CIÊNCIAS AGRÁRIAS, INDICADORES E SISTEMAS DE PRODUÇÃO SUSTENTÁVEIS

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Pedro Henrique Abreu Moura
Vanessa da Fontoura Custódio Monteiro
(Organizadores)

Atena Editora
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APRESENTAÇÃO

A agricultura faz parte da área do conhecimento denominada de Ciências Agrárias. Importante para garantir o crescimento e manutenção da vida humana no planeta, a agricultura precisa ser realizada de forma responsável, considerando os princípios da sustentabilidade.

Esta obra, intitulada "Ciências agrárias, indicadores e sistemas de produção sustentáveis 3", apresenta-se em três volumes que trazem uma diversidade de artigos sobre agricultura produzidos por pesquisadores brasileiros e de outros países.

Neste terceiro volume, encontram-se trabalhos que abordam as culturas do eucalipto, citros, pera, girassol, tomate, graviola e mandioca, sendo que alguns trabalhos estão relacionados ao controle de pragas e doenças, outros relacionados à propagação de plantas, além de trabalhos nas áreas de bovinocultura e piscicultura.

Agradecemos aos autores dos capítulos pela escolha da Atena Editora. Desejamos a todos uma ótima leitura e convidamos para apreciarem também os outros volumes desta obra.

Pedro Henrique Abreu Moura
Vanessa da Fontoura Custódio Monteiro

SUMÁRIO

CAPÍTULO 4

SURVIVAL OF *Xanthomonas citri* pv. *fuscans* IN THE PHYLLOPSHERE AND RHIZOSPHERE OF CROPS AND WEEDS

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ABSTRACT: *Xanthomonas citri* pv. *fuscans* (Xcf), the causal agent of common bacterial blight, causes high damage to the common bean (*Phaseolus vulgaris* L.) crop and the knowledge of its ecological survival niches is fundamental for the efficient disease management. This work evaluated the Xcf survival in the phyllosphere and rhizosphere of 17 crops, used in rotation with common bean, and 15 weeds, that occurring in bean cultivation areas. In the crops phyllosphere, the longest survival periods were obtained in white oat, common bean, common bean cv. jalo and wheat (63 days), forage turnip (56 days), maize (49 days), peanut and barley (42 days). In weeds, the longest periods were registered in the phyllosphere of *N. physalodes* (63 days), *C. benghalensis* and *L. virginicum* (49 days), and *C. bonariensis* (35 days). The survival periods in the rhizosphere were lower than those observed in the phyllosphere, with emphasis on ryegrass (21 days) and crotalaria (14 days), among the crops, and *C. benghalensis* (21 days), *A. viridis* and *D. stramonium* (14 days), among the weeds. Based on the results, the rhizosphere is not a potential Xcf survival niche. In addition to common bean, jalo bean, white oat, wheat, and forage turnip have been identified as possible alternative hosts of Xcf and should be avoided in succession with

common bean, especially in areas with a history of common bacterial blight. In weeds, except for *A. tenella*, *C. rotundus*, *P. oleraceae* and *S. americanum*, Xcf survived in the phyllosphere and/or rhizosphere of the other species for at least seven days, and eradication in bean crop fields is recommended.
KEYWORDS: *Phaseolus vulgaris*, common bacterial blight, disease management, ecology, and crop rotation.

SOBREVIVÊNCIA DE *Xanthomonas citri* pv. *fuscans* NO FILOPSFERA E RIZOSFERA DE PLANTAÇÕES E ERVAS DANINHAS

