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SHORT COMMUNICATION

On the assessment of the sources of inoculum of bacterial wilt in Brazil

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Abstract

Dispersal of *Ralstonia* spp. cells by water and contaminated plant material and the importance of weeds as inoculum sources have been poorly investigated. Water of rivers, soil from fields of diverse crops, and areas of natural vegetation both from the Amazônia (Amazon), Cerrado (Savanna), Pampa (Pampas), and Mata Atlântica (Atlantic rain forest) biomes, besides soil of the rhizosphere of weeds present in tomato fields with records of bacterial wilt were sampled and analyzed to detect *Ralstonia* spp. Seeds of tomato plants artificially and naturally infected with *Ralstonia* spp. were also processed. All samples were enriched a priori in selective medium South Africa (SMSA), and colonies were isolated in plates containing solid SMSA. Detection of *Ralstonia* spp. was confirmed by polymerase chain reaction with specific primers. The Co-operational PCR (CO-PCR) was also used to detect *Ralstonia* spp. Colonies were obtained from soil samples. Five soil samples from eggplant fields, one from coffee field, two soil samples from the rhizosphere of *Amaranthus* spp., one from *Bidens pilosa*, and one from *Solanum americanum*, tested positive for *Ralstonia* spp. when using CO-PCR. Five water samples of rivers were also positive for CO-PCR detection: two samples from Amazônia, one from Cerrado, and two samples from irrigation water collected from tomato fields located in the Mata Atlântica biome. *Ralstonia* spp. were not detected in tomato seeds. These results revealed potential inoculum sources, especially weeds, in areas with historical records of bacterial wilt. Additionally, rivers may act as dispersal agents of inoculum of *Ralstonia* spp.

Keywords *Ralstonia solanacearum* · *R. pseudosolanacearum* · Detection · Epidemiology

Bacterial wilt is a destructive disease that reduces yield of many plant species of economic importance. Plants classified in at least 54 botanical families can be affected by bacterial wilt, and epidemics can develop anywhere, but the disease incidence is usually higher in the tropics (Hayward 1994; Elphinstone 2005). Different species of *Ralstonia* can cause wilt: *Ralstonia solanacearum* (formerly classified as Phylotype II), *R. pseudosolanacearum* (Phylotypes I and III), *R. syzygii* subsp. *syzygii* (Phylotype IV), *R. syzygii* subsp. *celebesensis* (Phylotype IV), and *R. syzygii* subsp. *indonesiensis* (Phylotype IV) (Safni et al. 2014). In Brazil, two species are known to cause bacterial wilt in different crops: *R. solanacearum* and *R. pseudosolanacearum* (Santiago et al. 2016).

Management of bacterial wilt is difficult because the pathogen can thrive in different environments/substrates (Kelman 1953; Graham et al. 1979; Graham and Lloyd 1979; van Elsas et al. 2001). Possible routes of transport of *Ralstonia* spp. are by soil, water, contaminated machines, and propagative materials (Graham et al. 1979; Graham and Lloyd 1979; Coutinho 2005; Álvarez et al. 2008; Parkinson et al. 2013). The role of infected propagative materials such as seed tubers and transplants is well-known (Elphinstone 1996; Mafia et al. 2012). Interestingly, transmission of *Ralstonia* spp. by tomato seeds is still uncertain. Although *Ralstonia* spp. was detected in tomato fruits of infected plants (Sanchez Perez et al. 2008), the pathogen could not be detected by PCR in seeds collected from symptomatic eggplants (Ramesh et al. 2011). For this reason, more studies need to be developed to prove the capacity of *Ralstonia* spp. to infect seeds by colonization of the vascular tissue.

