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DANIELE MARIA DO NASCIMENTO

NICHOS DE SOBREVIVÊNCIA DE *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*

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Tese apresentada à Faculdade de Ciências Agronômicas da Unesp Câmpus de Botucatu, para obtenção do título de Doutor em Agronomia (Proteção de Plantas)

Orientador: Prof. Dr. Antonio Carlos Maringoni

Coorientador: Prof. Dr. Tadeu Antônio Fernandes da Silva Júnior

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TÍTULO DA TESE: NICHOS DE SOBREVIVÊNCIA DE *Curtobacterium flaccumfaciens* PV. *flaccumfaciens*

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Botucatu, 18 de dezembro de 2020

Aos meus amados pais,

Maria Josilene e Jorge (in memorian)

A minha irmã e meu esposo,

Dedico

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RESUMO

Curtobacterium flaccumfaciens pv. *flaccumfaciens* (Cff), agente causal da murcha de curtobacterium, é um dos principais patógenos bacterianos do feijão comum, tendo sido relatado também em soja, incitando a mancha bacteriana marrom. O conhecimento dos nichos de sobrevivência de Cff é essencial para o manejo eficiente desta doença. Este trabalho avaliou a sobrevivência de Cff no filosfera e rizosfera de 15 plantas cultivadas e 21 plantas daninhas, entre os anos de 2017 e 2019, assim como, a sobrevivência de isolados de Cff de feijão e soja em solo, em ambiente controlado e a campo. Em todos os experimentos, a sobrevivência de Cff foi avaliada a cada sete dias, por até 70 dias, através de plaqueamentos em meio de cultura semi-seletivo. *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* sobreviveu por pelo menos sete dias na filosfera e rizosfera de aveia branca, aveia preta, azevém, cevada, canola, nabo forrageiro, milho, milheto, sorgo, soja, girassol, mucuna e trigo. Em plantas daninhas, Cff também sobreviveu por no mínimo sete dias nos dois nichos avaliados, com destaque para as espécies *Amaranthus viridis, Conyza bonariensis, Emilia fosbergii, Galinsoga parviflora, Gnaphalium purpureum, Raphanus sativus, Lepidium virginicum, Commelina benghalensis, Ipomoea triloba, Cyperus rotundus, Senna obtusifolia, Digitaria insularis, Nicandra physalodes* e *Solanum americanum.* No solo, sob condições controladas, o isolado do feijão Feij. 2628A sobreviveu por um período máximo de 147 dias e, para os três isolados de soja, esse perídio variou entre 87 a 108 dias. No campo, a sobrevivência de ambos os isolados, do feijão e da soja, não ultrapassaram os 91 dias, independente do tipo de solo. Com base nos resultados obtidos, não se recomenda o plantio das espécies cultivadas avaliadas em sucessão ao feijão comum, em áreas com histórico de ocorrência de murcha bacteriana. As plantas daninhas, por serem potenciais hospedeiras de Cff, devem ser erradicadas dos campos de cultivos. Devido aos elevados períodos de sobrevivência dos isolados de Cff de feijão e da soja, o solo se apresenta como um nicho de sobrevivência, necessitando de uma maior atenção ao manejo da murcha de curtobacterium, em feijão e da mancha bacteriana marrom, em soja.

Palavras-chave: Murcha de curtobacterium. Mancha bacteriana marrom. Plantas daninhas. Plantas cultivadas. Solo.

ABSTRACT

Curtobacterium flaccumfaciens pv. *flaccumfaciens* (Cff), causal agent of bacterial wilt, is one of the main bacterial pathogens of common beans, having also been reported in soybeans, inciting bacterial tan spot. Knowledge of Cff survival niches is essential for the efficient management of this disease. This work evaluated the survival of Cff in the phyllosphere and rhizosphere of 15 cultivated plants and 21 weeds, between the years 2017 and 2019, as well as the survival of Cff isolates from common bean and soybean in soil, in a controlled environment and in the field. In all experiments, Cff survival was evaluated every seven days, for up to 70 days. *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* survived for at least seven days in the phyllosphere and rhizosphere of white oat, black oat, ryegrass, barley, canola, turnip, maize, pearl millet, sorghum, soybean, sunflower, mucuna and wheat. In weeds, Cff also survived for at least seven days in the two niches evaluated, with emphasis on the species *Amaranthus viridis, Conyza bonariensis, Emilia fosbergii, Galinsoga parviflora, Gnaphalium purpureum, Raphanus sativus, Lepidium virginicum, Commelina benghalensis, Ipomoeaper rotundus, Senna obtusifolia, Digitaria insularis, Nicandra physalodes* and *Solanum americanum*. In the soil, under controlled conditions, the isolate Feij. 2628A survived for a maximum period of 147 days and, for the three soybean isolates, this period varied between 87 to 108 days. In the field, the survival of both isolates, bean and soybean, did not exceed 91 days, regardless of the type of soil. Based on the results obtained, it is not recommended to plant the cultivated species evaluated in succession to common beans, in areas with a history of bacterial wilt. Weeds, as potential hosts for Cff, and must be eradicated from the crop fields. Due to the high survival periods of Cff isolates from bean and soybean, the soil presents itself as a survival niche, requiring greater attention to the management of bacterial wilt, in common bean and bacterial tan spot, in soybean.

Keywords: Bacterial wilt. Bacterial tan spot. Weeds. Crops. Soil.

SUMÁRIO

INTRODUÇÃO GERAL

O feijão comum (*Phaseolus vulgaris*) e a soja (*Glycine* max) estão entre as principais culturas produzidas no mundo, desempenhando um papel fundamental na alimentação humana (PAGANO; MIRANSARI, 2016; MYERS; KMIECIK, 2017). Na safra 2018, a produção mundial foi de 30 e 348 milhões de toneladas, para o feijão e a soja, respectivamente (FAOSTAT, 2020). O Brasil é o terceiro maior produtor mundial de feijão, com uma produtividade de 1,03 T/ha, e segundo maior produtor mundial de soja com produtividade de 3,20 T/ha, na safra 2018/2019 (CONAB, 2020). A produtividade de ambas culturas pode ser afetada por diversos fatores, entre eles, a ocorrência de doenças, como a murcha de curtobacterium, no feijoeiro, e a mancha bacteriana marrom, na soja, causadas pelo mesmo agente causal, *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Cff).

Curtobacterium flaccumfaciens pv. *flaccumfaciens* foi relatada pela primeira vez em 1920, nos Estados Unidos, causando murcha em plantas de feijão e, desde então, tem sido registrada em diversas regiões do mundo (HEDGES, 1922; BRADBURY, 1986; HARVESON et al., 2015). No Brasil, foi descrita primeiramente em 1995, em lavouras de feijão no estado de São Paulo e, posteriormente, observada em outras áreas de produção de feijão, nas regiões sul, sudeste e centro-oeste (MARINGONI; ROSA, 1997; SOARES, 2017).

Em 1963, Cff foi observada causando os mesmos sintomas típicos do feijoeiro, na soja, nos Estados Unidos, sendo descrita como agente causal da doença denominada "*bacterial tan spot*", com perdas de produtividade na soja de até 18,8% em cultivares suscetíveis (DUNLEAVY, 1963; DUNLEAVY, 1983; DUNLEAVY, 1984). Foi detectada também em lavouras de soja no Canadá, Rússia e Alemanha e, na safra 2011/2012, sua ocorrência foi constatada em soja no Brasil, no estado do Paraná, causando a doença denominada mancha bacteriana marrom (SOARES et al., 2013; SOARES, 2017).

Em relação aos sintomas, o mais característico no feijoeiro é a murcha, devido ao bloqueio, causado pelas células bacterianas, do movimento da água no sistema vascular da planta (HEDGES, 1922; HARVESON et al., 2015; WENDLAND, 2016). Pode ocorrer também o amarelecimento e, posteriormente, lesões necróticas, sendo possível observar na mesma planta, folhas sadias em contraste com folhas murchas

e necrosadas. Os vasos do xilema, por sua vez, não adquirem coloração escura, típica de doenças vasculares (THEODORO; MARINGONI, 2010). Sementes infectadas podem tornar-se enrugadas e adquirirem coloração laranja ou púrpura, como também podem não ocorrer sintomas visíveis (THEODORO; MARINGONI, 2010; VALENTINI et al., 2010). Na soja, ocorrem lesões cloróticas nos folíolos que evoluem para lesões necróticas, com ou sem a presença de halo amarelo, posteriormente, estas lesões secam e se tornam marrons, podendo o tecido seco se soltar com o vento. A murcha, típica do feijoeiro, também pode ocorrer na soja (HARVESON, 2015; HARVESON; VIDAVER, 2007; SOARES, 2017).

O controle dessas doenças é através do uso de sementes sadias, rotação de cultura com não hospedeiras, cultivares resistentes e incorporação de restos culturais ao solo (HARVESON et al., 2015; WENDLAND, 2016). Cff sobreviveu por 240 dias em restos culturais de feijão na superfície do solo, mas por apenas 30 dias quando estes foram incorporados, a 20 cm de profundidade (SILVA JÚNIOR et al., 2012). Estudos demonstraram que excesso de nitrogênio na forma de uréia, com doses 50% acima da recomendada, influenciou positivamente no desenvolvimento da doença em feijoeiro, sendo recomendado evitar o excesso de adubação nitrogenada (THEODORO; MARINGONI, 2006). Em relação as cultivares resistentes, IAC Diplomata, IAC Carioca Akytã, IAC Carioca Pyatã, IAC Imperador, IAC Alvorada, IAC Carioca Aruã, são algumas das cultivares de feijão que apresentam níveis de resistência à murcha de curtobacterium (MARINGONI et al., 2015). Para soja, a cultivar BR388RR apresenta nível moderado de resistência (SOARES, 2018).

