h dark) in B.O.D. All plates were incubated at 28 ± 2 °C. The bacterial growth was evaluated after 48 hours, while actinomycetes and fungi were evaluated after 5 days. Each population was determined from the mean of number of colonies on three plates and expressed in CFU g^{-1} of soil.

Survival of *A. citrulli* in soils at different temperatures

To evaluate the influence of temperature on survival of *A. citrulli Aac1*^{Rif} in seven different types of soil (Table 1), each soil sample (200 g) was placed in gerbox and 50 mL of bacterial suspension (3.4 x 10^9 CFU mL^{-1}) was added and homogenized using a glass stirring rod. The gerbox were incubated in B.O.D. at constant temperatures of 10, 15, 20, 25, 30 and 35 °C. The samples were processed daily as previously described. The experimental design was completely randomized with a factorial arrangement of 7 x 6 representing seven types of soil x six temperatures, with four replicates, where one gerbox constituted a replicate.

Survival of *A. citrulli* in soil with different moisture levels

To evaluate the influence of moisture on survival of *A. citrulli Aac1*^{Rif} in seven different types of soil (Table 1), each soil sample (200 g) was placed in gerbox and maintained at field capacity (100%) and 50% of field capacity, determined by constant weight. Then 50 mL of bacterial suspension (3.4 x 10^9 CFU mL^{-1}) was added and homogenized using a glass rod. The boxes were incubated in B.O.D. at 30 ºC and the samples processed daily, as previously described. The experimental design was completely randomized, with a factorial arrangement of 7 x 2 representing seven types of soil x two levels of moisture, with four replicates, where one box constituted a replicate.

Statistical analysis

All assays were replicated to determine the consistency of the results. Given that significant differences in the variances of the experimental replicates were not observed (P<0.05), the data were evaluated as replicates in time. The relative extinction rates obtained in the experiments were submitted to ANOVA and the Duncan's test or the Student's t-test (P<0.05) were used to separate the means. Standard deviations of the means were also calculated. To compare results of pathogen survival in the soil and all the soil characteristics, Pearson's correlation analysis was used. All the statistical analyses were performed using SAEG software (System for Statistical and Genetic Analysis, version 9.0, 2005, Universidade Federal de Viçosa, Brazil).

Results

Survival of *A. citrulli* in infected melon tissues buried in the soil at different depths

In both experiments the greenhouse conditions were similar: average air temperature (30 ± 2 °C), soil temperature (28 ± 2 °C) and the relative air moisture (90 ± 3%).

A. citrulli Aac1^{Rif} survived in the tissues of melon for 21 days at depths of 0 (surface), 5 and 10 cm and for 14 days at a depth of 15 cm (Figure 1a and b). Larger bacterial populations were found in melon fruit tissues at 7 and 14 days in depths of 0 and 5 cm than at depths of 10 and 15 cm. At 21 days, populations in this tissue at 0, 5 and 10 cm were similar, with values of 3.86 x 10^3, 3.66 x 10^3 and 3.3 x 10^3 CFU g^{-1} of tissue, respectively, whereas *A. citrulli Aac1*^{Rif} was no longer detected at 15 cm (Figure 1a).

In leaf tissues at 7 and 14 days, the populations were similar at all depths, whereas the *A. citrulli Aac1*^{Rif} population at 10 cm declined considerably at 21 days (3.38 x 10^3 CFU g^{-1} of tissue) in relation to the 0 and 5 cm depths (4.27 x 10^4 and 4.16 x 10^4 CFU g^{-1} of tissue) and was not detected at 15 cm (Figure 1b). On the 28th day, no population was detected at any depth in the tissues (Figure 1a and b).

The highest and lowest RERP for *A. citrulli Aac1*^{Rif} occurred respectively in fruit [0.15 and 0.14 log (CFU) day^{-1}] and leaf tissues [0.03 and 0.04 log (CFU) day^{-1}] at depths of 0 and 5 cm. The RERP in the two types of tissue were very similar at 10 and 15 cm (Figure 2). It is important to point out that the highest RERP for the leaf tissues [0.08 log (CFU) day^{-1}] were observed at 10 cm (Figure 2).