Survival of the bacterium has been associated with roots of host or non-host plants, presence of infected plant debris,

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in the soil, and in volunteer propagation organs, mainly tubers (Graham et al. 1979). In non-host plants, the pathogen cannot infect the roots, but it can multiply and survive associated with exudates in the rhizosphere (Álvarez et al. 2008; Uwamahoro et al. 2020). For this reason, weeds and invasive plants may be important to the management of bacterial wilt. In Europe, perennial bittersweet (*Solanum dulcamara*), an aquatic plant, is a problem in irrigated areas, because *Ralstonia* spp. can survive associated with its root system (Wenneker et al. 1999; Stevens and van Elsas 2010). Using several methods of detection, it was possible to associate the presence of bittersweet with the incidence of bacterial wilt in surrounding areas (Elphinstone 1996; Elphinstone et al. 1998; Elphinstone and Stanford 1998; Janse et al. 1998; Stevens and van Elsas 2010). Other natural solanaceous weeds are described as hosts of *Ralstonia* spp.: *S. nigrum* (Olsson 2008) and *S. cinereum* (Graham and Lloyd 1978). Non-solanaceous weeds that allow latent infection are *Amaranthus* spp., *Bidens pilosa*, *Galinsoga parviflora*, *Oxalis latifolia*, *Spergula arvensis*, *Tagetes minuta*, *Rumex abyssinicus*, *Physalis minima*, *Euphorbia hirta*, and *Stellaria sennii* (Tusiime et al. 1998; Dittapongpitch and Surat 2003; Wicker et al. 2009). In Brazil, species of *Lepidium virginicum*, *Nicandra physalodes*, *S. americanum*, *Portulaca oleracea*, *Physalis angulata*, *Amaranthus* spp., *Euphorbia heterophylla*, *Crotalaria spectabilis*, and *B. pilosa* are listed as potential hosts to *Ralstonia* spp. (Miranda et al. 2004; Bringel et al. 2001). Even though the tests were conducted under controlled conditions, there is evidence that weeds can be an important inoculum source to the forthcoming crops in the same area.

Ralstonia solanacearum can survive in soil either associated or not with plant debris. Cells of *Ralstonia* spp. were detected in the soil up to 2 years after potato crops were affected by bacterial wilt (Elphinstone 1996; Olsson 2008). However, survival of *Ralstonia* spp. can vary as reported by Stander et al. (2003). Bacterial wilt developed in crops established in fields kept without plants (fallow) for 5 years (Stander et al. 2003). The ability to survive in deeper soil layers can be one explanation for the long survival period of *Ralstonia* spp. (Graham and Lloyd 1979), once superficial soil is more exposed to desiccation than deep soil layers (Graham and Lloyd 1979; van Elsas et al. 2000). In addition to non-host plants, crop residues can contribute to the survival of *Ralstonia* spp. (Felix et al. 2012).

Besides the sources of inoculum associated with soil (roots, crop debris, soil particles), there are reports of water sources, mainly rivers and irrigation canals, acting as sources of inoculum or dispersal agents from where or which bacterial cells could be introduced into fields (Hayward 1991; Wenneker et al. 1999; Pradhanang and Momol 2001; Coelho Netto et al. 2004; Parkinson et al. 2013). Along the Solimões and Amazonas rivers, in Amazonas state, Brazil, high incidence of Moko disease was observed and can be related with the dispersal of *Ralstonia* spp. (Coelho Netto et al. 2004). But, other than these studies, no other investigation was conducted and published on the role of dispersal agents and inoculum sources of the pathogen in Brazil.

The detection of *Ralstonia* spp. in water from rivers and other sources close to solanaceous crops reinforces that these water resources are one of the main means of spreading the pathogen. The pathogen has been detected in irrigation water, and the spread of race 2 biovar 3 of *R. solanacearum* in Europe confirms the importance of monitoring water quality to avoid infestation of clean areas or increasing inoculum levels in already affected fields (Elphinstone et al. 1998; Janse et al. 1998; Wenneker et al. 1999; Stevens and van Elsas 2010; Parkinson et al. 2013). Brazil has the largest river network in the world, and the biodiversity of different biomes are noticeable. Rivers have been shown to be potential means to disperse inoculum. However, no studies were conducted to assess the contribution of water bodies as inoculum sources for bacterial wilt epidemics.

The objective of this study was to determine the contribution of rivers, weeds, seeds and soil, associated to different biomes of Brazil as inoculum sources of *Ralstonia* spp. For this, we proposed to collect samples of possible inoculum sources and to detect *Ralstonia* spp. in these samples.

