Eur J Plant Pathol (2017) 148:357–365
DOI 10.1007/s10658-016-1094-4

Alternative hosts of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, causal agent of bean bacterial wilt

Ricardo M. Gonçalves · Carlos A. Schipanski · Lincom Koguishi · José M. Soman · Renate K. Sakate · Tadeu A. F. Silva Júnior · Antonio C. Maringoni

Accepted: 7 November 2016 / Published online: 11 November 2016

Abstract Alternative hosts are an important way of phytopathogenic bacteria survival between crop seasons, constituting a source of inoculum for the following crops. Bacterial wilt, caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Cff), is one of the most important diseases for common bean, and little information is available about the host range of the bacterium. In this study, we assessed possible alternative hosts for Cff, especially those cultivated during winter, in rotation systems with common bean. Plants of barley, black oat, canola, radish, ryegrass, wheat and white oat, were assessed under field and greenhouse conditions. Cff colonized epiphytically all plant species and endophytically black oat, ryegrass, wheat and white oat plants assessed in the greenhouse assays. Under field conditions, Cff colonized all plant species by except radish. All bacterial strains re-isolated from the plants were pathogenic to common bean and identified as Cff by PCR with specific primers. Based on our results, the cultivation of bean crop in succession with barley, black oat, canola, ryegrass, wheat and white oat should not be recommended, mainly in areas with a history of bacterial wilt occurrence. In these cases, the better option for crop rotation during the winter is radish, a non-alternative host for Cff.

Keywords Crop rotation · Disease management · Ecology · Survival · Winter crops · *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*

Electronic supplementary material The online version of this article (doi:10.1007/s10658-016-1094-4) contains supplementary material, which is available to authorized users.

R. M. Gonçalves (✉)
Universidade Estadual de Londrina (UEL), Rod PR 445, Km 380, Cx. P. 6001, Londrina, PR 86051-980, Brazil
e-mail: rmgoncalves@icloud.com

C. A. Schipanski · L. Koguishi
Fundação ABC, Rod PR 151, Km 288, Cx. P. 1003, Castro, PR 84166-981, Brazil

J. M. Soman · R. K. Sakate · A. C. Maringoni
Faculdade de Ciências Agronômicas, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Rod Alcides Soares, Km 3, Botucatu, SP 18610-307, Brazil

T. A. F. S. Júnior
Universidade do Sagrado Coração, Rua Irmã Arminda, 10-50, Bauru, SP 17011-160, Brazil

Introduction

The common bean (*Phaseolus vulgaris* L.) is an important source of protein and has high levels of carbohydrates, vitamins, minerals and fibers, constituting an important aliment in the human diet of many developing countries, as Brazil (Abreu and Biava 2005; Broughton et al. 2003). The common bean cultivation is very diffuse in all Brazilian territory, in single systems or intercropped with other crops. Due to the adaptation of a wide range of edapho-climatic conditions, the crop can be cultivated in three seasons per year, a fact that contributes to an increase in the occurrence of diseases (Abreu and Biava 2005; Sartorato et al. 2003; Schwartz et al. 2005).

Plant diseases are one of the main factors associated with the low productivity of common bean in Brazil, and bacterial wilt, caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Hedges) Collins and Jones 1983, is one of the main bacterial diseases for this crop (Sartorato et al. 2003; Schwartz et al. 2005). The disease was first reported in 1920, in South Dakota, USA (Hedges 1922), and in Brazil in 1995, in São Paulo State (Maringoni and Rosa 1997). Since its first report in Brazil, the importance of Cff for common bean has increased, being reported also in Paraná, Santa Catarina, Goiás, Federal District of Brazil and Mato Grosso do Sul States (Leite Junior et al. 2001; Uesugi et al. 2003; Theodoro and Maringoni 2006; Herbes et al. 2008; Theodoro et al. 2010). The early symptoms of the disease are leaf yellowing, which can progress to necrotic lesions. A typical symptom is the wilting of aerial parts of the plant, caused by the colonization and block of xylem vessels by Cff. These symptoms can affect the development of the plant, as well as decreasing its size and production (Theodoro and Maringoni 2010).