Na ausência da cultura principal, bactérias fitopatogênicas podem sobreviver em sementes, restos culturais infectados, hospedeiros alternativos e no solo (LEBEN, 1981; VIDAVER; LAMBRECHT, 2004). No caso de Cff, sua transmissão por sementes é responsável pela disseminação e introdução da bactéria em novas áreas, com uma porcentagem de transmissão de semente para planta de mais de 70%, podendo também permanecer viável por até 24 anos em sementes de feijoeiro armazenadas (BURKHOLDER, 1945; SAETTLER; PERRY, 1972; CAMARA; VIGO; MARINGONI, 2009). Em soja, a taxa de transmissão planta-semente é baixa, de até 1% em cultivares suscetíveis, sugerindo que, neste caso, em um sistema de rotação de cultura entre feijão e soja, a safra anterior é a mais provável fonte de introdução de Cff (SOARES et al., 2018).

Além do feijoeiro e da soja, outras plantas foram identificadas como hospedeiras de Cff, como o feijão mungo (*Vigna radiata*), feijão caupi (*V. unguiculata)*, feijão preto (*V. mungo*), feijão azuki (*V. angularis*), entre outras (WOOD; EASDOWN, 1990; TRIPEPI; GEORGE, 1991; SCHWARTZ et al., 2005; HUANG et al., 2009). Culturas usadas em rotação com o feijoeiro, quando hospedeiras de Cff, tornam-se fonte de inóculo para a cultura subsequente. No Brasil, cevada, aveia preta, aveia branca, azevém, trigo e canola foram relatadas como hospedeiras de Cff, assim como, já foi verificada maior incidência de murcha de curtobacterium em feijoeiro após a sucessão com aveia preta, aveia branca e trigo (GONÇALVES, 2015; GONÇALVES et al., 2017). Estudos semelhantes foram conduzidos nos Estados Unidos, identificando trigo, girassol e alfafa, como hospedeiras (HARVESON et al., 2015). Cff também já foi relatada sobrevivendo em berinjela, pimenta e tomate (OSDAGHI et al., 2018).

Sobre as plantas daninhas hospedeiras de Cff, até o momento, a bactéria foi identificada em ervilhaca peluda (*Vicia vilosa*), uma das principais plantas daninhas associada a cultivos de feijoeiro no Irã (OSDAGHI et al., 2015). Alguns autores sugerem que Cff possa sobreviver em plantas daninhas, mas há poucas informações disponíveis (OSDAGHI et al., 2015; HARVESON et al., 2015).

O solo é um dos maiores reservatórios de microrganismos, mas a maioria das bactérias fitopatogênicas apresenta dificuldades em sobreviver neste nicho, uma vez que, geralmente, não produzem estruturas de resistência, e também pelo efeito antagônico exercido por alguns microrganismos (SCHUSTER; COYNE, 1974; ROUSK et al., 2010; FIERER; LENNON, 2011). A sobrevivência no solo por fitobactérias pode ocorrer de três modos: a) a bactéria permanece em estado hipobiótico, sendo assim menos suscetível aos agentes degradantes; b) sobrevivem associada à rizosfera de plantas hospedeiras ou não hospedeiras e c) permanecem em estágio viável mas não culturável (“*viable but non cuturable”* - VBNC), ou seja, apesar das células bacterianas estarem viáveis, são incapazes de crescer em meio de cultura não seletivo, ao menos em quantidade suficiente para sua detecção visual (LEBEN, 1981; GREY; STECK, 2001). Apesar de não ter sido ainda detectada ocorrendo naturalmente em solos, estudos demonstraram a capacidade de Cff sobreviver neste habitat por até 80 dias, sob condições de campo (GONÇALVES et al., 2018).

É evidente a escassez de relatos sobre a sobrevivência de Cff em plantas daninhas e, no caso da mancha bacteriana marrom, nenhum estudo foi ainda

desenvolvido. Apesar de ainda não terem sidos avaliados os danos em lavouras de soja no Brasil, a experiência nos Estados Unidos, onde já foi relatada há mais tempo, demonstrou que essa doença é de ocorrência esporádica, que pode ficar anos sem causar perdas econômicas, mas quando ocorre, possui um potencial de dano considerável. Portanto, o conhecimento dos nichos de sobrevivência de Cff pode evitar situações que favorecem sua ocorrência.

Este trabalho avaliou a sobrevivência de um isolado de Cff, proveniente de feijoeiro, em plantas daninhas ocorrentes em campos de cultivo (Capítulo 1); em plantas cultivadas utilizadas em rotação com o feijoeiro (Capítulo 2), e em solo, bem como a sobrevivência de isolados de Cff de soja, neste nicho, em condições controladas e de campo (Capítulo 3).

CAPÍTULO 1

SURVIVAL OF *Curtobacterium flaccumfaciens* PV. *flaccumfaciens* IN WEEDS

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Abstract

Weeds are important alternative hosts of pathogens, responsible for the survival and spread of phytopathogenic bacteria. Our study evaluated the potential of weeds as hosts of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Cff), causal agent of bacterial wilt, one of the main diseases of common beans. Cff survival was evaluated in the phyllosphere and in the rhizosphere of 21 weeds, in four experiments under field conditions, during the years 2018 and 2019. The aerial part of the plant was inoculated by spraying bacterial suspension (10^7 cfu/ml) of Cff, while the soil of the growing pots was infested with the same suspension. Cff survival was evaluated every 7 days, for 70 days. The identity of the bacterium was confirmed by PCR with the specific primers CffFOR2 and CffREV4, from strains recovered from all samples. Principal component analysis (PCA) showed that high temperatures and rainfall reduced Cff survival in the phyllosphere, while high temperatures reduced the survival of the bacterium in the rhizosphere. Our results demonstrated that *Amaranthus viridis* (family Amaranthaceae), *Conyza bonariensis*, *Emilia fosbergii*, *Galinsoga parviflora*, *Gnaphalium purpureum* (Asteraceae), *Raphanus sativus*, *Lepidium virginicum* (Brassicaceae), *Commelina benghalensis* (Commelinaceae), *Ipomoea triloba* (Convolvulaceae), *Cyperus rotundus* (Cyperaceae), *Senna obtusifolia* (Fabaceae), *Digitaria insularis* (Poaceae), *Nicandra physalodes*, and *Solanum americanum* (Solanaceae) are potential hosts for Cff. Their eradication in common bean fields is recommended, especially in fields with a history of bacterial wilt occurrence.

Keywords: bacterial wilt, common bean, disease management, ecology, phytobacteria, principal component analysis.

1.1. Introduction

Bacterial wilt, caused by *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Cff), is an important disease of the common bean worldwide (Harveson et al., 2015). The management of the disease is based on the use of certified seeds, resistant cultivars, and crop rotation with nonhost species. When present in the field, Cff is able to survive on bean debris, soil, and alternative hosts, making eradication difficult (Silva Júnior et al., 2012; Harveson et al., 2015; Gonçalves et al., 2017, 2018).

Most phytopathogenic bacteria can survive as residents on nonhost plants. The phylloplane, a region that comprises the leaves of the plant, can be a survival niche for phytopathogenic bacteria. The survival of phytopathogenic bacteria is influenced by the plant species and their genotype, and more specifically, by the chemical composition of the cuticle, the presence of conducting vessels, and natural openings in the phyllosphere. Moreover, environmental factors, such as ultraviolet radiation (UVR), relative humidity, geographical localization, growing season, and agrochemicals are also influencing factors (Vorholt, 2012; Moulas et al., 2013; Rastogi et al., 2013).

In the rhizosphere, bacterial populations are attracted by the exudation of compounds from the roots, which are used as nutrients, favouring the survival and maintenance of an inoculum source (Berendsen et al., 2012; Vorholt, 2012).

It is known that crops used in rotation with common bean, such as barley, black oat, white oat, canola, ryegrass, wheat, maize, and sunflower, are alternative hosts for Cff (Harveson et al., 2015; Gonçalves et al., 2017). In relation to weeds, hairy vetch (*Vicia vilosa*) has been reported as a host, suggesting that other species may be potential reservoirs of Cff (Osdaghi et al., 2015).

The lack of data about host weeds for Cff motivated this study, which aimed to: (a) investigate the survival capacity and population dynamics of Cff in the phyllosphere and rhizosphere of 21 weeds; and (b) correlate this information with climatic data through principal component analysis (PCA).

1.2. Materials and methods

Bacterial strains, culture conditions and preservation

Cff strain 2628A, resistant to 100 µg/ml rifampicin and pathogenic to common bean, was obtained from the Plant Bacteriology Laboratory Collection, São Paulo State University (Unesp), School of Agriculture, Botucatu, Brazil, and used for all experiments. The strain was grown on NSAR medium, consisting of nutrient agar (Merck), 5 g/L sucrose (Sigma) (Schaad et al., 2001), and 100 µg/ml rifampicin (Rifaldin). The strain was incubated at 28°C for 48 hr. For long-term preservation, the strain was maintained in 30% glycerol at − 80°C.

Origin of weeds

Twenty-one weed species, belonging to 13 families, were selected according to their prevalence in common bean-growing areas (Table 1). Seeds were sown in 10 L plastic pots containing a mixture of soil, sand, and organomineral substrate (Tropstrato HT) (ratio 1:1:1), supplemented with 0.6 kg ammonium sulphate, 1.7 kg superphosphate, 0.6 kg potassium chloride, and 0.8 kg dolomitic lime per cubic metre of mixture. Fertilizations were carried out according to the soil analysis. All pots were initially maintained in a greenhouse (22–27°C at 60%–90% relative humidity) and then

taken to the experimental area 40 days after emergence, for 70 days, and given supplementary irrigation when necessary.

Table 1 Weeds used in the survival experiments of *Curtobacterium flaccumfaciens* pv*. flaccumfaciens* in the phyllosphere and rhizosphere.