Samples of river water, soil, rhizosphere soil, and seeds were collected to attempt the detection of *Ralstonia* spp. The soil samples were collected in three biodiversity-rich biomes of Brazil: Amazônia, Cerrado, and Mata Atlântica. Soil samples were taken at a depth of 10 cm in fields with and without reports of bacterial wilt epidemics and also from areas of native vegetation (Table S1). One sample of 200 g was collected at each sampling site from the region under the influence of the rhizosphere, placed in clean plastic bags, and taken to the laboratory. The water samples were collected from the surface of rivers, near the margins, and irrigation sources in different states of Brazil: Acre, Amazonas, Tocantins, Bahia, Distrito Federal, Goiás, Minas Gerais, São Paulo, Paraná, and Rio Grande do Sul (Fig. 1). At least 500 mL of water was collected from a source using a clean plastic bottle. The rhizosphere soil of weeds was collected in areas with incidence of bacterial wilt in tomato crop fields. The soil sample was stored in plastic bags, and the weeds were photographed. Plant species identification was based on photographs taken *in situ*. Fruits were collected from either artificially or naturally infected tomato plants. In the laboratory, fruits were washed with detergent and rinsed in running tap water. Seeds were extracted and processed with and without fermentation. The seeds were dried under shade and room temperature conditions. All samples used in this study were georeferenced using a portable Garmin GPS, and all were maintained at room temperature in the Laboratório

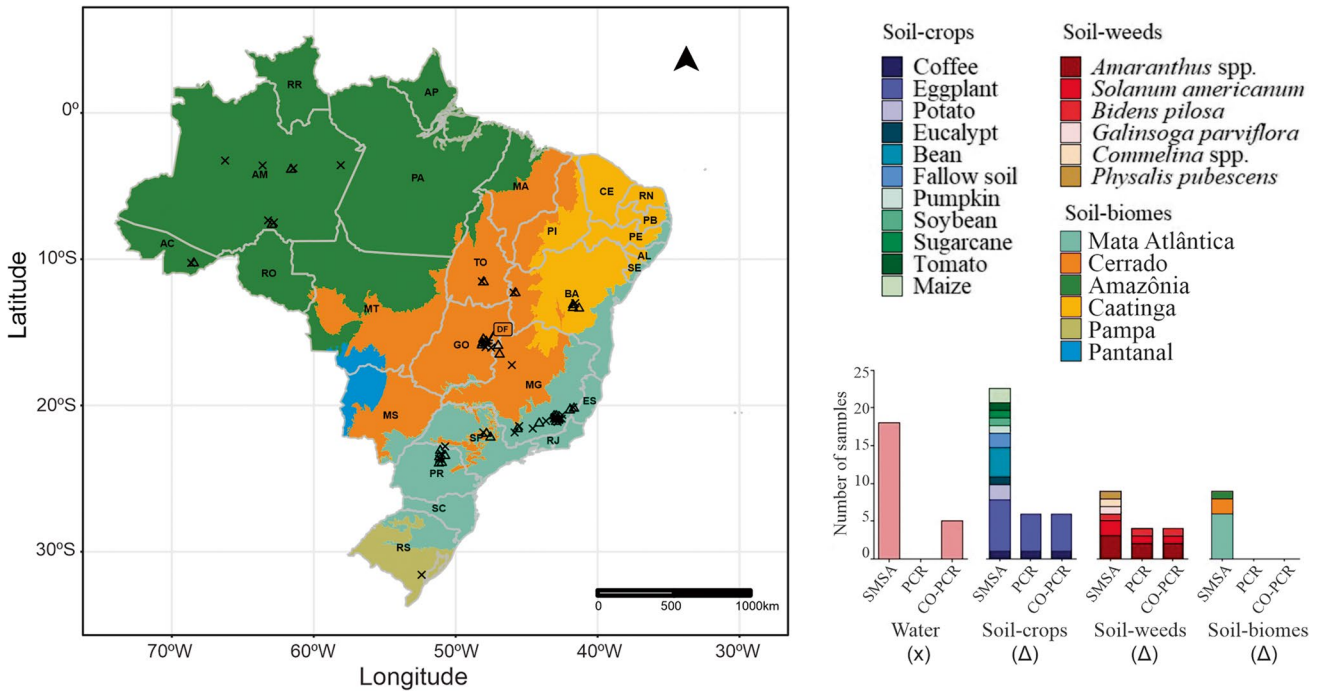

Fig. 1 Map of Brazil showing the origin of water (×) and soil (Δ) samples collected in different regions. The graph represents the number of samples tested positive according to each of three methods of detection: SMSA, PCR, and CO-PCR