Phytopathogenic bacteria can survive associated with seeds, organs of vegetative propagation, infected crop debris, alternative hosts, as free cells in the soil, or in insect vectors (Leben 1981; Vidaver and Lambrecht 2004). Cff can be transmitted by seeds (Saettler and Perry 1972) and is able to survive on common bean debris (Silva Júnior et al. 2012b) and in alternative hosts (Saettler 1991; Silva Júnior et al. 2012a), such as *Glycine max* (L.) Merr. (Hedges 1926; Dunleavy 1983; Sammer and Reiher 2012; Soares et al. 2013), *Vigna* sp. (Schuster and Sayre 1967; Wood and Easdown 1990; Tripepi and George 1991; Schwartz et al. 2005; Huang et al. 2009), *Phaseolus* sp. (Schuster and Sayre 1967; Huang et al. 2009), *Dolichos lablab* L. (Huang et al. 2009), *Lupinus polyphyllus* Lindl., *Pisum sativum* L. (Schuster and Sayre 1967), *Amaranthus retroflexus* L., *Chenopodium album* L. (Schuster 1959), *Triticum aestivum* L. (Silva Júnior et al. 2012a), *Cicer arietinum* L., *Vicia faba* L., *V. villosa* Roth. and *Lens culinaris* Medik. (Osdaghi et al. 2015).

The management of bean bacterial wilt is essentially based on using seeds with high sanitary quality and crop rotation with non-host species of Cff. The adoption of these practices has been shown to be efficient for eradication of the pathogen from areas cultivated with the common bean or to maintain Cff populations at low levels (Harveson et al. 2011). However, in some areas cultivated with common bean in the USA, where bacterial wilt was not detected for almost 25 years, the disease recurred, even with the adoption of recommended management measures (Harveson et al. 2011). Field observations in Brazil evidenced that the incidence of the disease was higher after the cultivation of common bean in areas cultivated previously with oat crop in São Paulo State, but without scientific report (Theodoro and Maringoni 2010).

The knowledge of the alternative hosts of a pathogen is essential for efficient disease management. In spite of the importance of bacterial wilt to the common bean crop, there are few studies about the host range of Cff. In this study, we evaluated seven plant species as alternative hosts for Cff cultivated as winter crops in rotation system with the common bean crop in Paraná State, Brazil.

Materials and methods

Greenhouse experiments

Plant species cultivation and experimental design

Two assays were developed under greenhouse conditions with the following winter crops: barley cv. BRS Cauê; black oat cv. Agro Coxilha; canola hybrid Hyola 61; radish cv. CATI AL 1000; ryegrass cv. Barjumbo; wheat cvs. BRS 328, TBio Tibagi and Topázio; and white oat cvs. Agro Ijuí and IPR 126 (Table 1). Assay 1 was installed on 07/15/2013 and assay 2 on 12/12/2013. Each plant species was sowed in 5 L pots with autoclaved soil (constituted of mixture of sand, ravine clay soil and commercial substrate Bioplant® - ratio 1:1:1).

Cff strain Feij-2628A, resistant to 100 μg/mL of rifampicin and pathogenic to the common bean, from the Collection of the Laboratory of Plant Bacteriology of FCA/UNESP, was selected for the assays. For inoculation, a bacterial suspension was adjusted to 10^8 CFU/mL (OD_{600} = 0.1) in phosphate buffer. Two inoculation methods were evaluated: a) adding 300 mL of bacterial suspension to each pot at the moment of the sowing (T1) and b) adding 300 mL of bacterial suspension to each pot 20 days after sowing (DAS), with the roots injured previously with a knife (T2). Control plants received 300 mL of distilled water instead of bacterial suspension. The assay was conducted using four repetitions, with each experimental unit being comprised of one pot

Table 1 Plant species investigated as alternative hosts of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*

Botanical family	Common name	Scientific name	Cultivar/hybrid
Brassicaceae	Canola	*Brassica napus* L.	Hyola 61
	Radish	*Raphanus sativus* L.	CATI AL 1000
Poaceae	Barley	*Hordeum vulgare* L.	BRS Cauê
	Black oat	*Avena strigosa* Schreb.	Agro Coxilha
	Ryegrass	*Lolium multiflorum* Lam.	Barjumbo
	Wheat	*Triticum aestivum* L.	BRS 328, CD 108, TBio Tibagi, Topázio
	White oat	*Avena sativa* L.	Agro Ijuí, IPR 126

with three plants. For assay 1, epiphytic and endophytic isolations of Cff were performed, while in assay 2, only endophytic isolation was assessed.

Epiphytic isolation of Cff

The epiphytic isolation of Cff from the plants was performed 75 DAS. From each repetition, 10 g of the aerial part of plants was transferred to 250 mL Duran flasks with 100 mL of sterilized phosphate buffer, followed by shaking (250 rpm/30 min), under laboratory conditions. The suspensions were serial diluted (10^0 to 10^{-4}) and 100 μL of each suspension was plated in culture medium (3 plates/dilution/repetition).

In assay 1, Cff isolation was performed by streaking the bacterial suspensions in plates with MSCFF culture medium (Maringoni et al. 2006). In assay 2, the suspensions were plated in nutrient-sucrose-agar culture medium (NSA), supplemented with 100 μg/mL of rifampicin, 50 μg/mL of thiophanate methyl and 50 μg/mL of chlorothalonil. All samples were incubated at 28 °C for 96 h and assessed for the presence of Cff colonies. For confirmation of the presence of Cff, colonies were transferred to NSA medium with 7% NaCl (incubation at 28 °C for 96 h) and stored at −80 °C, until PCR characterization and pathogenicity tests (as described below).