RESUMO: *Xanthomonas citri* pv. *fuscans* (Xcf), agente causal do crestamento bacteriano comum, causa grandes danos ao feijão comum (*Phaseolus vulgaris* L.) e o conhecimento de seus nichos ecológicos de sobrevivência é fundamental para o manejo eficiente da doença. Esse trabalho avaliou a sobrevivência de Xcf na filosfera e rizosfera de 17 plantas cultivadas, usadas em rotação de cultura com o feijão comum, e 15 plantas daninhas, que ocorrem em áreas de cultivo de feijoeiro. Na filosfera das plantas cultivadas, maiores períodos de sobrevivência foram obtidos na aveia branca, feijão comum, feijão cv. Jalo e trigo (63 dias), nabo forrageiro (56 dias), milho (49 dias), amendoim e cevada (42 dias). Nas plantas daninhas, maiores períodos de sobrevivência foram registrados na filosfera de *N. physalodes* (63 dias), *C. benghalensis* e *L. virginicium* (49 dias), e *C. bonariensis* (35 dias). Os períodos de sobrevivência na rizosfera foram maiores que os observados na filosfera, com ênfase para azevém (21 dias) e crotalária (14 dias), dentre as plantas cultivadas, e C. benghalensis (21 dias), A. viridis e D. stramonium (14 dias), dentre as plantas daninhas. Com base nesses resultados, a rizosfera não é um potencial nicho de sobrevivência para Xcf. Além do feijão comum, o feijão cv. Jalo, aveia branca, trigo e nabo forrageiro foram identificados como possíveis hospedeiros alternativos de Xcf e devem ser evitados em sucessão ao feijão comum, especialmente em áreas com histórico de crestamento bacteriano comum. Em plantas daninhas, com exceção de *A. tenella, C. rotundus, P. olereacae* e *S. americanum*, Xcf sobreviveu na filosfera e/ou rizosfera das outras espécies por pelo menos sete dias, e sua erradicação das lavouras de feijoeiro é recomendada.
PALAVRAS-CHAVE: *Phaseolus vulgaris*, crestamento bacteriano comum, manejo de doenças, ecologia, rotação de culturas.

1 | INTRODUCTION

Common bean (*Phaseolus vulgaris* L.) has great importance in the agricultural scenario due to profitability, in addition to nutritional benefits and social aspects, acting as a protein source in human food, especially in developing countries, such as Brazil (BARBOSA; GONZAGA 2012; MYERS; KMIECIK, 2017).

Diseases of bacterial etiology are one of the most important factors that can limit the common bean cultivation, due to the high damage, especially in countries with tropical climate (ROMEIRO, 2005). The common bacterial blight (CBB), caused by *Xanthomonas phaseoli* pv. *phaseoli* (Xpp), and the fuscans variant *Xanthomonas citri* pv. *fuscans* (Xcf), is one of the main bacterial diseases in major bean producing countries, with losses around 70% (BOERSMA et al. 2015; WENDLAND et al. 2016; CHEN et al., 2021).

During extended periods of warm and humid weather, the disease can be highly destructive, causing losses in both yield and seed quality. Symptoms manifest in the aerial part of the plant, affecting leaves, stems, pods, and seeds. On the leaves, symptoms initially appear as water-soaked spots that gradually enlarge, become flaccid, and then necrotic surrounded or not by a strict yellow halo. As they develop, the tissues become dry and brittle. Lesions may be sparse on the limbus, or the marginal part of the leaf. In the pods, little balls appear, small watery spots, which progressively increase in size, being coated with yellowish encrustations that then become depressed and reddish. In seeds, once the pod infection occurs, rotting or wrinkling appears (SCHWARTZ et al., 2005; WENDLAND et al. 2016).

The disease management is based on the use of health seeds, incorporation of crop debris in the soil, use of bean cultivars with resistance levels, crop rotation with non-host species for the bacteria, and elimination of alternative hosts, such as weeds. Chemical control is restricted to the application of copper-based solutions in fields (WENDLAND et al., 2016; AGROFIT, 2021).

Xpp can survive in *Beta vulgaris* (beet), *Glycine max* (soybean), *Vigna unguiculata* (cowpea), and weeds *Amaranthus hybridus* (slender amaranth), *Bidens pilosa* (hairy beggarticks), *Cyperus rotundus* (purple nutsedge), and *Oxalis latifolia* (purple-leaved sorrel) (CAFATI; SAETTLER 1980; ANGELES-RAMOS et al., 1991; KARAVINA et al. 2011). The knowledge of alternative hosts is fundamental for the efficient management of CBB.

Our study aimed to evaluate the survival of Xcf in the phyllosphere and rhizosphere of crops commonly used in rotation system with common bean, and in weeds that are frequently present in bean crop fields.

2 | MATERIAL AND METHODS

The experiment was conduced at the Departamento de Proteção Vegetal, Faculdade de Ciências Agronômicas, Universidade Estadual Paulista ‘Júlio de Mesquita Filho’ – FCA/UNESP, Botucatu, São Paulo, Brazil.