Botanical family	Scientific name	Common name	Experiments
Amaranthaceae	*Alternanthera tenella*	Joyweed	2, 3
	Amaranthus viridis	Slender amaranth	2, 4
Asteraceae	*Conyza bonariensis*	Hairy fleabane	1, 4
	Emilia fosbergii	Florida tasselflower	1, 4
	Gnaphalium purpureum	Purple cudweed	1, 4
	Galinsoga parviflora	Gallant soldier	1, 3
	Bidens pilosa	Hairy beggarticks	2, 3
Brassicaceae	*Lepidium virginicum*	Virginia pepperweed	2, 4
	Raphanus sativus	Turnip	1, 3
Commelinaceae	*Commelina benghalensis*	Benghal dayflower	1, 3
Convolvulaceae	*Ipomoea triloba*	Littlebell	1, 3
Cyperaceae	*Cyperus rotundus*	Purple nutsedge	1, 3
Fabaceae	*Senna obtusifolia*	Sicklepod	1, 3
Lamiaceae	*Leonurus sibiricus*	Siberian motherwort	2, 3
Malvaceae	*Sida rhombifolia*	Arrowleaf sida	2, 3
Poaceae	*Rhynchelytrum repens*	Natal grass	2, 3
	Digitaria insularis	Sourgrass	2, 4
Portulaceae	*Portulaca oleracea*	Common purslane	2, 3
Rubiaceae	*Richardia brasiliensis*	Brazilian pusley	2, 3
Solanaceae	*Solanum americanum*	Black nightshade	2, 4
	Nicandra physalodes	Apple of Peru	1, 4

Inoculation experiments and sampling

Cff survival in weeds was evaluated in four experiments performed on 1 September 2018 (Experiment 1), 5 November 2018 (Experiment 2), 11 October 2019 (Experiment 3), and 9 May 2019 (Experiment 4). The aerial part of the weeds was sprayed with a 10^7 cfu/ml suspension of Cff until the point of run-off. The soil of each pot was infested with 700 ml of the same bacterial suspension. Pots containing only soil were infested to compare Cff survivability in the absence of plants. Samplings were carried out at the inoculation time to determine the initial Cff population and then every 7 days for 70 days. For sampling, two plants per species were removed from the pots

at each evaluation, obtaining a composite sample. The leaves and the soil clinging to roots (rhizosphere soil) were transferred, separately, to plastic bags for homogenization.

Sample processing

To determine the Cff population in the phyllosphere and rhizosphere of weeds, 5 g plant tissue and 10 g rhizosphere soil were used. Samples were transferred to Duran flasks containing 100 ml autoclaved water and shaken at 300 rpm for 30 min. After shaking, soil samples remained at rest for 30 min to sediment the soil particles. The suspensions were serial-diluted (10^0–10^{-4}), and 100 µl of each was plated in duplicate on NSAR medium supplemented with chlorothalonil (0.01 g/L) and thiophanate methyl (0.01 g/L), followed by incubation at 28°C for 96 hr. The colonies that were morphologically identical to Cff were quantified.

Characterization of bacterial strains

For survival-period confirmation, colonies morphologically identical to Cff were selected from all treatments and transferred to NSA medium, consisting of nutrient agar (Merck), 5 g/L sucrose (Sigma) with 7% NaCl, followed by incubation at 28°C for 96 hr (Maringoni and Camara, 2006). Strains were characterized by PCR, using specific primers for Cff, CffFOR2 (5′-GTTATGACTGAACTTCACTCC-3′) and CffREV4 (5′-GATGTTCCCGGTGTTCAG-3′) (Tegli et al., 2002). Total DNA of each strain was extracted by adjusting a suspension to 10^8 cfu/ ml and submitting it to 95°C for 15 min.

Each PCR was carried out in a total volume of 25 µl, containing 12.5 µl GoTaq Green Master Mix (Promega), 0.5 µl of each primer, 8.5 µl milli-Q water, and 3 µl DNA. The PCR was performed in a Mastercycler Gradient model thermocycler (Eppendorf) using the following PCR programme: 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; and a final incubation at 72°C for 10 min. The amplicons obtained were submitted to horizontal electrophoresis an 1% agarose gel prepared with 0.5 × TBE buffer and ethidium bromide (Sigma) (4 µl/120 ml). The gels were visualized and recorded on the BioDoc-It Imaging System for gel documentation (UVP).

Climate data

The maximum, minimum, and average temperatures, rainfall, and relative humidity that occurred during the experimental periods were collected daily from a meteorological station located approximately 300 m from the experimental area and are described in Table 2. Soil moisture was determined in all evaluations and treatments, using an infrared moisture analyser (Gehaka, Model IV 2500).

Table 2 Average, maximum and minimum temperature, rainfall, relative humidity and soil moisture occurring during the survival experiments of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* in the phyllosphere and rhizosphere of weeds.

Period (days)	Average temp. (°C)	Average min. temp. (°C)	Average max. temp. (°C)	Rainfall (mm)	Relative humidity (%)	Soil moisture (%)
Experiment 1						
0-7	18.0	12.9	24.7	3.0	68.4	15.1
8-14	18.8	14.2	25.4	14.4	70.6	27.0
15-21	18.9	15.5	24.1	51.5	87.9	13.3
22-28	22.9	17.4	29.4	1.5	66.8	21.2
29-35	22.6	18.4	28.2	1.1	76.8	24.4
36-42	20.4	16.9	25.1	97.4	87.1	23.7
43-49	22.1	17.9	27.7	0.0	86.9	25.4
50-56	19.4	15.7	24.5	34.4	83.6	20.4
57-63	21.1	16.3	27.6	3.4	74.4	23.6
64-70	20.0	16.0	25.8	3.0	81.6	21.6
Experiment 2						
0-7	19.9	16.5	25.3	3.0	84.4	16.5
8-14	24.1	19.1	30.3	84.6	74.3	26.0
15-21	19.9	16.8	24.3	83.2	89.7	20.0
22-28	22.0	17.7	27.8	76.4	78.5	9.2
29-35	21.7	16.7	27.9	0.0	67.6	17.8
36-42	25.6	20.3	32.0	7.0	58.3	24.2
43-49	25.2	20.9	31.6	57.9	75.2	6.7
50-56	23.2	19.1	28.6	10.3	79.6	11.4
57-63	23.8	20.5	29.8	49.0	85.2	15.7
64-70	25.5	20.4	31.5	50.1	71.9	10.1
Experiment 3						
0-7	25.1	20.1	31.3	96.5	73.4	19.1
8-14	25.8	21.1	32.5	5.3	73.8	19.1
15-21	25.8	20.9	32.0	11.8	63.8	12.5
22-28	23.7	19.4	29.2	21.8	70.9	12.5
29-35	24.2	20.0	29.4	68.8	71.8	15.0
36-42	22.2	19.3	26.9	5.1	89.1	18.8
Experiment 4						
0-7	15.6	17.6	26.3	0.0	82.7	21.2
8-14	21.4	15.2	23.2	0.0	80.9	17.9
15-21	18.6	13.3	23.7	1.2	73.4	23.7
22-28	18.5	15.5	23.5	33.3	81.1	21.7
29-35	19.1	12.7	23.2	0.0	67.4	17.9
36-42	17.8	15.4	25.9	0.0	62.8	28.7
43-49	20.9	14.8	25.2	0.8	64.2	19.7
50-56	20.0	16.9	26.9	80.8	61.5	21.7
57-63	21.6	8.8	18.6	0.0	72.6	21.7
64-70	13.3	11.9	23.5	2.5	61.2	23.2

Experimental design and statistical analysis

The experimental design consisted of randomized blocks with the treatment number varying between experiments: 21 treatments in Experiment 1 (10 weeds × 2 survival niches [phyllosphere and rhizosphere] +1 soil without plants as negative control [NC]); 23 treatments in Experiment 2 (11 weeds × 2 survival niches + 1 NC);

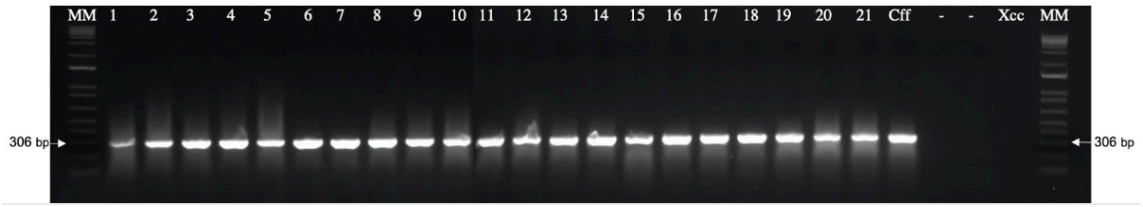
27 treatments in Experiment 3 (13 weeds × 2 survival niches + 1 NC), and 17 treatments in Experiment 4 (8 weeds × 2 survival niches + 1 NC). Pots containing only soil infested with bacteria were used as a negative control for rhizosphere survival. Each experimental plot was represented by one pot, containing five plants, for a total of eight replicates.

On the basis of Cff survival periods (days) in each experiment, the average survival periods were calculated. Data $\log^{10}$ of the number of cfu/g tissue and the number of cfu/g dry soil were used to calculate the area under the curve (AUC). Because of the interdependency and interrelationship of the variables studied, PCA was performed using Minitab 17 statistical software.

1.3. Results

Characterization of bacterial strains

Strains selected from the phyllosphere and rhizosphere of the weeds, and from the soil, were characterized in all experiments and evaluation periods were characterized. The strains grew on NSA culture medium (supplemented with NaCl). All strains were confirmed as Cff-positive by PCR with specific primers through amplification of a 306 bp DNA fragment (Figure S1).



Supplemental Figure 1 Agarose gel 1% of PCR products of DNA extracted from bacteria isolated from phyllosphere and rhizosphere of weeds, and from soil, using the specifics primers for *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, CffFOR2 and CffREV4. MM: molecular marker (Invitrogen 1 Kb Plus DNA Ladder); from 1 to 10: samples from phyllosphere; from 11 to 20: samples from rhizosphere; 21: sample from soil; Cff: *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* as positive control; (-): negative control; Xcc: *Xanthomonas campestris* pv. *campestris* as negative control.