Endophytic isolation of Cff

The endophytic isolation of Cff was also performed 75 DAS, using 10 g of the aerial part of each plant. To this aim, plant samples were surface-disinfested before processing, according to Zinneil et al. (2002). Aliquots of the last water rinse were plated in triplicate in NSA medium to check the efficiency of the disinfestation process. All samples were transferred to 250 mL Duran flasks containing 100 mL of sterile phosphate buffer and macerated in a Turrax homogenizer. Each sample was serial diluted (10^0 to 10^{-4}) and 100 μL of each dilution was plated in MSCFF culture medium (Assay 1) or NSA with the addition of rifampicin, chlorothalonil and thiophanate methyl (Assay 2), followed by incubation at 28 °C for 96 h.

Plating assessment and strains characterization

The evaluation consisted of the observation of Cff colonies in culture medium (qualitative evaluation for the assay 1) and colony counting (quantity evaluation for assay 2). For each plant species and isolation method (endophytic and epiphytic), 18 colonies were selected and characterized by PCR, using specific primers for Cff, *Cff*FOR2 (5′- GTT ATG ACT GAA CTT CAC TCC -3′) and *Cff*REV4 (5′- GAT GTT CCC GGT GTT CAG -3′) (Tegli et al. 2002). The total DNA of each strain was extracted by adjusting a suspension at 10^8 CFU/mL and submitted to 95 °C for 15 min, followed by fast cooling on ice. Each PCR reaction was carried out in a total volume of 12.5 μL, containing 6.25 μL GoTaq Green Master Mix (Promega, USA), 0.25 μL of each primer, 4.25 μL of MilliQ water and 1.5 μL of DNA. The PCR was performed in a Mastercycler Gradient model thermocycler (Eppendorf, USA) using the following PCR program: 94 °C for 3 min, followed by 30 cycles of 94 °C for 1 min, 60 °C for 45 s and 72 °C for 30 s. Finally, the reactions were incubated for 10 min at 72 °C. The amplified DNAs were submitted to horizontal electrophoresis, at 6 V/cm^2 on an agarose gel (1%) with 1X TBE buffer, using Neotaq Brilliant Green Plus dye (7 μL/100 mL). The gels were visualized and recorded on the BioDoc-It Imaging System for gel documentation (UVP, CA).

Assays in field conditions

Experimental design, crop management and sampling – 2012 assays

The assays were conducted in two experimental fields from ABC Foundation, in the municipalities of Tibagi and Ponta Grossa/Paraná State, Brazil. Both areas had a history of occurrence of bean bacterial wilt. In each field, the area was subdivided into randomized blocks with eight repetitions of 16 × 20 m and with three treatments: sowing of black oat (T1), wheat (T2) and the fallow treatment (absence of winter crop) (T3). The sowing in Ponta Grossa field occurred at 05/14/2012 and in Tibagi at 05/18/2012, with spacing of 17 cm between lines; in both areas, chemical fertilizer had not been applied in soil.

Two samplings of winter crops were performed 105 DAS (phenological stage of grain filling) and 135 DAS (plants close to maturation) for assessment of the presence of Cff. The sampling consisted of the collection of five single samples (aerial part of three tillers) per repetition in distinct points of the area, obtaining one composite sample per repetition.

Experimental design, crop management and sampling – 2013 assay

The assay was conducted in the experimental field of Ponta Grossa, which was the same area as the 2012 assay. The area was subdivided into three blocks and each one was cultivated with common bean cv. Andorinha, soybean cv. SYN1163RR and corn hybrid Fórmula TL (sowing of all three crops in 12/18/2012). The harvest of common bean was performed in 03/22/2013, corn in 05/08/2013 and soybean in 05/10/2013. Seven winter crops were sowed in each block: barley cv. BRS Cauê; black oat cv. Agro Coxilha; canola hybrid Hyola 61; radish cv. CATI AL 1000; ryegrass cv. Barjumbo; wheat cv. CD 108; white oat cv. IPR 126 (Table 1) and fallow (absence of crop), being distributed in 24 treatments of 5 × 30 m (three repetitions per treatment). All winter crops were sowed in 05/15/2013. The samplings of crops for analysis of endophytic and epiphytic colonization by Cff were performed 60, 90, 115 and 145 DAS, following the methodology of 2012 assay.