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Isolation of *Ralstonia* spp. was attempted with the SMSA composed of 10 g peptone, 5 g glucose, 1 g hydrolyzed casein, 12 g bacteriological agar, 1 L distilled water, 25 mg bacitracin, 100 mg of polymyxin B sulfate (600,000 U), 5 mg of chloramphenicol, 0.5 mg of penicillin G, 5 mg of violet crystal, and 50 mg of triphenyl tetrazolium 2,3,5-hydrochloride (Elphinstone et al. 1996). All samples were enriched a priori in selective medium South Africa (SMSA), and colonies were isolated in plates containing solid SMSA. Additionally, for water samples, 5 g of sodium pyruvate were added to each liter of SMSA medium (Imazaki and Nakaho 2009). Five grams of tomato seeds from each sample were placed in 50 mL of saline solution in a rotary shaker for 24 h. Aliquots from a ten-fold serial dilution from 10–1 to 10–5 were then transferred to SMSA and incubated in a rotary shaker at 28 °C for 24 h. Three plates were used for each sample of water, soil, or seeds. Plates were maintained for 5 to 10 days at 28 °C until the development of bacterial colonies. The bacterial strains isolated from the different substrates were maintained in cryogenic storage tubes containing sterilized saline solution, 0.85% NaCl, under room temperature.

The adjusted methodology of Pradhanang et al. (2000) was used for the soil samples, and the methodologies described by Caruso et al. (2005); Wicker et al. (2009) were used for water samples.

Each 500 mL of water sample was filtered through a 0.22-µm Millipore membrane filter. The filter was aseptically cut into smaller pieces and placed in 20 mL of SMSA broth and kept in a rotary shaker (80 rpm) at 28 °C for 8 h. As with the soil samples, 100 µL was spread onto SMSA agar. Three plates were used for each water sample. Plates were maintained at 28 °C until the development of bacterial colonies. Colonies with the appearance of *Ralstonia* spp. were streaked in another plate with solid Casamino acid-Peptone-Glucose agar (CPG) medium (Dhingra and Sinclair 1995) for 48 h, at 28 °C to obtain pure cultures.

"Santa Clara" tomato seedlings were inoculated with a sterile toothpick laden with bacterial cells of each strain. The base of the stem of the tomato seedlings was punctured with the infested toothpick. Each strain was inoculated in three plants. The tomato plants were maintained in a growth chamber at 28 °C until the development of symptoms. Strains that did not cause symptoms in inoculated plants after 3 weeks were considered non-pathogenic. A positive control consisted of a virulent strain of *R. solanacearum* (UFV 245), and the negative control was a set of plants punctured with a clean toothpick.

From colonies formed SMSA after 24 h of incubation at 28 °C, a loopful of each pure colony was placed in a microtube containing 50 µL of ultrapure water. The tubes were centrifuged for 20000 g to form a pellet to extract the DNA and the supernatant discarded. The Wizard® genomic DNA purification kit (Promega) was used

according to the manufacturer's instructions for Gram-negative bacteria. The quality of DNA was analyzed by gel electrophoresis. The DNA was quantified with a NanoDrop 2000 spectrophotometer (Thermo Fisher Scientific) and adjusted to 50 ηg.

Species identification was accomplished using the primer pair 759/760 (Opina et al. 1997). Polymerase chain reactions were performed using the GoTaq G2 kit from Promega®. Reactions were run in the T100M Bio Rad thermocycler. PCRs were conducted at a final volume of 25 µL, containing 1 µL of DNA, 1X Colorless GoTaq Reaction Buffer, 0.2 mM each dNTP, 0.5 µM of each primer, 1.25 U of GoTaq®G2 DNA polymerase (5 U. μL^{-1}), and ultrapure water to complete the final volume. The amplified fragments were analyzed by electrophoresis in 1% (wt/v) agarose gel, stained with GelRed (Biotium) and visualized under UV light. The comparison was done with the DNA marker of 100 base pairs (bp) and with the DNA of one strain of *R. solanacearum* (RS 279) that was submitted to the same conditions of PCR with specific primer (positive control). Isolates of *Ralstonia* spp. were identified based on the presence of a single band of 282 bp.