Cff survival in the phyllosphere

The strain Feij 2628A showed the highest average survival periods in the phyllosphere of *Emilia fosbergii*, *Nicandra physalodes*, and *Gnaphalium purpureum* (70 days), *Conyza bonariensis* (63 days), and *Raphanus sativus* and *Cyperus rotundus* (56 days) (Table 3). Lower average survival periods, between 21 and 28 days, were observed in the phyllosphere of *Portulaca oleracea*, *Sida rhombifolia*, *Richardia brasiliensis*, *Rhynchelytrum repens*, *Bidens pilosa*, and *Leonorus sibiricus* (Table 3). Regarding the AUC, the highest average values were observed in the phyllosphere of *N. physalodes* (348), *E. fosbergii* (346), and *G. purpureum* (336) (Table 3). For the other weeds, these values varied from 273 to 107.

Table 3 Survival periods and area under the curve (AUC) of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* in the phyllosphere of weeds.

Weeds species	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Average
Emilia fosbergii	70[a] (290)[b]	-	-	70 (403)	70 (346)
Nicandra physalodes	70 (319)	-	-	70 (377)	70 (348)
Gnaphalium purpureum	70 (309)	-	-	70 (363)	70 (336)
Conyza bonariensis	70 (259)	-	-	56 (228)	63 (243)
Raphanus sativus	70 (329)	-	42 (216)	-	56 (273)
Cyperus rotundus	70 (365)		42 (161)		56 (263)
Amaranthus viridis	-	35 (162)	-	70 (332)	53 (247)
Digitaria insularis	-	35 (155)	-	70 (311)	53 (233)
Lepidium virginicum	-	35 (182)	-	70 (330)	53 (256)
Commelina benghalensis	70 (343)	-	35 (135)	-	53 (239)
Alternanthera tenella	-	42 (155)	28 (115)	-	49 (135)
Ipomoea triloba	70 (344)	-	28 (141)	-	49 (242)
Solanum americanum	-	28 (146)	-	70 (368)	49 (257)
Galisonga parviflora	70 (301)	-	28 (137)	-	49 (219)
Senna obtusifolia	56 (195)	-	21 (93)	-	39 (144)
Portulaca oleracea	-	35 (174)	21 (102)	-	28 (138)
Sida rhombifolia	-	35 (165)	21 (88)	-	28 (126)
Richardia brasiliensis	-	35 (156)	21 (108)	-	28 (132)
Rhynchelytrum repens	-	35 (188)	35 (147)	-	25 (168)
Bidens pilosa	-	35 (149)	14 (66)	-	25 (107)
Leonorus sibiricus	-	35 (169)	7 (52)	-	21 (111)

[a]Survival periods (days) of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* in the phyllosphere of weeds.

[b]Area under the curve (AUC) of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* in the phyllosphere of weeds.

For Experiments 1 and 2, the PCA for Cff survival in the phyllosphere showed that the survival periods and AUC increased towards the negative values of the first component (x axis), while the minimum, maximum, and average temperature, as well as precipitation, increased with positive values of the same component (Figure 1a). As the vectors (lines) increased in opposite directions, it could be inferred that Cff survival in the phyllosphere was favoured by lower temperatures and lower rainfall and was reduced by higher temperatures and greater rainfall.

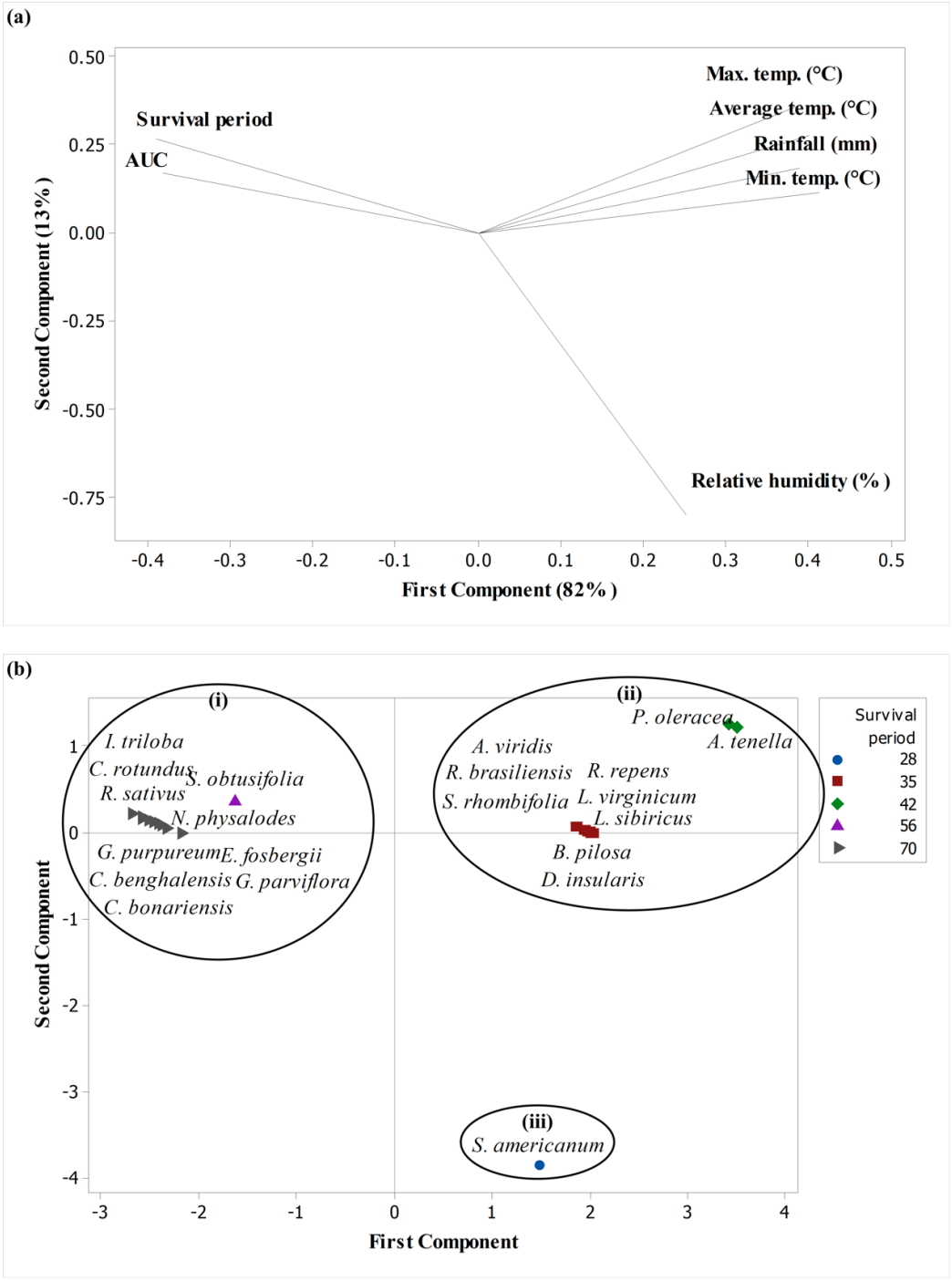
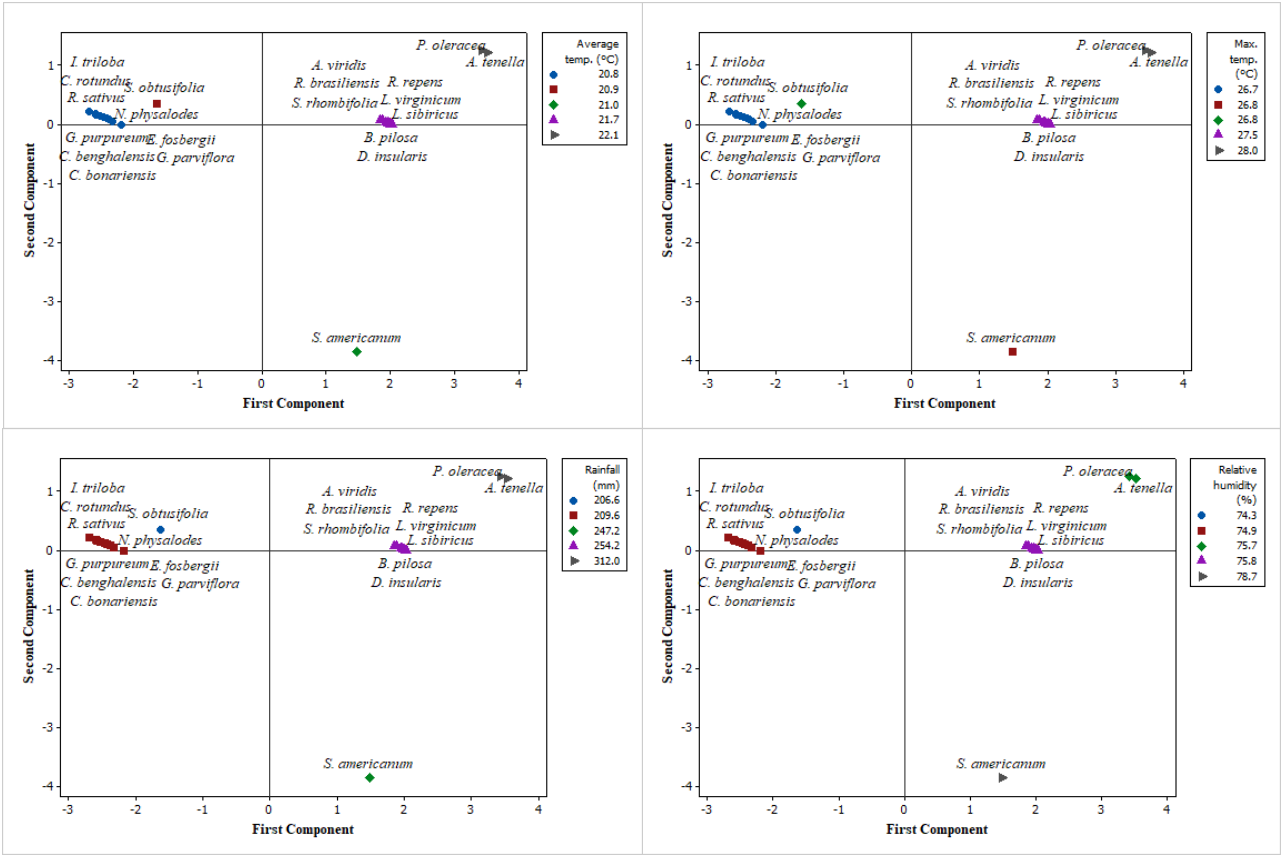


Figure 1 Principal component analysis (PCA) of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* survival in the weeds phyllosphere on experiments 1 and 2. (a) Projection of vectors of traits: survival period, area under the curve (AUC), maximum, minimum and average temperature (°C), rainfall (mm) and relative humidity (%); (b) Biplot graph with dispersion of 21 weeds according to the principal components.