Survival of *A. citrulli* in different types of soil

Regardless of the type of soil, *A. citrulli Aac1*^{Rif} survived only three days in the seven melon crop soils. However, RERP ranged from 0.51 to 0.91 log (CFU) day^{-1}, with the formation of three groups of soil. The highest RERP occurred in Soil C and the lowest occurred in Soils E and G (Table 1).

In the analysis of possible indicators that influenced *A. citrulli Aac1*^{Rif} survival in the different soils, significant correlations (P=0.05) were found between RERP and the chemical, physical and microbiological characteristics of the soil. RERP were positively correlated with the concentration of Na^+ (r = 0.86) and silt (r = 0.86) as well as the population of actinomycetes (r = 0.93) and *Trichoderma* (r = 0.82) in the soils. These factors extinguished the bacterial population in the soil more quickly (higher RERP), which is verified by comparing Soils C, E and G (Table 1). The efficiency of these factors in the extinction of the bacterial population were also either positively or negatively correlated with other characteristics of the soil: Na^+ with silt (r = 0.82), sand with silt (r = -0.82), clay with silt (r = 0.71), silt with actinomycetes (r = 0.83), *Pseudomonas* with actinomycetes (r = -0.79) and actinomycetes with *Trichoderma* (r = 0.082).

Survival of *A. citrulli* in soils at different temperatures

Temperature did not affect the survival time of *A. citrulli Aac1*^{Rif} in the different soils and, as confirmed in the previous experiments, the time needed for the extinction of the bacterial population were three days. However, temperature affected the RERP of *A. citrulli Aac1*^{Rif}, indicating the best and worst temperature ranges for the survival of this pathogen (Figure 3).

For most of the soils, the lowest RERP were reached at 10 or 15 ºC and the highest were reached at 30 or 35 ºC. The bacterium behaved similarly to that of the previous experiments, exhibiting higher RERP in Soil C [mean value of 1.11 log (CFU) day^{-1}] and the lowest in Soils E [mean value of 0.89 log (CFU) day^{-1}] and G [mean value of 0.78 log (CFU) day^{-1}]. In Soil C, the RERP increased with the temperature, with greater survival of *A. citrulli Aac1*^{Rif} at 10 °C and lowest survival at 30 °C (Figure 3).

Survival of *A. citrulli* in soil with different moisture levels

In the analysis of variance, no significant interaction (P=0.05) were found between the soils and moisture. Regardless of the moisture level, there were a significant difference in the RERP of *A. citrulli Aac1*^{Rif} in the seven soils (data not presented), confirming previously obtained results.

Significant difference (P=0.05, Student's t-test) between RERP at moisture levels of 100% [0.67 log (CFU) day^{-1}] and 50% [0.50 log (CFU) day^{-1}] of field capacity, with a more rapid extinction of the bacterial population when the soil was at 100% moisture, regardless of the moisture level, *A. citrulli Aac1*^{Rif} also only survived in the soils for three days.

Table 1 - Relative extinction rate of the *A. citrulli Aac1*^{Rif} population (RERP) and chemical, physical and microbiological characteristics of seven melon crop soils from northeastern Brazil.

		CHEMICAL									PHYSICAL			MICROBIOLOGICAL				
SOIL^z	RERP	pH	P	Na^+	K^+	$Ca^{2+} + Mg^{2+}$	Ca^{+2}	Al^{+3}	H + Al	OC^y	OM	SAND	CLAY	SILT	FUNGI	BACT	PSEU	ACT	TRIC	BAC
		(water – 1:2,5)	(mg (dm^{-3})$^{-1}$)	----------(cmol(dm^{-3})$^{-1}$)----------						-----(g kg^{-1})----		----------(%)----------			----------CFU g^{-1} soil----------				
A	0.6582 b^x	8.3	39	0.71	0.77	16	13	0	1.79	12.6	21.72	47.5	47.2	5.28	80.67	39.67	19.33	37.33	19,67	159
B	0.6205 b	6.9	19	0.51	0.38	3.25	1.4	0	1.90	5.43	9.37	75.52	21.2	3.28	104	78.67	33	46.67	18,67	174,67
C	0.9062 a	7.0	12	0.82	0.35	16.75	12.8	0	2.14	13.25	22.85	51.52	37.2	11.28	59.67	74.67	26.33	121.67	30	210
D	0.6700 b	7.1	111	0.75	0.12	3.1	1.8	0	1.69	3.82	6.59	47.52	43.2	9.28	59.67	53.33	104	42	3	102,33
E	0.5061 c	7.6	1	0.42	0.87	12.95	10.3	0	2.28	10.93	18.84	88.76	10.1	1.14	25.33	26.67	54.67	18.67	0,67	78
F	0.6550 b	6.3	15	0.69	0.26	2.25	1.4	0.5	1.79	2.93	5.04	85.12	13.24	1.64	37	154.33	97.67	22	8	283
G	0.5235 c	6.8	35	0.4	0.2	3	2	0.5	1.9	4.48	7.72	69.22	29.5	1.28	16	144.33	121.67	20.67	1,33	173