Ralstonia spp. strains were classified in phylotypes from primers, Nmult 21:1F, Nmult 21:2F, Nmult 22:Inf, and Nmult 23:AF, and a reverse primer, Nmult 22:RR (Fegan and Prior 2005). Amplicons of 144, 372, 91, and 213 bp correspond to phylotypes I, II, III, and IV, respectively. The thermocycling conditions were 96 °C for 5 min for initial denaturation, 30 cycles of 94 °C for 15 s, 59 °C for 30 s, 72 °C for 30 s, and a final extension of 72 °C for 10 min. Final products of PCR were stained with GelRed ™ and subjected to 1% agarose gel electrophoresis. Confirmation of phylotype was done by visual inspection and comparison with bands of a 1 kb plus DNA ladder (Invitrogen). Phylotypes I and III belong to *R. pseudosolanacearum*, phylotype II corresponds to *R. solanacearum*, and phylotype IV to *R. syzygii*. The isolates used in this study were classified based on phylotype information.

The Co-operational PCR (CO-PCR) was performed to detect *Ralstonia* spp. in samples that were incubated in liquid SMSA medium, under rotation (80 rpm) at 28 °C for 24 h. Primers OLI-1(GGGGGTAGCTTGCTACCTGCC), OLI-2(CGTCATCCACTCCAGGTATTAACCG), and JE-2(GTGGGGGATAACTAGTCGAAAGAC) were used in the Co-PCR using a volume of 5 µL as described elsewhere (Caruso et al. 2003). The PCRs were performed in a total volume of 25 µL, in the T100M Bio Rad thermocycler. The amplified fragments were analyzed in electrophoresis in 1% (wt/v) agarose gel, stained with GelRed (Biotium), and photographed under UV light. The comparison was done with the DNA marker of 100 base pairs (bp) and with DNA of one strain of *R. solanacearum* (RS 279) that was submitted to the same conditions of CO-PCR (positive control). Confirmation of the detection was based on the presence of a single 408 bp amplicon.

In total, 99 samples were analyzed attempting to isolate and detect *Ralstonia* spp.: 35 water samples, four samples of tomato seeds, 18 soil samples from three biomes, 33 soil samples of crops, and nine soil samples associated with weeds. Localization of each water and soil sample is represented in Fig. 1 and Table S1.

From 35 water samples, 18 samples resulted in colonies grown in SMSA; however, PCR with specific primers were negative for *Ralstonia* spp. (Fig. 1). The CO-PCR was positive for five water samples: one sample each of the Madeira and Purus rivers, both in Amazonas state, one sample from Brasília, Distrito Federal (Federal District), and two samples from Coimbra, Minas Gerais state (Table S1).

Thirty-three soil samples from agricultural areas were collected in different regions of Brazil. Five soil samples from eggplant fields located in Brasília, Distrito Federal, and one soil sample from a coffee field in Coimbra, Minas Gerais, were positive for *Ralstonia* spp. from PCR, CO-PCR, and isolation with SMSA (Fig. 1 and Table S1). CO-PCR is expected to be as sensitive as qPCR. The detection limit of CO-PCR for *R. solanacearum* in river water was estimated to be 1 cell/mL (Caruso et al. 2003). In another study involving *R. solanacearum* concluded that the detection limit of LAMP, qPCR, and conventional PCR was similar: 90% of samples were detected by LAMP and qPCR and 86% of the samples detected by conventional PCR (Okiro et al. 2019). *R. pseudosolanacearum* was detected in three samples of eggplant and *R. solanacearum* in two other samples from the same host plant. Samples of crop fields in Coimbra, Minas Gerais state, were from areas with historical records of bacterial wilt. However, *Ralstonia* spp. was detected in only one sample from a coffee field. Strains obtained from this sample were classified as *R. pseudosolanacearum*. Samples from bean, maize, zucchini, cucumber, and pumpkin fields tested negative for *Ralstonia* spp. (Table S1). These samples were collected in fields with no previous record of bacterial wilt.