On the basis of the survival periods and climatic data of Experiments 1 and 2, PCA separated the weeds into three groups (Figure 1b): (a) high survival (56–70 days) of Cff in the phyllosphere, with 209 mm of accumulated precipitation, average temperature of 20.8°C, and 74.5% relative humidity; (b) intermediate survival (35–42 days), with an average temperature of 21.8°C, relative humidity of 75.7%, and accumulated precipitation varying from 254 to 312 mm; and (c) low survival (28 days), with accumulated precipitation of 247 mm, average temperature of 21°C, and relative humidity of 78.7%, which was the variable that most influenced Cff survival in the *Solanum americanum* phyllosphere (Figure S2).



Supplemental Figure 2 Biplot graphics with dispersion of 21 weeds according to the principal components of experiments 1 and 2 of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* survival in the weed phyllosphere, grouped by: a) average temperature (°C); b) maximum temperature (°C); c) rainfall (mm); and d) relative humidity (%).

In Experiments 3 and 4, the temperature vectors increased in the opposite direction to the survival period and AUC vectors, indicating, again, an inverse relationship (Figure 2a). Three weed groups were formed (Figure 2b): (a) high survival (56–70 days) of Cff in the phyllosphere, due to the lower temperatures (average temperature of 19°C) and lower rainfall (118 mm accumulated); (b) intermediate survival (35–42 days), average temperature of 24.5°C and higher ac- cumulated precipitation (250.4 mm for *R. repens* and *Commelina benghalensis* phyllosphere, and 394 mm for *R. sativus* and *C. rotundus*); and (c) low survival (7–28 days), average temperature of 25°C, and accumulated precipitation ranging from 145 to 245 mm (Figure S3).

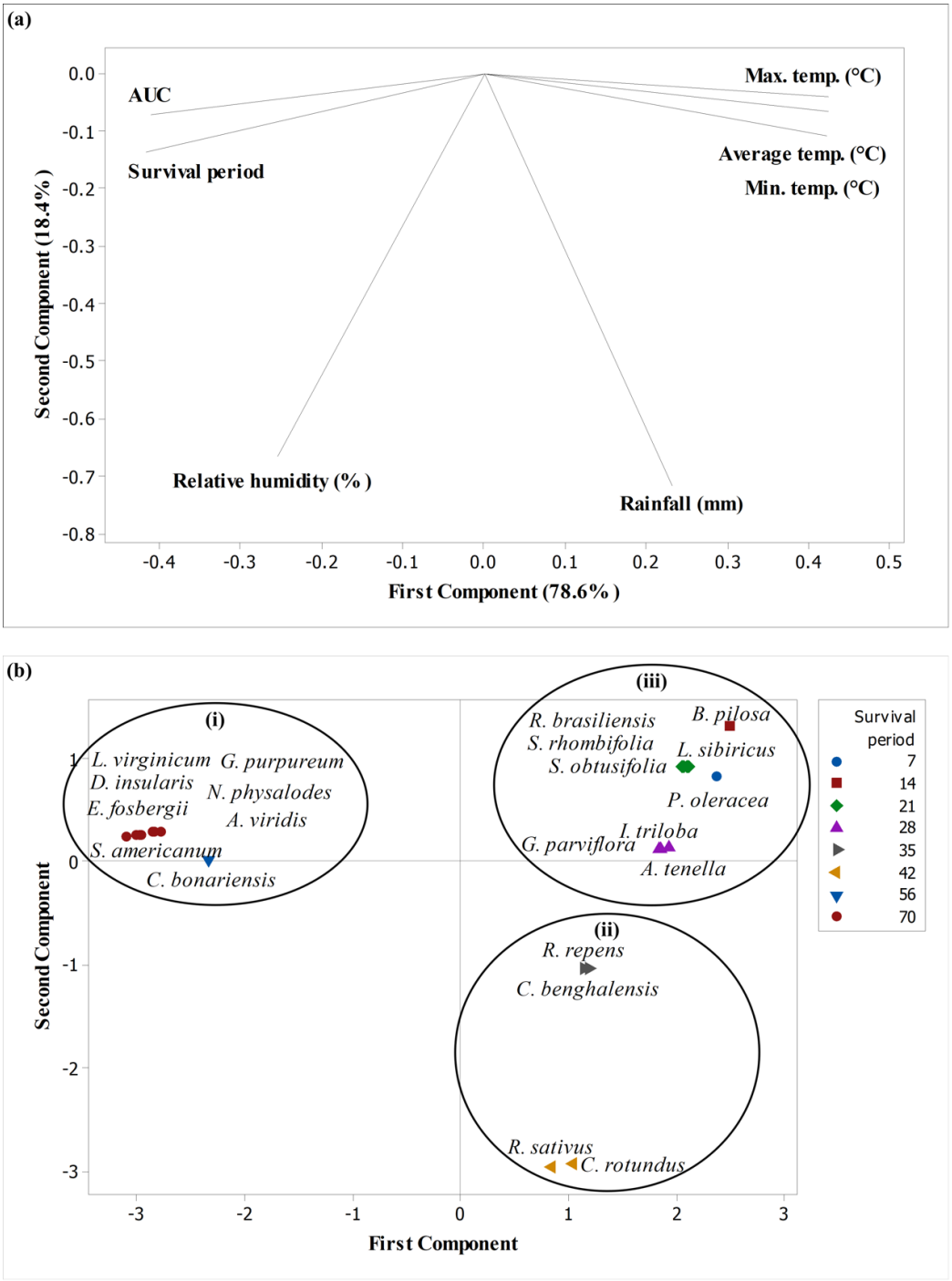
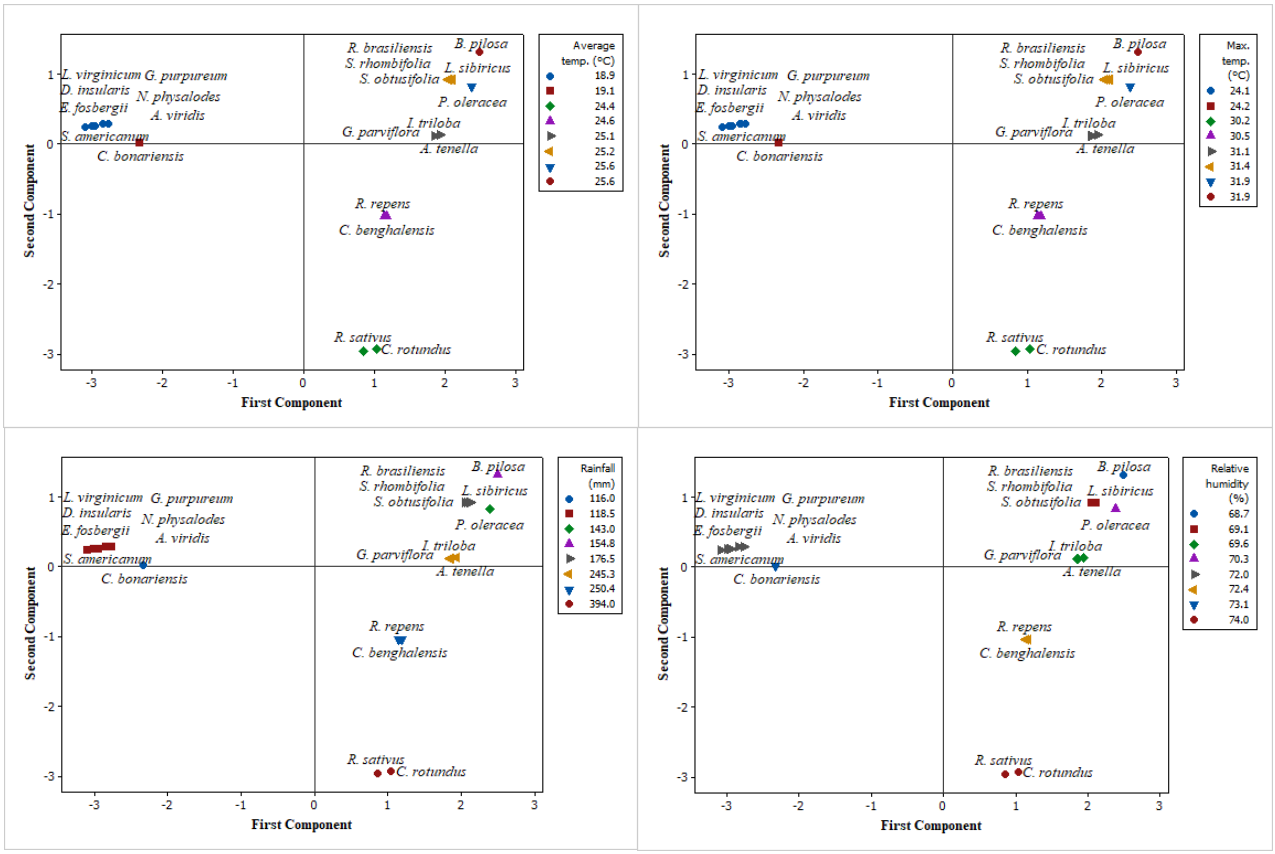


Figure 2 Principal component analysis (PCA) of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* survival in the weeds phyllosphere on experiments 3 and 4. (a) Projection of vectors of traits: survival period, area under the curve (AUC), maximum, minimum and average temperature (°C), rainfall (mm) and relative humidity (%); (b) Biplot graph with dispersion of 21 weeds according to the principal components.