^{z}(Soil A: clay-sandy texture, Quixeré – CE; Soil B: loamy-clay-sandy texture, Mossoró – RN; Soil C: clay-sandy texture, Quixeré – CE; Soil D: clay-sandy texture, Mossoró – RN; Soil E: sandy-loamy, Baraúna – RN; Soil F: sandy-loamy, Mossoró – RN; Soil G: loamy-clay-sandy texture, Mossoró – RN).

^{y} OC = Organic C; OM = organic matter; BACT = Bacteria; PSEU = Fluorescent pseudomonads; ACT = Actinomycetes; TRI = *Trichoderma* spp.; BAC = *Bacillus* spp.

^{x} Mean of four repetitions. Means followed by the same letter do not differ significantly from each other (Duncan's test; P=0.05).

Discussion

In the present study, it was demonstrated that fruit and leaf tissues can be potential inoculum sources of *A. citrulli* for new crops, depending on the depth of incorporation into the soil, surviving the bacteria for up to 21 days when tissues are at a maximum depth of 10 cm.

In fruit tissues, the largest bacterial populations were detected at 0 and 5 cm and the highest RERP of *A. citrulli Aac1*^{Rif} were also observed at these depths, logically because the final populations at 0, 5 and 10 cm were similar on 21th day. This means that probably the population decline at 0 and 5 cm was more pronounced. There is a small degree of microorganism activity at 0 and 5 cm (MOREIRA; SIQUEIRA, 2006), which enabled greater colonization of *A. citrulli Aac1*^{Rif} at these depths than at 10 and 15 cm through to 14th day. However, with the gradual decomposition of the fruit over time, all that were left were the dehydrated rind, which is inhospitable to the bacterial survival, especially on the surface of the soil. At other depths, the maintenance of the moisture content may have favored the saprophytic activity of the inhabitants in the soil throughout the experimentation period. Thus, there was an accentuated decrease in population on 21th day at all depths and total extinction by 28th day. In leaf tissues, the lesser influence of the depth of incorporation on the populations and RERP of *A. citrulli Aac1*^{Rif}, especially on days 7 and 14, may be attributed to the greater uniformity of the leaf tissue (midrib and main veins) in comparison to fruit tissue (rind and pulp), as the fruit pulp is easily degradable due to the greater water content. The short survival time observed in the present study of *A. citrulli Aac1*^{Rif} in infected tissues may vary depending on the microbiota, type of soil, cucurbitaceae host and climatic conditions. Latin et al. (1995) found greater survival of *A. citrulli* in fragments of watermelon rinds buried for ten months at a depth of 20 to 30 cm.

The highest RERP occurred when the leaf tissue was incorporated to 10 cm of soil. This effect was likely due to the greater contact of the infected tissues with the soil environment, which exhibits fewer abrupt environmental fluctuations beginning at a depth of 10 cm, thereby providing greater equilibrium in the population and microbial activity, resulting in a more accelerated decomposition of the crop residues and lower survival of the pathogen. Furthermore, incorporation at 10 cm or deeper favors a quicker loss of cell viability by submitting the tissue to the greater moisture of the soil, which rises with the increase in depth. The lower RERP of *A. citrulli Aac1*^{Rif} in the leaf tissues deposited at 0 and 5 cm may result from the small degree of degrading associated with microorganism activity, which gradually increases by depths of 10 cm (MOREIRA; SIQUEIRA, 2006).