Bacterial isolation and identification

Epiphytic and endophytic isolations for Cff detection were performed using the same methodology described for greenhouse assays. However, for epiphytic isolation, only MSCFF culture medium was used, with five repetitions per treatment, followed by incubation at 28 °C for 120 h. Colonies similar to Cff (18 colonies per treatment) were replicated to NSA medium with 7% of NaCl, incubated at 28 °C for 144 h and preserved in nutrient broth medium with 30% glycerol (*v*/v) at −80 °C. The bacterial isolates were then characterized

Table 2 Detection of epiphytic and endophytic colonization of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* in plant species under greenhouse conditions

Alternative host	Cultivar/hybrid	T1 Inoculation		T2 Inoculation	
		Epiphytic	Endophytic	Epiphytic	Endophytic
Barley	BRS Cauê	und[a]	und	+[b]	und
Black oat	Agro Coxilha	+	+	+	und
Canola	Hyola 61	und	und	+	und
Radish	CATI AL 1000	und	und	+	und
Ryegrass	Barjumbo	+	+	+	+
Wheat	BRS 328	+	und	und	und
	TBio Tibagi	+	+	und	und
	Topázio	und	und	und	und
White oat	Agro Ijuí	und	und	+	+
	IPR 126	+	+	+	+

[a] Undetected

[b] Positive PCR for Cff

Table 3 Percentage of epiphytic and endophytic detection of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* from bacterial colonies selected from black oat and wheat plants under field conditions (2012 assays)

Alternative host	Area	1st Sampling		2nd Sampling
		Epiphytic	Endophytic	Endophytic
Black oat	Ponta Grossa	3.85[a]	4.00	52.38
	Tibagi	80.00	96.00	56.00
Wheat	Ponta Grossa	12.00	und[b]	15.38
	Tibagi	86.36	92.86	5.00

[a] Percentage (%) of Cff detected by PCR from bacterial colonies selected

[b] Undetected

by PCR (data shown in Tables 3 and 4 are the percentage of Cff detected by PCR from bacterial colonies selected), as already described.

Pathogenicity test

The isolates identified as Cff by PCR, obtained from greenhouse and field experiments, were submitted to pathogenicity tests under greenhouse conditions. Plants of common bean cv. Pérola were cultivated in 3-L pots (three plants per pot) containing autoclaved soil. For each strain, three plants (phenological stage of first trifoliate leaf totally expanded) were inoculated by puncturing the stem in the region between cotyledons and the primary leaves with entomological needles moistened in each strain bacterial colony (Maringoni 2002). All Cff strains were cultivated in NSA medium at 28 °C for 48 h.

Disease assessment was performed 30 days after the inoculation observing the absence or the presence of wilt symptoms. Symptomatic bean plants inoculated with Cff strains obtained from all treatments were selected for re-isolation of Cff in NSA culture medium with 7% NaCl, according to Dhingra and Sinclair (1995). All samples were incubated at 28 °C for 144 h followed by the evaluation of the presence of Cff colonies.

Results

Alternative hosts of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* in greenhouse conditions

Culture media MSCFF and NSA supplemented with rifampicin and fungicides were effective for the isolation and detection of Cff. In treatment 1 (T1), Cff was detected colonizing black oat, ryegrass, wheat cvs. BRS 328 and TBio Tibagi, and white oat cv. IPR 126 epiphytically and black oat, ryegrass, wheat cv. TBio Tibagi and white oat cv. IPR 126 endophytically (Table 2). In treatment 2 (T2), Cff was detected

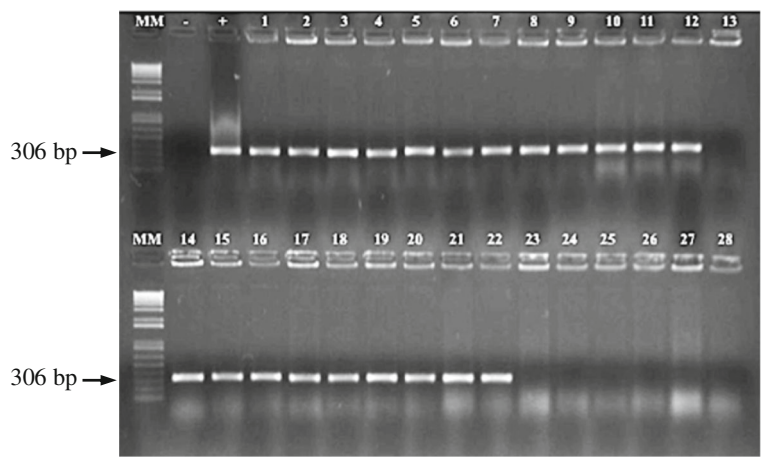

Fig. 1 Agarose gel 1 % of PCR products of DNA extracted from endophytic bacteria isolated from black oat, using specific primers (Tegli et al. 2002) for *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*. MM: molecular marker (1 Kb plus, Invitrogen); (− and +): negative and positive control, respectively; from 1 to 22: samples from Tibagi/PR; 23 to 28: samples from Ponta Grossa area

epiphytically in barley, black oat, canola, radish, ryegrass and white oat cvs. Agro Ijuí and IPR 126, and endophytically in ryegrass and white oat cvs. Agro Ijuí and IPR 126 (Table 2, Supplemental Table 1).