Supplemental Figure 3 Biplot graphics with dispersion of 21 weeds according to the principal components of experiments 3 and 4 of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* survival in the weed phyllosphere, grouped by: a) average temperature (°C); b) maximum temperature (°C); c) rainfall (mm); and d) relative humidity (%).

Cff survival in the rhizosphere

The strain Feij 2628A showed the highest average survival periods in the rhizosphere of *E. fosbergii* (70 days), *Digitaria insularis*, and *S. americanum* (67 days), *G. purpureum* (63 days), *N. physalodes* (60 days), and *Lepidium virginicum* and *C. rotundus* (56 days) (Table 4). Lower average periods were observed in the rhizosphere of *L. sibiricus* and *S. rhombifolia* (39 days) and *B. pilosa* (35 days) (Table 4). However, in Experiment 2, the average survival period for Cff in these three weeds varied between 49 and 63 days (Table 4). In soil, Cff survived for an average period of 42

days, superior only to that observed in the rhizosphere of *L. sibiricus*, *S. rhombifolia*, and *B. pilosa*. Regarding the AUC, the highest average value was observed in the rhizosphere of *E. fosbergii* (292) (Table 4). For the other weeds, these values varied from 130 to 274.

Table 4 Survival periods and area under the curve (AUC) of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* in the rhizosphere of weeds.

Weeds species	Experiment 1	Experiment 2	Experiment 3	Experiment 4	Average
Emilia fosbergii	70[a] (242)[b]	-	-	70 (343)	70 (292)
Digitaria insularis	-	63 (231)	-	70 (297)	67 (264)
Solanum americanum	-	63 (227)	-	70 (321)	67 (274)
Gnaphalium purpureum	56 (220)	-	-	70 (266)	63 (243)
Nicandra physalodes	49 (195)	-	-	70 (290)	60 (243)
Lepidium virginicum	-	42 (162)	-	70 (242)	56 (202)
Cyperus rotundus	70 (275)	-	42 (171)	-	56 (223)
Conyza bonariensis	49 (178)	-	-	56 (240)	53 (209)
Rhynchelytrum repens	-	63 (236)	42 (173)	-	53 (205)
Raphanus sativus	70 (264)	-	35 (130)	-	53 (197)
Amaranthus viridis	-	42 (142)	-	63 (283)	49 (212)
Alternanthera tenella	-	70 (226)	28 (124)	-	49 (175)
Ipomoea triloba	70 (323)	-	28 (124)	-	49 (203)
Commelina benghalensis	70 (243)	-	28 (128)	-	49 (185)
Richardia brasiliensis	-	70 (224)	28 (80)	-	49 (152)
Portulaca oleraceae	-	63 (211)	28 (114)	-	46 (163)
Senna obtusifolia	70 (234)	-	21 (77)	-	46 (155)
Galinsoga parviflora	70 (202)	-	21 (97)	-	46 (169)
Leonurus sibiricus	-	49 (191)	28 (126)	-	39 (159)
Sida rhombifolia	-	63 (184)	14 (76)	-	39 (130)
Bidens pilosa	-	49 (202)	21 (99)	-	35 (151)
Soil	35 (109)	42(172)	35 (135)	56 (227)	42 (160)

[a]Survival periods (days) of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* in the phyllosphere of weeds.
[b]Area under the curve (AUC) of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* in the phyllosphere of weeds.

In Experiments 1 and 2, it was also possible to discriminate three weed groups on the basis of the Cff survival periods in the rhizosphere (Figure 3b): (a) high survival (63 to 70 days), accumulated precipitation between 206.6 and 312 mm, average temperature between 20.8 and 22.1°C, and soil moisture between 15.8% and 25.7%; (b) intermediate survival (42 to 56 days), accumulated precipitation between 209.6 and 254.2 mm, average temperature between 20.8 and 21.7°C, and soil moisture between 14.4% and 24.3%; and (c) low survival (35 days), accumulated precipitation of 71.5 mm, average temperature of 21.6°C, and soil moisture of 18.7% (Figure S4). However, as the PCA indicates, it was not possible to correlate the climatic variables with the Cff survival in the rhizosphere (Figure 3a).

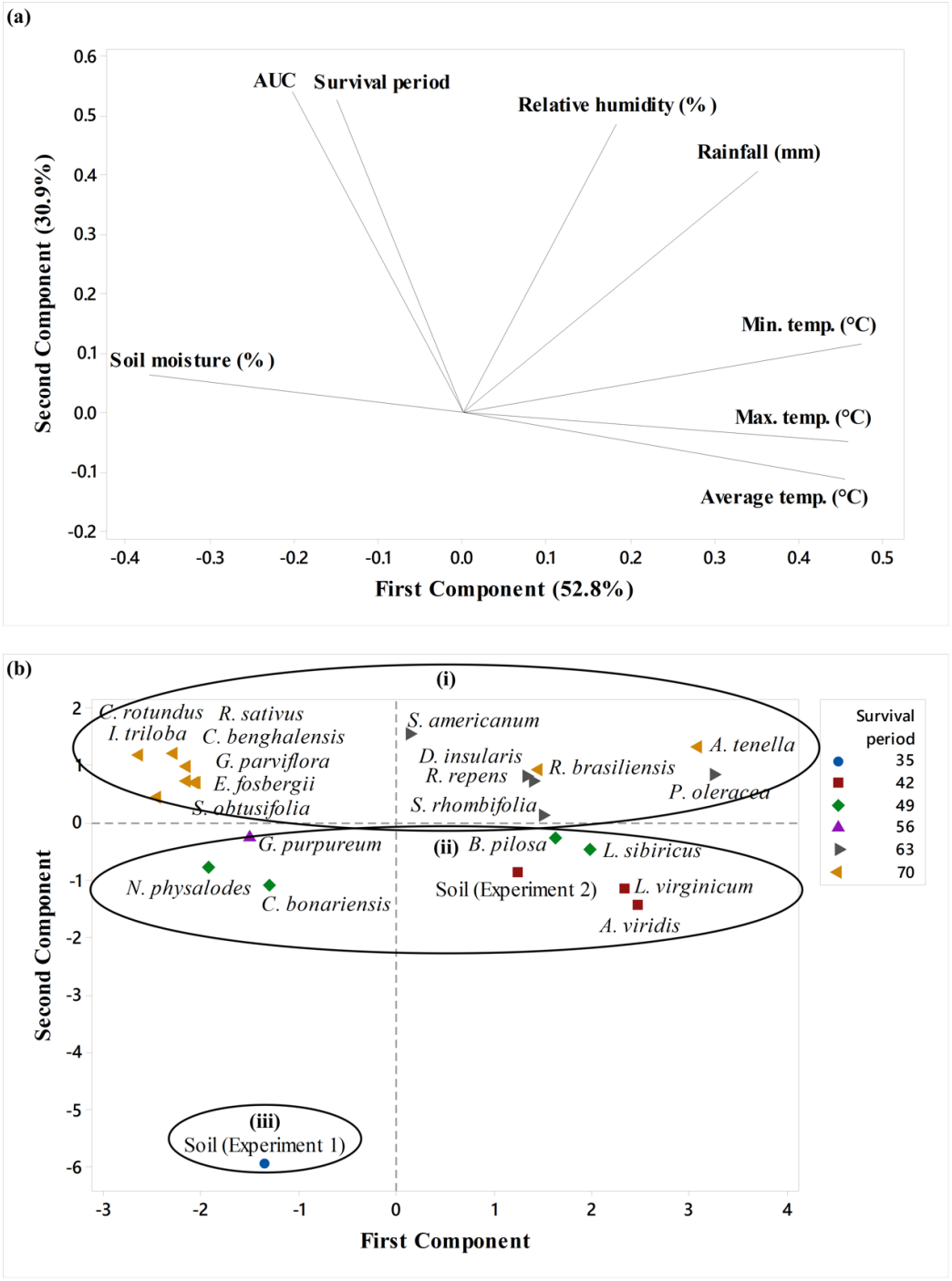
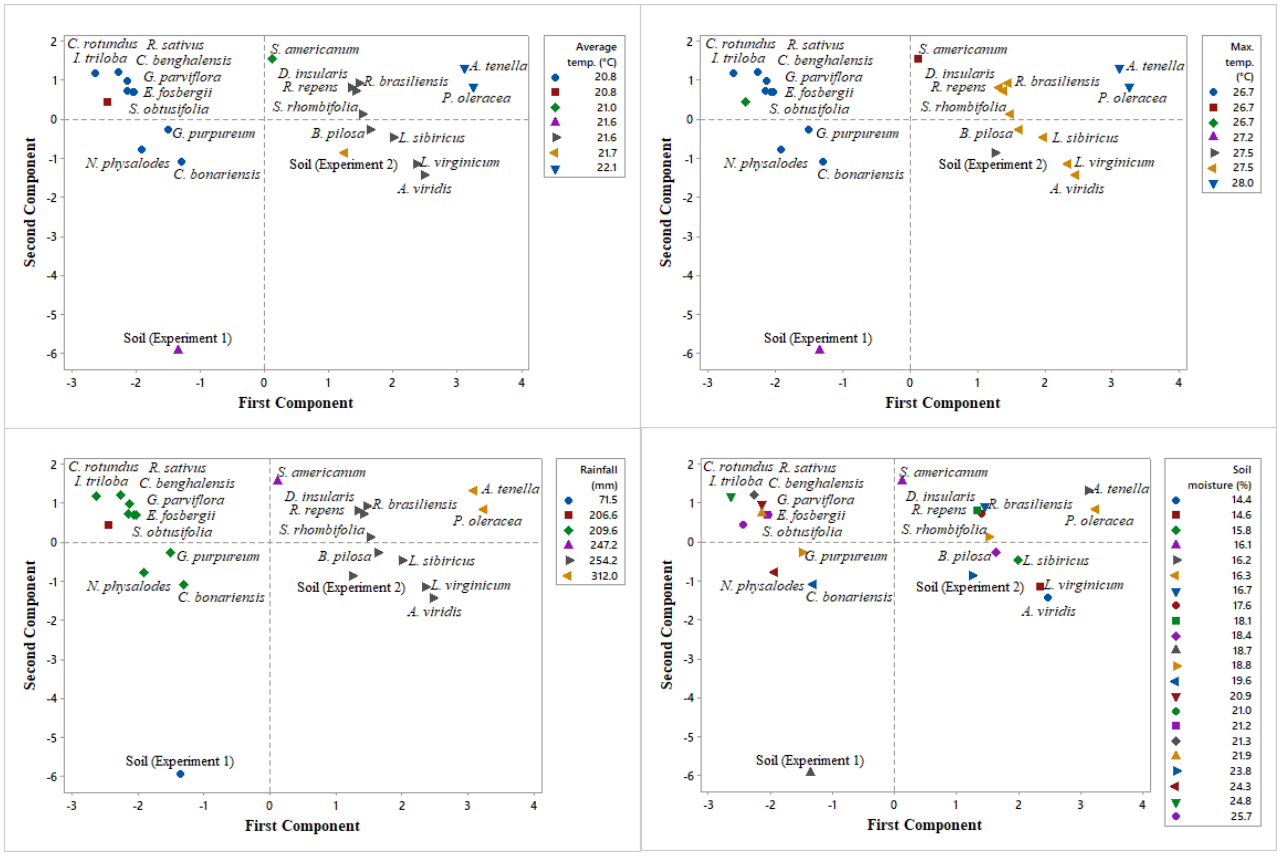