A number of reports on bacteria that cause leaf damage confirm greater survival on the surface of the soil. The leaf pathogens of the cruciferous *Xanthomonas campestris* pv. *armoraciae* (McCulloch) Dye and *Pseudomonas syringae* pv. *maculicola* (McCulloch) Young et al. survived in crop residues of turnips (*Brassica rapa* L.) and cabbage (*B. oleracea* L.) buried for 60 days, even when the residues were decomposed, whereas residues on the surface were recovered through to 150 days (ZHAO et al., 2002). Rifampicin-resistant mutant of *X. axonopodis* pv. *allii* Roumagnac et al. (strain O177) was recovered from infected onion leaves (*Allium cepa* L.) about 9 months after they were placed on the soil surface or buried to a depth of 25 cm (GENT et al., 2005). *X. campestris* pv. *citri* (Hasse) Dye was detected after 120 days in lesions of grapevine leaves placed on the soil surface but only up to 85 days when leaves were buried 10 cm deep, in the Argentina but the decline rate in bacterial populations was significantly greater in buried leaves than in surface leaves (GRAHAN et al., 1987).

The incorporation depth of the infected melon fruit and leaves directly influenced the populations and RERP of *A. citrulli Aac1*^{Rif}, reducing the capacity of infested residuals to support the pathogen survival. Despite the fact that higher RERP for fruit and leaves occurred at 0-5 and 10 cm, respectively, it is more important to consider that bacterial populations were no longer detected after 21 days only at the 15-cm depth.

While *A. citrulli Aac1*^{Rif} survived in the infected tissues for 21 days in the absence of a host plant, regardless of the type, temperature and moisture of the soil, the pathogen was only detected for three days, indicating a limited survival capacity in the soil under tropical conditions. Silva et al. (2006) found that *A. citrulli* survived better on melon plant leaves than the roots and rhizosphere under greenhouse and field conditions. These results suggest that *A. citrulli* is probably a leaf pathogen, belonging to Group A of the Buddenhagen classification (BUDDENHAGEN, 1965), the populations of which are nearly exclusively developed on the host, undergoing rapid decline in the soil, which does not contribute toward the propagation of the disease from one season to the next.

Based on the RERP of *A. citrulli Aac1*^{Rif}, the seven soils studied were separated into three groups. However, it were not pertinent to classify them as suppressive or conducive, as the bacterium only survived for three days, regardless of the soil type. Nonetheless, some chemical, physical and biological characteristics of the soils were correlated to RERP and may have been the causes of the more rapid decline in the population of the pathogen in Soil C. These characteristics were high concentrations of sodium (Na^+) and silt as well as large populations of actinomycetes and *Trichoderma*, which merit mention. The planktonic survival in the soil is short for many bacterial pathogens. There is a rapid decline in population, most probably because they compete poorly with the soil microflora and due to lack of appropriate nutrients (JANSE, 2005).

Bacterial cells maintain osmotic balance through the constant pumping of protons and other ions (Na^+) to the exterior of the cell through transport proteins located in the cytoplasmic membrane (TORTORA et al., 2015). Thus, a larger amount of Na^+ in the soil may lead to an imbalance in the cell metabolism.

Soils with high silt content hinder the deep infiltration of water and consequently affect the amount of water available to microorganisms. In the present study, the presence of silt was positively correlated to the amount to clay, which has a high ability for retaining water and promoting soil expansion. This characteristic decreases the effect of irregular rains and increases microbial life (DAVET, 2004). It was also determined that Soil C had a larger amount of clay (47.20%) than Soils E and G.