The endophytic population of Cff recovered from these hosts ranged between 10^4 and 10^5 CFU/g of plant tissues (Supplemental Table 2). Cff was constantly detected in any sample of ryegrass cv. Barjumbo, independently from the inoculation method adopted.

Alternative hosts of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* under field conditions.

In both samplings performed in 2012 assays, Cff was detected epiphytically and endophytically colonizing plants of black oat and wheat without the development of symptoms. Colonies sampling recovered in MSCFF culture medium and cultivated in NSA + 7% NaCl were identified as Cff by PCR (Tegli et al. 2002) (Table 3, Supplemental Tables 3 and 4). Accordingly the characteristic amplicon of 306 base pairs (bp) was obtained (Fig. 1).

In the 2013 assay, from areas previously cultivated with common bean and soybean, Cff was recovered epiphytically and endophytically from barley, black oat, canola, ryegrass, wheat and white oat. In the area previously cultivated with corn, Cff was recovered epiphytically from barley, black oat, ryegrass, white oat and endophytically from barley, black oat, wheat, white oat (Table 4, Supplemental Tables 5, 6 and 7). By emphasizing the successions common bean/radish, soybean/radish, corn/canola and corn/radish, Cff was not epiphytically or endophytically detected. All colonies recovered were characterized as Cff using the same methodologies as for the 2012 assays.

Discussion

The results observed here provide knowledge that helps to explain the initial observations that the bean bacterial wilt incidence tends to be higher after succession with oat crop (Theodoro and Maringoni 2010). Cff survived epiphytically and endophytically in ryegrass, wheat, black and white oat in assays performed in the field and greenhouse. Wheat crop already had been described with capacity to harbor *C. flaccumfaciens* on the phyllosphere (Legard et al. 1994), as well as endophytically in stem (Silva Júnior et al. 2012a). Harveson et al. (2011) also detected Cff in common bean plants rotated with wheat, which raised the hypothesis of survival in these crops as well as alfalfa, corn, soybean and sunflower.

Although the botanical family Fabaceae has been already reported as alternative hosts for Cff (Bradbury 1986), our study is the first to report the occurrence of Cff in barley, ryegrass, wheat, black and white oat in Brazil, all belonging to the family Poaceae. Cff was detected colonizing white oat cvs. Agro Ijuí and IPR 126 epiphytically and endophytically in greenhouse conditions. Cff was detected epiphytically and endophytically in wheat cv. TBio Tibagi, and only epiphytically in the cv. BRS

Table 4 Percentage of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* from bacterial colonies selected from winter crops areas previously cultivated with common bean, corn and soybean in Ponta Grossa (2013 assay)

Previous crop	Alternative host	Epiphytic	Endophytic
Common bean	Barley	52.2[a]	35.0
	Black oat	23.1	32.2
	Canola	6.7	11.1
	Fallow[b]	np[c]	79.0
	Radish	und[d]	und
	Ryegrass	46.7	77.8
	Wheat	48.5	78.3
	White oat	85.4	80.4
Corn	Barley	7.7	2.3
	Black oat	4.4	2.9
	Canola	und	und
	Fallow	np	2.8
	Radish	und	und
	Ryegrass	16.7	und
	Wheat	und	14.6
	White oat	13.3	7.3
Soybean	Barley	3.3	32.6
	Black oat	11.1	9.6
	Canola	8.3	15.8
	Fallow	np	20.7
	Radish	und	und
	Ryegrass	36.4	11.8
	Wheat	22.2	27.0
	White oat	7.1	2.4

[a] Percentage (%) of Cff detected by PCR from bacterial colonies selected

[b] Absence of winter crop (presence of crop debris of common bean, corn or soybean)

[c] Not performed

[d] Undetected

328. The same cultivars of wheat used in our study were also used previously (Silva Júnior et al. 2012a), and both studies showed the capacity of Cff to colonize these cultivars. However, Silva Júnior et al. (2012a) did not detect the colonization of white oat cv. IAC 7 and yellow oat cv. São Carlos by Cff. In our study, Cff colonized the black oat and two white oat cultivars examined, suggesting that there is a difference in the colonization capacity of oat genotypes by the bacterium.