Figure 3 Principal component analysis (PCA) of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* survival in the weeds rhizosphere on experiments 1 and 2. (a) Projection of vectors of traits: survival period, area under the curve (AUC), maximum, minimum and average temperature (°C), rainfall (mm) and relative humidity (%); (b) Biplot graph with dispersion of 21 weeds according to the principal components.



Supplemental Figure 4 Biplot graphics with dispersion of 21 weeds according to the principal components of experiments 1 and 2 of Curtobacterium flaccumfaciens pv. flaccumfaciens survival in the weed rhizosphere, grouped by: a) average temperature (°C); b) maximum temperature (°C); c) rainfall (mm); and d) soil moisture (%).

In Figure 4a, there is an inverse relationship between the soil moisture, Cff survival, and AUC vectors and the minimum, maximum, and average temperature vectors. In this case, two weed groups could be separated (Figure 4b): (a) high survival (56–70 days) of Cff in the rhizosphere, with average temperature varying from 18.9 to 19.7°C, soil moisture between 20.5% and 24.7%, and accumulated precipitation between 116 and 118.5 mm; and (b) lower survival (14–42 days), average temperatures varying from 24.4 to 25.6°C, soil moisture between 14.8% and 18.6%, and accumulated precipitation varying from 143 to 394 mm. The highest Cff survival

period in the soil (56 days) occurred due to the lower temperature (19.1°C), higher soil moisture (22%), and less rainfall (116 mm) recorded, while under higher temperature (25.1°C), lower soil moisture (17.4%), and higher precipitation (245.3 mm), Cff survival in the soil was lower (35 days) (Figure S5).

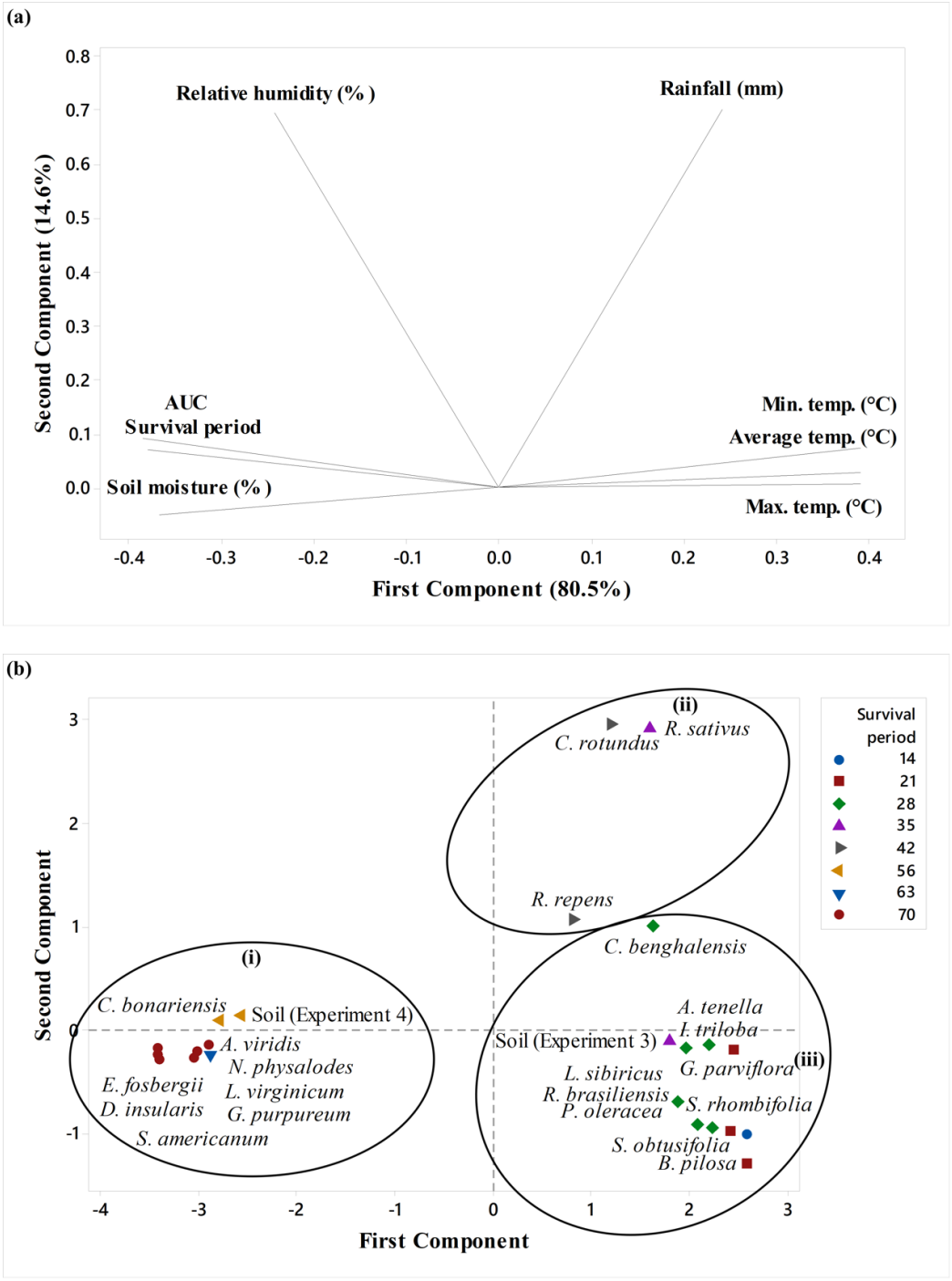
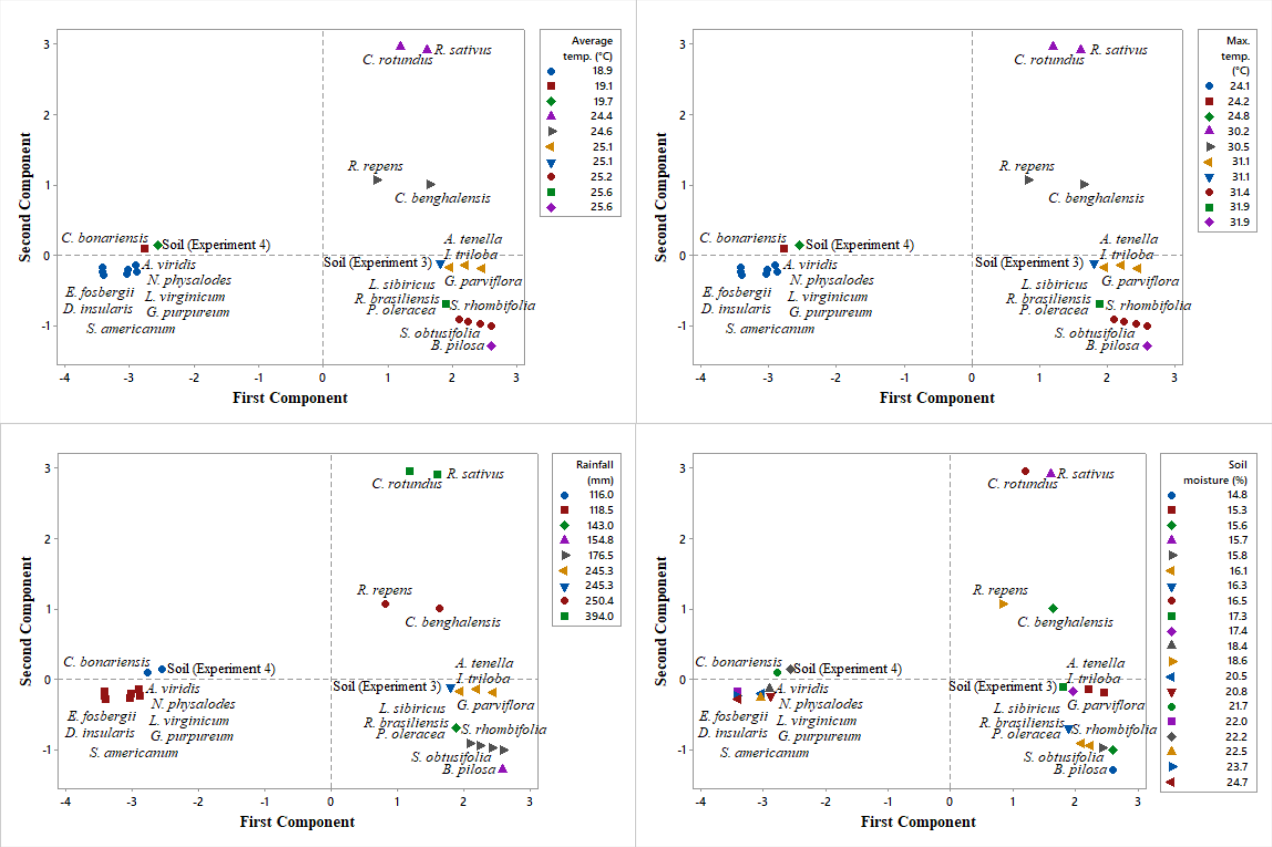