2.1 Crops and weeds used

For the experiment, 17 crops and 15 weeds were used. The crop species were: *Arachis hypogae* (peanut cv. IAC 503), *Avena sativa* (white oat cv. IPR 126), *Avena strigosa* (black oat cv. Embrapa 29), *Brassica napus* (rapeseed hybrid Hyola 61), *Cajanus cajan* (pigeon pea cv. Crioula), *Crotalaria juncea* (sunn hemp cv. IAC-1), *Glycine max* (soybean cv. M6410 PRO), *Gossypium hirsutum* (cotton cv. IAC 23), *Helianthus annuus* (sunflower cv. IAC Iarama), *Hordeum vulgare* (barley cv. BRS Cauê), *Lolium multiflorum* (ryegrass cv.

Barjumbo), *Mucuna pruriens* (velvet bean cv. mucuna preta), *Phaseolus vulgaris* (common bean cv. IPR Campos Gerais), *Phaseolus vulgaris* (cv. Jalo MG-65), *Raphanus sativus* (turnip cv. IPR 116), *Triticum aestivum* (wheat cv. BRS374), and *Zea mays* (maize cv. IAC 8390).

The weed species were: *Alternanthera tenella* (hairy joyweed), *Amaranthus viridis* (slender amaranth), *Bidens pilosa* (hairy beggarticks), *Commelina benghalensis* (benghal dayflower), *Conyza bonariensis* (hairy fleabane), *Cyperus rotundus* (purple nutsedge), *Datura stramonium* (jimsonweed), *Galinsoga parviflora* (gallant soldier), *Gnaphalium purpureum* (spoonleaf purple everlasting), *Lepidium virginicum* (Virginia pepperweed), *Nicandra physalodes* (apple of Peru), *Portulaca oleraceae* (common purslane), *Sida rhombifolia* (arrowleaf sida), *Solanum americanum* (american black nightshade) and *Sonchus oleraceus* (common sowthistle).

All plants were cultivated in 3 L pots containing soil mixture (organo-mineral substrate Tropstrato HT® and sand, in the ratio of 1:1:1), plus 0.6 kg/m^3 of ammonium sulfate, 1.7 kg/m^3 of simple superphosphate, 0.6 kg/m^3 of potassium chloride and 0.8 kg/m^3 of dolomitic limestone.

2.2 Plant inoculation and sampling

The experiment was performed 40 days after sowing. Plants were inoculated with the Xcf strain Feij. 7732R, resistant to rifampicin, from the Laboratório de Bacteriologia Vegetal - FCA/UNESP. The strain was cultivated in NSAR culture medium, consisted by nutrient-agar (NA, Merck), plus 5 g.L^{-1} of sucrose (Synth), and 100 µg.ml^{-1} of rifampicin (Rifaldin) (incubation 28°C /48 h) (NASCIMENTO et al., 2021).

The aerial part of the plants was sprayed with bacterial suspension (1x10^7 CFU.mL^{-1}) until the point of run-off. For rhizosphere assessment, the soil was infested with 300 mL of a bacterial suspension at the same concentration.

The plants were kept in a greenhouse with average temperature ranging from 22.8 °C to 28.6 °C. The plant samplings were carried out for phyllosphere and rhizosphere every 7 days for 70 days.

2.3 Sample processing

Xcf recovery from the phyllosphere and rhizosphere was performed by collecting three plants of each specie at each evaluation period.

For the phyllosphere, the aerial part of the plants was sectioned and homogenized, obtaining a composite sample for each plant specie. Five grams of each composite sample were transferred to Duran® flasks containing 100 mL of sterile distilled water followed by agitation (300 rpm/30 min).

For the rhizosphere, the soil adhered to the roots was collected individually in sterilized beckers, obtaining composite samples. Five grams of each composite sample were transferred to Duran® flasks containing 100 mL of sterile distilled water followed by agitation (300 rpm/30 min) and sedimentation (30 min).

After the sample processing, 100 µL of the supernatants were plated in NSAR medium supplemented with chlorothalonil (0.01 $g.L^{-1}$) and methyl thiophanate (0.01 $g.L^{-1}$). The Petri dishes were incubated (28 °C/72 h) and evaluated for the presence or absence of Xcf colonies.