In the field, the endophytic survival has greater impact as a source of inoculum because bacteria can be protected inside the plant tissues, suffering less interference from abiotic and biotic factors, enabling longer survival periods in the hypobiotic state (De Boer 1982; Leben 1981; Patrick 1954; Schuster and Coyne 1974, 1975). Besides barley, black oat, ryegrass, wheat and white oat, Cff was also detected endophytically colonizing canola under field conditions. It was verified that all alternative hosts enabled the survival of the Cff epiphytically, being less frequent in canola and radish, where in the latter it was only detected under greenhouse conditions and both plant species belonging to the family Brassicaceae.

Alternative hosts were inoculated in greenhouse assays by Cff suspension in soil and the bacteria colonized the plants endophytically. Similar fact occurs with the bacteria *Burkholderia* sp. that could spread systematically from roots to aerial parts of *Vitis vinifera* possibly through the transpiration stream (Compant et al. 2005). The population of Cff as endophytic in alternative hosts varied from 10^4 to 10^5 CFU/g of plant tissue. Similar results were obtained by Araújo et al. (2002) where *Curtobacterium flaccumfaciens* colonized endophytically citrus plants, ranging from 10^3 to 10^4 CFU/g of fresh branch. The population of Cff as pathogen from chlorotic spots of soybean was 8×10^4 CFU/cm^2, while in common bean varied from 10^6 to 10^7 CFU/cm^2 (Sammer and Reiher 2012), higher values than Cff endophytic populations.

Regarding the effect of crop rotations with bean, corn and soybean, a very low presence of Cff on the winter crops was observed, mainly when the corn hybrid Fórmula TL and soybean cv. SYN1163RR was previously planted, although studies have shown the capacity of Cff to cause disease in soybean in Brazil (Soares et al. 2013). Studies showed that different levels of resistance can exist between cultivars of soybean against Cff, from susceptibility to higher levels of resistance (Bracale and Soares 2014; Maringoni and Souza 2003). This could explain the reason for the lower recovery of Cff when common bean was rotated with soybean cv. SYN1163RR, as well as the possibility of variance of aggressiveness between Cff strains. It is emphasized that both corn and soybean, when preceding radish and canola, failed to show the presence of Cff colonizing the alternative hosts, making this a possible management strategy for the disease.

The tillage system, which has often been applied in the south Brazilian region, shows many agronomic and conservation benefits; however, regarding phytopathological terms, it can contribute to the worsening of some pathosystems. In the present study, it was verified that some winter crops, applied in rotation with common bean in Paraná, can harbor Cff in vegetable tissue both epiphytically and endophytically, as a source of inoculum for successive common bean crop. A possibility of disease management would be the incorporation of crop debris after harvesting, because Cff survived on the debris of common bean for 240 days when it remained on the soil surface, and only for 30 days when the crop debris was incorporated 20 cm into soil (Silva Júnior et al. 2012b).

Cff survived on plants of barley, black oat, canola, ryegrass, wheat and white oat. As such, winter crops could work as a source of inoculum of Cff for successive common bean crop, and not susceptible crops should be used for Cff in rotation with common bean, mostly where the tillage system is used. Cff was not detected in the succession crops common bean/radish, corn/radish, soybean/radish and corn/canola.

Acknowledgements We would like to kindly acknowledge the CAPES (Coordination for the Improvement of Higher Education Personnel) and Fundação ABC (ABC Fundation) for financial supports.

References

Abreu, A. F. B., & Biava, M. (2005). The first and second crops of common bean in southern region of Minas Gerais. Embrapa Arroz e Feijão, Sistemas de Produção n. 6.

Araújo, W. L., Marcon, J., Maccheroni Jr., W., van Elsas, J. D., van Vuurde, J. W. L., & Azevedo, J. L. (2002). Diversity of endophytic bacterial populations and their interaction with *Xylella fastidiosa* in citrus plants. *Applied and Environmental Microbiology, 68*, 4906–4914. doi:10.1128/AEM.68.10.4906-4914.2002.

Bracale, M. F., & Soares, R. M. (2014). Resistance of soybean cultivars to bacterial tan spot, caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*. In: *IX Jornada Acadêmica*

da Embrapa Soja – Resumos expandidos, Documentos 352, Londrina/PR.

Bradbury, J. F. (1986). *Guide to plant pathogenic bacteria*. Slough, UK: CAB International.

Broughton, W. J., Hernández, G., Blair, M., Beebe, S., Gepts, P., & Vanderleyden, J. (2003). Beans (*Phaseolus* spp.) – model food legumes. *Plant and Soil, 252*, 55–128. doi:10.1023/A:1024146710611.

Collins, M. D., & Jones, D. (1983). Reclassification of *Corynebacterium flaccumfaciens, Corynebacterium betae, Corynebacterium oortii* and *Corynebacterium poinsettiae* in the genus *Curtobacterium*, as *Curtobacterium flaccumfaciens* comb. Nov. *Journal of General Microbiology, 129*, 3545–3548. doi:10.1099/00221287-129-11-3545.