The high correlation between RERP and microbial populations in the soil were expected. Bacteria from the group of actinomycetes and diverse species of *Trichoderma* are abundant in tropical soils. Both are considered saprophytes and efficient in acting as antagonists of a number of phytopathogens of economic importance through antibiosis, parasitism, production of other toxic substances or volatile inhibitors and enzymatic activity inhibitors, competition for nutrients and / or substrate (Di FRANCESCO et al., 2015; HARMAN, 2011; VINALE et al., 2008). However, the microbial balance and efficiency of an antagonist are dependent on the physiochemical characteristics of the soil and it is difficult to separate the factors involved in suppression (WELLER et al., 2002), which were evident in the present study through the significant correlations between the concentration of silt and actinomycetes ($r = 0.83$), *Pseudomonas* and actinomycetes ($r = - 0.79$) and actinomycetes and *Trichoderma* ($r = 0.082$). As *A. citrulli Aac1*^{Rif} did not survive in the melon crop soil in the absence of the host plant for more than three days, the soil is not a potential source of primary inoculum for infections in the shoots of the plants. The majority of pathogenic bacteria that cause non-systemic diseases in the phyloplane are believed to encounter difficulty surviving in the soil without the host plant, as the microbial flora in the soil, as a rule, exercises considerable antagonism on these bacteria (JANSE, 2005).

Soil is an irregular, diverse and structured environment where bacteria could face stresses caused by restrictions in nutrient availability or fluctuations in time of physical and chemical factors (ELSAS et al., 2000). The temperature range around 30 to 35 ºC is considered optimum for the development of bacterial fruit blotch in melons (SILVEIRA et al. 2004) and the growth of *A. citrulli* on a culture medium. However, a higher RERP of the bacterium were generally determined at temperatures between 30 and 35 ºC as well as a lower temperature

of 10 ºC. Mild temperatures reduce microbial activity, whereas high temperatures do the opposite (BAIRD et al., 1999). Thus, both the pathogen and the antagonists in the soil would have greater activity at 30 to 35 ºC, but, as the latter are more adapted to this habitat, they may have been favored in detriment to the survival of *A. citrulli*. This would probably not occur in melon fruit or a culture medium in which the pathogen has all the most favorable conditions for the infection of tissue and cell growth.

The lower survival of *Aac1*^{Rif} in the soil with 100% field capacity may be attributed to the lesser aeration of the soil. Aerobic microorganisms require O_2 as a terminal receptor of electrons in the metabolism (TORTORA et al., 2015) and, in inundated soils, there is an accumulation of products from fermentative processes, such as short-chain organic compounds and reduced organic compounds that can accumulate in toxic concentrations, causing harm to microorganisms (MOREIRA; SIQUEIRA, 2006). Once again, the soil that exhibited the highest RERP was Soil C, which contained high silt and clay content (Table 1) and, consequently, a greater water retention capacity, as mentioned earlier.

In conclusion, the soil should not be considered a source of primary inoculum of *A. citrulli*, as this pathogen only survived for three days in the absence of the host plant. It is recommended that the management of bacterial fruit blotch include the incorporation of infected melon leaves and fruits tissues for tilling and maintenance of a minimum interval of 28 days between crops.