From 18 samples of soil from different biomes, colonies similar to *Ralstonia* spp. were recovered from nine samples: two from the Cerrado, one from the Amazônia, and six from the Mata Atlântica; however, none tested positive in the PCR assay. CO-PCR from enriched samples did not detect the presence of the pathogen (Fig. 1).

Nine samples of soil from the rhizosphere of weeds were analyzed, and *R. solanacearum* was detected in two samples from *Amaranthus* spp., and *R. pseudosolanacearum* was detected associated with *S. americanum* and *B. pilosa*. CO-PCR for these samples were positive in all cases (Fig. 1 and Table S1).

Four batches of tomato seeds were processed to detect and isolate *Ralstonia* spp. Three samples were from Brasília, Distrito Federal, from artificially inoculated tomato plants.

Another sample was obtained from a naturally infected plant in Coimbra, Minas Gerais state. All samples of seeds tested negative for *Ralstonia* spp. regardless of the detection method (Table S1).

We investigated samples of soil of crop fields, native plants, weeds, river waters, and seeds that can be associated with the occurrence of bacterial wilt epidemics in Brazil. Several methods to detect *Ralstonia* spp. and to obtain pure cultures have been tested to analyze soil, water, weeds, and propagative parts. The use of SMSA to isolate *Ralstonia* spp. has been demonstrated to be efficient (Pradhanang et al. 2000); however, in the current study, this method was not useful for soil samples taken from areas with no occurrence of bacterial wilt. Even with prior enrichment of samples, the use of SMSA was not sensitive enough. In soil samples analyzed, SMSA isolation and CO-PCR were efficient only for samples collected from areas with previous history of bacterial wilt epidemics. Colonies similar to *Ralstonia* spp. were detected in several soil and water samples; however, none was confirmed by PCR with specific primers. Similar results were reported previously, i.e., several strains obtained using selective medium were negative for PCR with specific primers (Ito et al. 1998).

Water resources can be inoculum reservoirs for bacterial wilt epidemics, but *Ralstonia* spp. were confirmed only when using CO-PCR. In Thailand, The Netherlands, Martinique, and Spain, the occurrence of *Ralstonia* spp. in water samples was reported after using SMSA (Wenneker et al. 1999; Álvarez et al. 2007; 2008; Stevens and van Elsas 2010). Although long-term survival of *Ralstonia* spp. in sterile water is a well-known phenomenon (Kelman 1956; van Elsas et al. 2001), its survival in natural water conditions is apparently reduced. The presence of lytic bacteriophages and indigenous microorganisms in river water can reduce the viability of *Ralstonia* spp. populations and decline the pathogen populations (Álvarez et al. 2007). Another major factor is the viable but nonculturable (VBNC) state of *Ralstonia* spp. in water. Low efficiency of detection also is associated with the VBNC. This form is induced mainly by nutrient deprivation, water, and soil without host, low temperature, and copper concentration (Grey and Steck 2001; van Elsas et al. 2001; van Overbeek et al. 2004). Overall, VBNC cells are difficult to detect, and addition of sodium pyruvate in SMSA is an alternative to increase the chances of isolation (Imazaki and Nakaho 2009). However, this modification of SMSA was not enough to obtain isolates of *Ralstonia* spp. in the present study. CO-PCR was better able to detect *Ralstonia* spp. than standard PCR assays. The technique has been shown to have high sensitivity for detection in river water samples (Caruso et al. 2003). In 35 water samples, CO-PCR confirmed the presence of the pathogen in five samples. Two of the five water samples were taken from rivers in the Amazon region, and the results corroborate the potential contribution of rivers in the Amazon to incidence of Moko disease in fields near Solimões and Amazonas rivers (Coelho Netto et al. 2004). One water sample from Brasília, in the Cerrado region, and two samples from Coimbra, Minas Gerais, collected near tomato fields with incidence of bacterial wilt also tested positive.