Compant, S., Reiter, B., Sessitsch, A., Nowak, J., Clément, C., & Barka, E. A. (2005). Endophytic colonization of *Vitis vinifera* L. By plant growth-promoting bacterium *Burkholderia* sp. strain PsJN. *Applied and Environmental Microbiology, 71*, 1685–1693. doi:10.1128/AEM.71.4.1685-1693.2005.

De Boer, S. H. (1982). *Survival of phytopathogenic bacteria*, *Pages 285–305, In: Phytopathogenic prokaryotes. M. S. Mount and G. H. Lacy, eds*. New York: Academic Press.

Dhingra, O. D., & Sinclair, J. B. (1995). *Basic plant pathology methods*. Boca Raton, FL: CRC Press.

Dunleavy, J. M. (1983). Bacterial tan spot, a new foliar disease of soybeans. *Crop Science, 23*, 473–476.

Harveson, R. M., Urrea, C. A., & Schwartz, H. F. (2011). *Bacterial wilt of dry beans in western Nebraska*. Lincoln Extension: University of Nebraska, Institute of Agriculture and Natural Resources http://extensionpublications.unl.edu/assets/pdf/g1562.pdf.

Hedges, F. (1922). A bacterial wilt of the bean caused by bacterium *flaccumfaciens* nov. sp. *Science, 55*, 433–434. doi:10.1126/science.55.1425.433.

Hedges, F. (1926). Bacterial wilt of beans (*Bacterium flaccumfaciens* hedges), including comparison with *Bacterium phaseoli*. *Phytopathology, 16*, 1–22.

Herbes, D. H., Theodoro, G. F., Maringoni, A. C., Piva, C. A., & Abreu, L. (2008). Detection of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* in seeds of common bean produced in Santa Catarina. *Tropical Plant Pathology, 33*, 153–156. doi:10.1590/S1982-56762008000200010.

Huang, H. C., Erickson, R. S., Balasubramanian, P. M., Hsieh, T. F., & Conner, R. L. (2009). Resurgence of bacterial wilt of common bean in North America. *Canadian Journal of Plant Pathology, 31*, 290–300. doi:10.1080/07060660909507603.

Leben, C. (1981). How plant-pathogenic bacteria survive. *Plant Disease, 65*, 633–637.

Legard, D. E., Mcquilken, M. P., Whipps, J. M., Fenlon, J. S., Fermor, T. R., Thompson, I. P., Bailey, M. J., & Lynch, J. M. (1994). Studies of seasonal changes in the microbial populations on the phyllosphere of spring wheat as a prelude to the release of a genetically modified microorganism. *Agriculture, Ecosystems and Environment, 50*, 87–101. doi:10.1016/0167-8809(94)90128-7.

Leite Junior, R. P., Meneguim, L., Behlau, F., Rodrigues, S. R., & Bianchini, A. (2001). Occurrence of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* on bean in the state of Paraná and Santa Catarina. *Fitopatologia Brasileira, 26*, 303–304.

Maringoni, A. C. (2002). Behaviour of dry bean cultivars to bacterial wilt. *Fitopatologia Brasileira, 27*, 157–162. doi:10.1590/S0100-41582002000200006.

Maringoni, A. C., & Rosa, E. F. (1997). Occurrence of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* on bean in state of São Paulo, Brazil. *Summa Phytopathologica, 23*, 160–162.

Maringoni, A. C., & Souza, E. L. C. (2003). Reaction of soybean cultivars to *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, common bean strain. *Pesquisa Agropecuária Brasileira, 38*, 777–781. doi:10.1590/S0100-204X2003000600015.

Maringoni, A. C., Camara, R. C., & Souza, V. L. (2006). Semi-selective culture medium for *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* isolation from bean seeds. *Seed Science and Technology, 34*, 117–124. doi:10.15258/sst.2006.34.1.12.

Osdaghi, E., Taghavi, S. M., Fazliarab, A., Elahifard, E., & Lamichhane, J. R. (2015). Characterization, geographic distribution and host range of *Curtobacterium flaccumfaciens*: an emerging bacterial pathogen in Iran. *Crop Protection, 78*, 185–192. doi:10.1016/j.cropro.2015.09.015.

Patrick, Z. A. (1954). The antibiotic activity of soil microorganisms as related to bacterial plant pathogens. *Canadian Journal of Botany, 32*, 705–735. doi:10.1139/b54-064.

Saettler, A. W. (1991). Diseases caused by bacteria. In R. Hall (Ed.), *Compendium of bean diseases* (pp. 29–32). The American Phytopathological Society: St. Paul.

Saettler, A. W., & Perry, S. K. (1972). Seed-transmitted bacterial diseases in Michigan navy (pea) beans, *Phaseolus vulgaris*. *Plant Disease, 56*, 378–381.