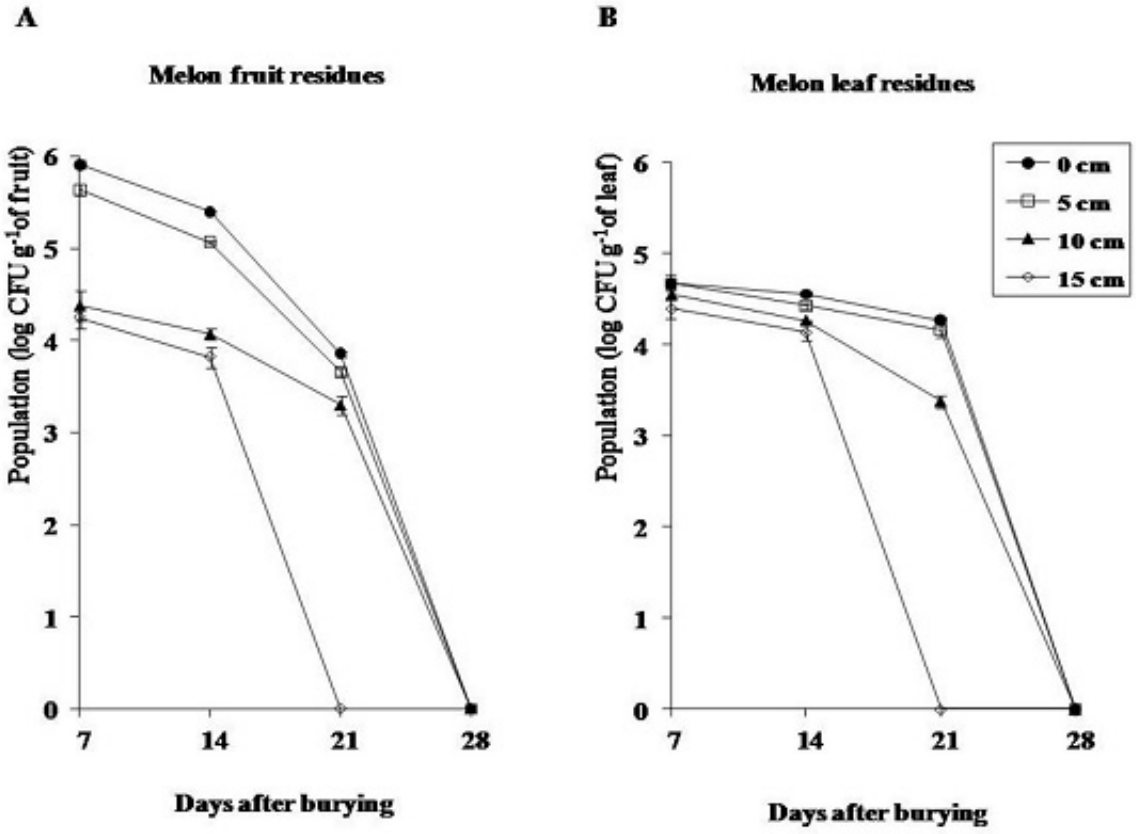

Figure 1 - Survival of *A. citrulli Aac1*^{Rif} in infected melon fruits (A) and leaves (B) incorporated to the soil at different depths, evaluated by the bacterial population (log CFU g^{-1}). Vertical bars represent the standard deviation of the mean.