Detection and isolation of *Ralstonia* spp. from weeds demonstrate the important epidemiological role these plants play as inoculum source (Tusiime et al. 1998; Wenneker et al. 1999; Dittapongpitch and Surat 2003; 2009). *Ralstonia* spp. were recovered from the rhizosphere soil of the weeds, *B. pilosa*, *S. americanum*, and *Amaranthus* spp. These species have been reported as plants that allow the survival of *Ralstonia* spp. (Tusiime et al. 1998; Miranda et al. 2004). *Ralstonia* spp. cannot infect roots of non-hosts plants, but they can survive associated with these plants. When host plants are introduced in a field, bacteria increase in number and can cause disease. It is already known that roots of alternate hosts, debris of infected plants, volunteer tubers, and deeper soil layers can harbor *Ralstonia* spp. contributing to its survival (Graham et al. 1979; Graham and Lloyd 1979). Detection methods are important to establish the contribution of weeds and native plants as inoculum sources. The report of the contribution of *S. dulcamara* is noteworthy (Elphinstone et al. 1998; Wenneker et al. 1999; Stevens and van Elsas 2010). Other species of weeds can also serve as inoculum sources. *Ralstonia* spp. were detected in four soil samples of the root system of weeds. Thus, these species may act as potential inoculum sources and contribute to pathogen survival. Future studies can explore more the importance of host and non-host plants in Brazil for survival of *Ralstonia* spp. In rare cases, *Ralstonia* spp. have been reported to be able to infect tomato fruits through the vascular system of the plant (Sanchez Perez et al. 2008). However, tomato seeds did not test positive for the pathogen even with prior enrichment, plating of the enriched samples in SMSA and CO-PCR. Failure to detect *Ralstonia* spp. associated with eggplant seeds was reported previously (Ramesh et al. 2011). As observed in the current study with tomato seeds, authors were not able to detect the pathogen in seeds of eggplant. Future studies are necessary to understand if *Ralstonia* spp. are able to reach the fruit via infected vascular tissue and colonize or contaminate the fruit and seeds.

Samples collected in areas of native plants in each biome did not test positive for the presence of *Ralstonia* spp. This result does not reject the hypothesis that the pathogen is not present. Other methodologies are necessary to test these samples taken from areas without historical records of bacterial wilt. We can conclude that SMSA and CO-PCR with prior enrichment of samples are sensitive to detect the pathogen in samples collected in and nearby fields. Different sources of inoculum should be inspected often so that growers can anticipate actions and prevent yield losses.

Soil samples collected from crop fields totaled 33; one sample from coffee field (Coimbra) and five samples from eggplant fields (Brasília) were positive. The presence of *R. pseudosolanacearum* in a sample collected from the rhizosphere of one coffee plant is evidence that coffee plants allow survival of this bacterial species. Lopes et al. (2015) showed, under controlled conditions, that coffee seedlings are potential hosts of *R. pseudosolanacearum*. However, the infection process in coffee plants has not been investigated in detail. The positive soil samples from eggplant crops were obtained from plants grown in a field with records of bacterial wilt. Other two samples from eggplant fields were negative: one from a fallow area and one from a field with resistant eggplant cultivar. Interestingly, among five samples (54–58 Table S1) from Brasília, three were of phylotype I and two of phylotype II which are associated with *R. pseudosolanacearum* and *R. solanacearum*, respectively. These results indicate the coexistence of both species in this area. Breeding programs have developed resistant cultivars and detected quantitative trait loci involved in the resistance, mainly to *R. pseudosolanacearum* (Salgon et al. 2017, 2018). This can explain the reduction of the population of *Ralstonia* spp. in the soil at levels undetectable by our methodology.

The occurrence of *R. solanacearum* in soil, water, and the rhizosphere of cultivated and non-cultivated plants in all regions in Brazil provides additional support to the hypothesis that the country is a putative center of origin of this bacterial species (Wicker et al. 2012; Santiago et al. 2020). In addition to the practical implications of the findings of the present study, i.e., the contribution to bacterial wilt epidemics of the potential inoculum sources, the results also allow us to infer about the role the different agents may have.

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Author contribution ESGM planned the study, collected some of the water samples, and wrote the manuscript. JKY conducted the analyses, collected samples, analyzed the data, and wrote the draft of the manuscript. TRS and CAL collected samples and helped with the conception of the study and with the analyses.

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Data availability The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

Declarations

Conflict of interest The authors declare no competing interests.

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