Sammer, U. F., & Reiher, K. (2012). *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* on soybean in Germany – a threat for farming. *Journal of Phytopathology, 160*, 314–316. doi:10.1111/j.1439-0434.2012.01902.x.

Sartorato, A., Rava, C. A., & Faria, J. C. (2003). *Common bean crop – Diseases and control methods*. Embrapa Arroz e Feijão. (Embrapa Arroz e Feijão. Sistemas de Produção, 2).

Schuster, M. L. (1959). Relation of root-knot nematodes and irrigation water to the incidence and dissemination of bacterial wilt of bean. *Plant Disease Report, 43*, 25–32.

Schuster, M. L., & Coyne, D. P. (1974). Survival mechanisms of phytopathogenic bacteria. *Annual Review of Phytopathology, 12*, 199–221. doi:10.1146/annurev.py.12.090174.001215.

Schuster, M. L., & Coyne, D. P. (1975). Survival factors of plant pathogenic bacteria. *Research Bulletin no. 268*, University of Nebraska, USA, 53.

Schuster, M. L., & Sayre, R. M. (1967). A coryneform bacterium induces purple-colored seed and leaf hypertrophy of *Phaseolus vulgaris* and other Leguminosae. *Phytopathology, 57*, 1064–1066.

Schwartz, H. F., Steadman, J. R., Hall, R., & Forster, R. L. (2005). *Compendium of bean diseases*. St. Paul, Minn: American Phytopathological Society.

Silva Júnior, T. A. F., Itako, A. T., Negrão, D. R., & Maringoni, A. C. (2012a). Pathogenicity of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* to several plant species. *Journal of Plant Pathology, 94*, 427–430. doi:10.4454/JPP.FA.2012.028.

Silva Júnior, T. A. F., Negrão, D. R., Itako, A. T., Soman, J. M., & Maringoni, A. C. (2012b). Survival of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* in soil and bean crop debris. *Journal of Plant Pathology, 94*, 331–337. doi:10.4454/JPP.FA.2012.025.

Soares, R. M., Fantinato, G. G. P., Darben, L. M., Marcelino-Guimarães, F. C., Seixas, C. D. S., & Carneiro, G. E. S. (2013). First report of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* on soybean in Brazil. *Tropical Plant Pathology, 38*, 452–454. doi:10.1590/S1982-56762013000500012.

Tegli, S., Sereni, A., & Surico, G. (2002). PCR-based assay for the detection of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* in bean seeds. *Letters in Applied Microbiology, 35*, 331–337. doi:10.1046/j.1472-765X.2002.01187.x.

Theodoro, G. F., & Maringoni, A. C. (2006). Bacterial wilt of common bean in Santa Catarina state, Brazil, and behavior of genotypes to *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*. *Summa Phytopathologica, 32*, 34–41. doi:10.1590/S0100-54052006000100005.

Theodoro, G. F., & Maringoni, A. C. (2010). Bacterial wilt of common bean. In: PRIA, M. D.; SILVA, O. C. *Cultura do Feijão:* doenças e controle. Paraná: UEPG. *Capsule, 2*, 23–30.

Theodoro, G. F., Maringoni, A. C., Chumpati, A. A., Correia, H. C., Theodoro, J. V. C., & Nogueira, R. J. (2010). First report of bacterial wilt of common bean caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* in Mato Grosso do Sul. *Journal of Plant Pathology, 92*, S4.107–S4.122.

Tripepi, R. R., & George, M. W. (1991). Identification of bacteria infecting seedlings of mung bean used in rooting bioassays. *Journal of the American Society for Horticultural Science, 116*, 80–84.

Uesugi, C. H., Freitas, M. A., & Menezes, J. R. (2003). First occurrence of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* on bean in the State of Goias and Federal District of Brazil. *Fitopatologia Brasileira, 28*, 324. doi:10.1590/S0100-41582003000300019.

Vidaver, A. K., & Lambrecht, P. A. (2004). Bacteria as plant pathogens. *The Plant Health Instructor.* doi:10.1094/PHI-I-2004-0809-01.

Wood, B. A., & Easdown, W. J. (1990). A new disease of mung bean and cowpea for Australia. *Australasian Plant Pathology, 19*, 16–21. doi:10.1071/APP9900016.

Zinneil, D. K., Lambrecht, P., Harris, N. B., Feng, Z., Kuczmarski, D., Higley, P., Ishimaru, C. A., Arunakumari, A., Barletta, R. G., & Vidaver, A. K. (2002). Isolation and characterization of endophytic colonizing bacteria from agronomic crops and prairie plants. *Applied and Environmental Microbiology, 68*, 2198–2208. doi:10.1128/AEM.68.5.2198-2208.2002.