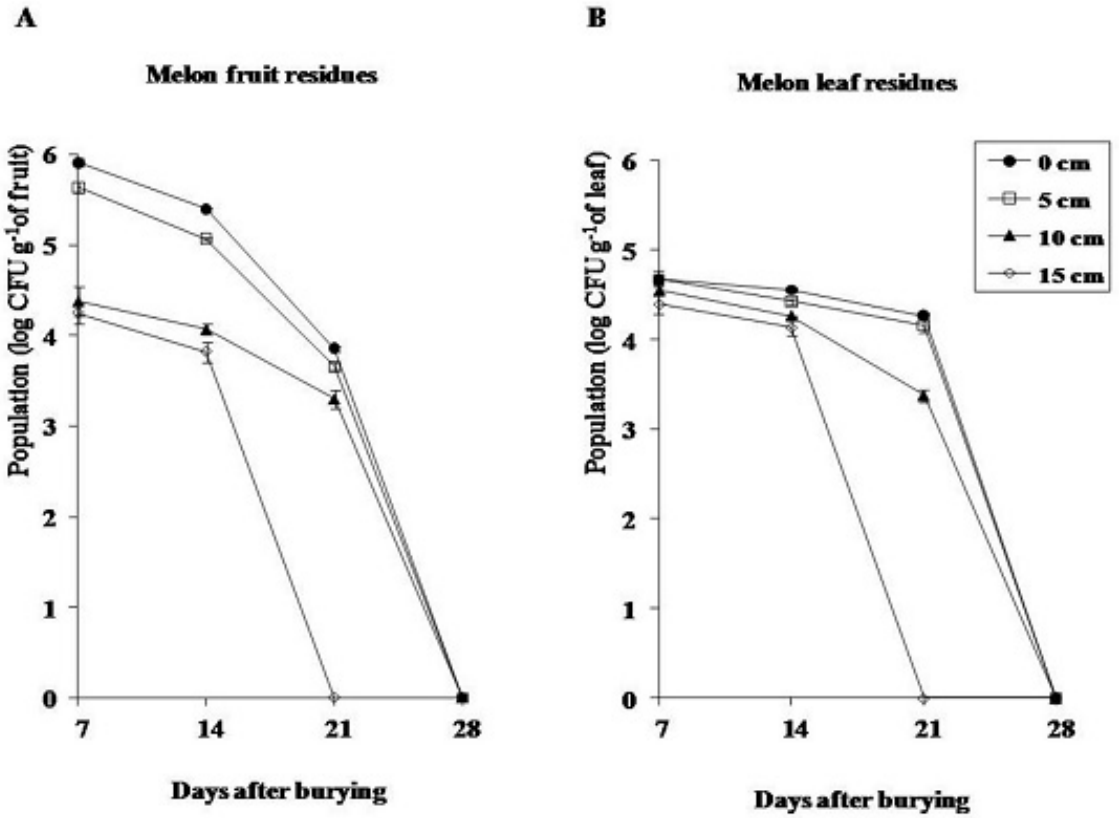

Figure 2 - Survival of *A. citrulli Aac1*^{Rif} in infected melon fruits and leaves incorporated to the soil at different depths, evaluated by the relative extinction rate of the bacterial population (RERP). Vertical bars represent the standard deviation of the mean.

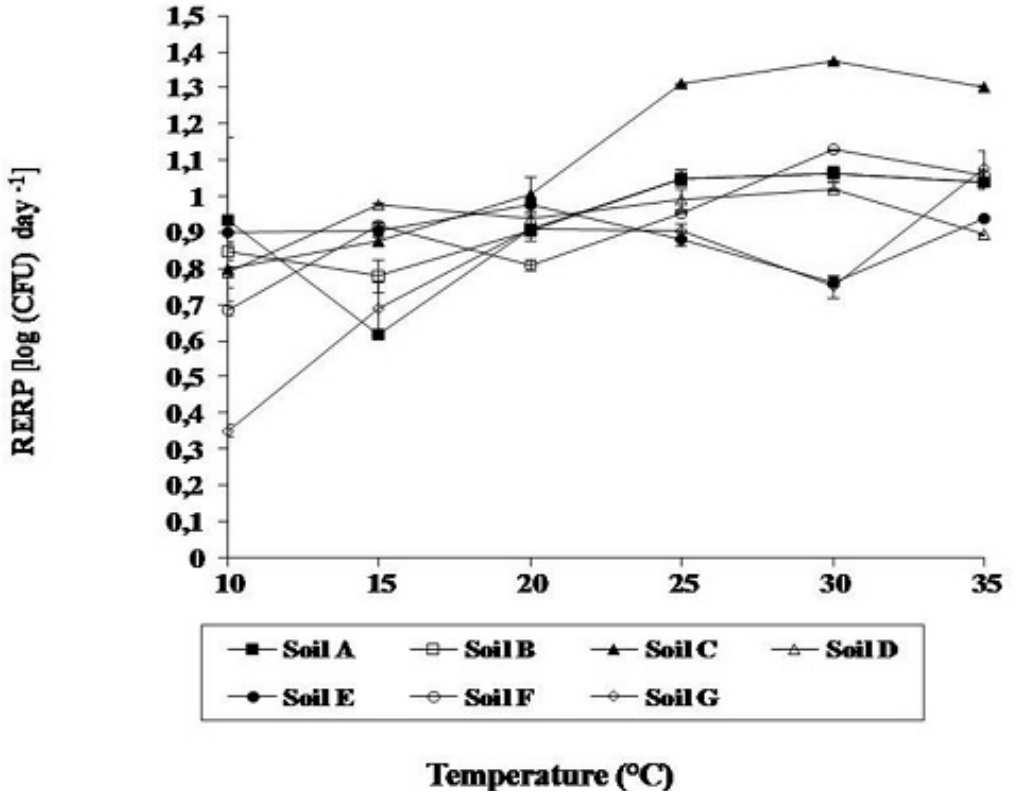

Figure 3 - Survival of *A. citrulli Aac1*^{Rif} in seven melon crop soils from northeastern Brazil submitted to different temperatures, evaluated by the relative extinction rate of the bacterial population (RERP). Vertical bars represent the standard deviation of the mean.

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