2.4 Experimental design

The experimental design was completely randomized with 64 treatments (32 plant species x 2 survival niches). In addition, the negative control for the rhizosphere was represented by soil pots without plants. Seven pots were used per treatment, each containing five plants of each specie. For positive control of the phyllosphere and rhizosphere, common bean plants, main host of Xcf.

2.5 Characterization of bacterial strains

To confirm the Xcf survival periods in all plants evaluated, colonies with Xcf morphological characteristics were selected from all treatments and identified by PCR using specific primers X3k (5′-GCTGTTGAT-CGCGCCGCCGTACC-3′) and X4e (5′ CGCCGGAA GCACGATCCTCGAAG-3′) (Audy et al. 1994). The total DNA of each strain was extracted from suspension (10^8 $UFC.mL^{-1}$) at 95°C/15 min, followed by rapid cooling in ice. Each PCR reaction was composed of 12.5 μL GoTaq Green Master Mix (Promega, USA), 0.5 μL of each primer, 8.5 μL of MiliQ water, and 3.0 μL of DNA. The PCR was performed in a ThermoCycler (Mastercyclers Gradient-Eppendorf, USA) and the PCR amplification conditions were as described by Audy et al. (1994). The amplified DNAs were submitted to horizontal electrophoresis, at 6 V/cm^2 in gel consisting of 1% agarose (w/v) with TBE buffer (90 mM Tris; pH 8.3; 90 mM of boric acid and 0.1 mM EDTA), plus Neotaq Brilliant Green Plus dye (7 μL/100 mL). The gels were visualized and registered in a Photodocumenter BioDoc-It Imaging System (UVP, CA).

3 I RESULTS AND DISCUSSIONS

The longest Xcf survival periods the crops phyllosphere were obtained in white oat, wheat, common bean, and common bean (cv. Jalo) (63 days), forage turnip (56 days), maize (49 days), peanut and barley (42 days) (Table 1). In the other crops, Xcf was recovered from the phyllosphere only at the inoculation time (0 days) (Table 1). Xcf survival periods in the rhizosphere were lower than those observed in the phyllosphere. The longest periods were obtained in ryegrass (28 days) and sunn hemp (21 days) (Table 1).

Crops	Survival period (days)	
	Phyllosphere	Rhizosphere
Barley	42	0
Black oat	0	0
Common bean	63	0
Common bean (cv. Jalo)	63	0
Cotton	0	0
Forage turnip	56	0
Maize	49	0
Peanut	42	0
Pigeon pea	0	0
Rapeseed	0	0
Ryegrass	0	28
Soybean	0	0
Sunflower	0	0
Sunn hemp	0	21
Velvet bean	0	0
Wheat	63	0
White oat	63	0
Soil	0	0

Table 1. Survival periods, in days, of *Xanthomonas citri* pv. *fuscans* in the phyllosphere and rhizosphere of crops, and in the soil.

In the weeds, the longest Xcf survival periods were obtained in the phyllosphere of *N. physalodes* (63 days), *C. benghalensis* and *L. virginicum* (49 days), and *C. bonariensis* (35 days) (Table 2). In the rhizosphere, the longest survival periods were in *L. multiflorum* and *C. benghalensis* (21 days), *C. juncea*, *A. viridis*, *D. stramonium,* and *S. oleraceus* (14 days). In the other weeds, the survival periods were considered low but higher than those observed in the soil (Table 2).

Species	Name	Survival period (days)	
		Phyllosphere	Rhizosphere
Alternanthera tenella	Joyweed	0	0
Amaranthus viridis	Slender amaranth	0	21
Bidens Pilosa	Hairy beggarticks	28	0
Commelina benghalensis	Benghal dayflower	49	21
Conyza bonariensis	Hairy fleabane	35	0
Cyperus rotundus	Purple nutsedge	0	0
Datura stramonium	Jimsonweed	7	14
Galinsoga parviflora	Gallant soldier	7	0
Gnaphalium purpureum	Purple cudweed	7	0
Lepidium virginicum	Virginia pepperweed	49	0
Nicandra physalodes	Apple of Peru	63	0
Portulaca oleraceae	Common purslane	0	0
Sida rhombifolia	Arrowleaf sida	7	0
Solanum americanum	Black mary	0	0
Sonchus oleraceus	Annual sowthistle	0	7
Soil	-	0	0

Table 2. Survival periods, in days, of *Xanthomonas citri* pv. *fuscans* in the phyllosphere and rhizosphere of weeds, and in the soil.

The plant surface is an environment susceptible to variations of solar radiation, temperature, humidity, and precipitation, factors that can be limiting to the survival of plant pathogens. However, CBB agents can form aggregates (biofilms) in the bean phyllosphere, protecting it from unfavorable conditions (JACQUES et al., 2005). Moreover, even in low humidity conditions, the biological cycle of the bacteria is completed on susceptible hosts without any symptoms (DARRASE et al., 2007). According to Darsonval et al. (2008), it could contribute to future CBB outbreaks, as it would be another inoculum source present in the cultivation area.

Until the development of our research there was little information available about Xcf hosts. Lima beans (*Phaseolus lunatus*) and species of the genus *Vigna* (*V. aconitifolia*, *V. angularis*, *V. mungo*, *V. radiata* and *V. umbellata*) can be considered natural hosts of Xcf and of Xpp. It is known that Xpp can survive epiphytically in some species crops and weeds, like soybean, maize, common bean cultivars, tepary bean, and representatives of the genus *Amaranthus*, *Bidens* and *Cyperus*, also evaluated in our study (CAFATI; SAETTLER, 1980; ANGELES-RAMOS, 1991; KARAVINA et al. 2011; CHEN et al., 2021). However, except for maize, the other species here evaluated were not identified as hosts of Xcf.

In our study, barley, maize, wheat, and white oat, which belong to the Poaceae family, behaved as possible alternative hosts of Xcf, since the bacterium had longer survival periods in these species. Different results were obtained by Silva et al. (2021) with *Xanthomonas campestris* pv. *campestris* (Xcc), the causal agent of black rot in crucifers, where Xcc had low survival in maize, which can be justified by the fact that the bacterium is not well adapted to the phyllosphere of this crop. According to Arias et al. (2000) the leaf surface of grasses hinders the adhesion of bacterial populations, which was not observed in our study.

The rhizosphere could be a favorable survival niche for many phytopathogenic bacteria, serving protection against climatic variations as well as providing carbon and minerals for their nutrition (BRENCIC; WINANS, 2005). However, in our study Xcf presented a low survival capacity in the rhizosphere, a fact also observed for Xcc in the rhizosphere of weeds (SILVA et al. 2017), and for *X. vesicatoria* in the rhizosphere of common bean, cucumber, pea, sorghum, tomato, and wheat (BASHAN et al. 1982). This low survival period can be explained by the fact that plants can benefit specific microorganisms populations in their rhizosphere by releasing root exudates, acting as a source of substrate for the development of certain microorganisms (GRAYSTON et al. 1998; YANG; CROWLEY, 2000; SMALLA et al. 2001; WU et al. 2015). This fact was proven by Vanura et al. (1969) as a direct relationship between the survival of Xcf in the rhizosphere and the exudation of biologically active peptides by the roots of barley, bean, and wheat. The authors also verified that in the early stages of plant development, Xcf was capable of multiplying in the rhizosphere and plants phenological stage can affect the bacterium survival. In our study, the rhizosphere of crops and weeds were inoculated 40 days after sowing, and a possible absence of biologically active peptides explain a reduced period of Xcf survival in this niche.

4 | CONCLUSIONS

Common bean is a relatively short cycle crop, varying between 65 and 110 days, and can be used in consortium and rotation systems with several annual crops. Thus, the knowledge of Xcf host range is important for efficient CBB management. Our results showed that Xcf presented a low survival period in black oat, cotton, pigeon pea, rapeseed, soybean, sunflower, velvet bean, being recommended the cultivation of these crops in rotation system or intercropping with common bean, especially in areas with history of occurrence of CBB.

In relation to weeds, *C. benghalensis* (Benghal dayflower), *C. bonariensis* (Hairy fleabane), *L. virginicum* (Virginia pepperweed), and *N. physalodes* (Apple of Peru) were species in which Xcf survived for a longer period, and their eradication from bean crop fields is recommended to reduce Xcf inoculum.

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ÍNDICE REMISSIVO

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C

D

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