Figure 4 Principal component analysis (PCA) of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* survival in the weeds rhizosphere on experiments 3 and 4. (a) Projection of vectors of traits: survival period, area under the curve (AUC), maximum, minimum and average temperature (°C), rainfall (mm) and relative humidity (%); (b) Biplot graph with dispersion of 21 weeds according to the principal components.



Supplemental Figure 5 Biplot graphics with dispersion of 21 weeds according to the principal components of experiments 3 and 4 of Curtobacterium flaccumfaciens pv. flaccumfaciens survival in the weed rhizosphere, grouped by: a) average temperature (°C); b) maximum temperature (°C); c) rainfall (mm); and d) soil moisture (%).

1.4. Discussion

Knowledge of Cff survival niches is essential for bean bacterial wilt management, because the bacterium has already been reported to survive in soil, bean debris, and crops of Fabaceae, Poaceae, and Solanaceae families (Silva Júnior et al., 2012; Harveson et al., 2015; Gonçalves et al., 2017, 2018; Osdaghi et al., 2018). On weeds, only hairy vetch (*V. vilosa*) has been reported to date as a host of Cff (Osdaghi et al., 2015), with no information available about the Cff survival in the phyllosphere and rhizosphere of other weed species.

In our study, based on the PCA classification, *C. benghalensis*, *A. viridis*, *C. bonariensis*, *C. rotundus*, *D. insularis*, *E. fosbergii*, *Galinsoga parviflora*, *G. purpureum*, *Ipomoea triloba*, *L. virginicum*, *N. physalodes*, *R. sativus*, *S. americanus*, and *Senna obtusifolia* showed the highest average survival periods of Cff in the phyllosphere. PCA made it possible to study the interrelationships between climatic variations that occurred during the experiments and the survival period and AUC of Cff. The first two main components of the PCA explained between 95% and 97% of all the variability in the data set of the Cff survival experiments. Variable number elucidated by the main components of a PCA are essential to define the number of components to be maintained, which should correspond to at least 70% (Rencher and Christensen, 2002).

As demonstrated by the PCAs, abundant rainfall reduced the Cff survival period and AUC, being one of the factors associated with its low and intermediate survival in the phyllosphere of *A. viridis*, *D. insularis*, *L. virginicum*, and *S. americanum*. The occurrence of higher temperatures, in addition to abundant rainfall, reduced the Cff survival in the phyllosphere of *R. sativus*, *C. rotundus*, *C. ben- ghalensis*, *I. trilobal*, and *G. parviflora*. Intense rainfall may favour the survival of bacteria belonging to the *Xanthomonadaceae* family in the phyllosphere, while species of the *Microbacteriaceae* family, to which the genus *Curtobacterium* belongs, have their populations reduced, probably due to the removal of bacterial cells from the leaf surface (Hirano and Upper, 2000; Gent et al., 2005; Allard et al., 2020).

Some weeds showed a shorter Cff survival period in their phyllosphere, in both experiments. This may be related to the plant-specific leaf characteristics, as well as phenological stage, cultivation environment, geographical area, and climatic conditions (Kinkel, 1997; Hirano and Upper, 2000; Hunter et al., 2010; Vorholt et al., 2012; Vacher

et al., 2016; Leveau, 2018). In our study, we pointed out climatic conditions as one of the possible factors that affect Cff survival. The PCA and the climatic data showed that Cff survived for shorter periods in the phyllosphere in the experiments with higher temperatures and precipitation.

The microbial community can inhibit the development and maintenance of high populations of bacteria in the soil (Mendes et al., 2011; Berendsen et al., 2012). However, Cff can survive for long periods in the soil (Gonçalves et al., 2018). The rhizosphere of most evaluated weeds increased the Cff survival period compared to weedless soil. The PCA showed the highest survival in the rhizosphere of *Alternanthera tenella*, *Amaranthus viridis*, *C. benghalensis*, *C. bonariensis*, *C. rotundus*, *D. insularis*, *E. fosbergii*, *G. parviflora*, *G. purpureum*, *I. triloba*, *L. virginicum*, *N. physalodes*, *P. oleracea*, *R. brasiliensis*, *R. repens*, *R. sativus*, *S. americanum*, *S. obtusifolia*, and *S. rhombifolia*.

The rhizosphere is a favourable environment for the survival of bacterial populations because it protects cells from rapid and drastic climatic variations, and for *Curtobacterium* spp., there are reports that exudates released by the roots can inhibit or stimulate the development of species of the genus. The fact that plants select bacterial populations associated with their rhizosphere may explain the variation in survival periods obtained in the rhizosphere of different weeds (Bennet and Lynch, 1981; Brencic and Winans, 2005; Wu et al., 2015). On the basis of the PCA, the occurrence of higher temperatures reduced the Cff survival periods in the weed rhizosphere. It is known that the survivability of Cff is reduced in soil at higher temperatures (Silva Júnior et al., 2012).

The common bean has a growing season of 60–120 days (Myers and Kmiecik, 2017). In some weed species, Cff can survive for 70 days in the phyllosphere or rhizosphere. In the soil, this period lasts up to 56 days. The survival of Cff in weeds, even for 7 days, may be sufficient for the bacteria to be transmitted to the common bean. Successive planting of beans can contribute to the maintenance of the disease inoculum in the field. Likewise, weeds, as well as soil, can act as inoculum source throughout the crop cycle.

With the PCA, it was possible to understand how the climatic variables influenced the Cff survival in the phyllosphere and rhizosphere of weeds. In the phyllosphere of some weeds, survival was reduced by accumulated precipitation and higher temperature, while in the rhizosphere, low Cff survival may be due to higher temperatures. The PCA allowed the clustering of evaluated weeds into groups, based on their Cff survival periods. Thus, *A. viridis* (family Amaranthaceae), *C. bonariensis*, *E. fosbergii*, *G. parviflora*, *G. purpureum* (Asteraceae), *R. sativus*, *L. virginicum* (Brassicaceae), *C. benghalensis* (Commelinaceae), *I. triloba* (Convolvulaceae), *C. rotundus* (Cyperaceae), *S. obtusifolia* (Fabaceae), *D. insularis* (Poaceae), *N. physalodes*, and *S. americanum* (Solanaceae) had the longest Cff survival periods in the phyllosphere and rhizosphere. Their eradication in common bean fields is recommended, especially in fields with a history of occurrence of bacterial wilt.

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Data availability statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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CAPÍTULO 2

SURVIVAL OF *Curtobacterium flaccumfaciens* PV. *flaccumfaciens* IN THE PHYLLOSPHERE AND RHIZOSPHERE OF CROPS

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Abstract

Knowledge of the ecological survival niches of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* (Cff), the causal agent of bean bacterial wilt, is essential for the efficient disease management. Our study evaluated the survival of Cff in the phyllospheres and rhizospheres of barley, black oat, canola, common bean, forage turnip, maize, pearl millet, ryegrass, sorghum, soybean, sunflower, velvet bean, wheat and white oat planted in a greenhouse. The aerial parts of the plants were inoculated by spraying a bacterial suspension (10^7 CFU.mL-1) from the strain Feij. 2628A, which is resistant to rifampicin and pathogenic to common bean. The soil of the pots was infested with 200 mL of the same suspension. Cff survival was evaluated every seven days for 70 days, and the survival periods were confirmed by selecting strains from all samples and performing PCR with specific primers. Cff survived for at least seven days in the phyllosphere, and 21 days in the rhizosphere of all evaluated crops. Based on our results, barley, black oat, canola, forage turnip, maize, pearl millet, ryegrass, sorghum, soybean, sunflower, velvet bean, wheat and white oat can be potential asymptomatic hosts for Cff, and their cultivation in succession with common bean is not recommended in areas with a history of bacterial wilt occurrence.

Keywords: alternative host, bacterial wilt, common bean, crop rotation, disease management, ecology of phytobacteria.

2.1. Introduction

Common bean (*Phaseolus vulgaris* L.) is one of the main crops produced in the world, with a fundamental role in human nutrition, wide edaphoclimatic adaptation and ease of production (Myers and Kmiecik 2017). Bacterial wilt, caused by *Curtobacterium*

flaccumfaciens pv. *flaccumfaciens* (Cff) is an important disease of common bean, reducing its productivity by up to 90% (Wendland et al. 2016). The disease management is based on the use of healthy seeds, soil incorporation of crop residues, resistant cultivars and crop rotation. The selection of non-host crops is essential for the efficient disease management, mainly in areas with a history of bacterial wilt occurrence (Silva Júnior et al. 2012b; Harveson et al. 2015; Wendland et al. 2016).

For rotation with common bean, crops from the Fabaceae and Poaceae families are recommended, and many of these have already been identified as Cff hosts (Posse et al. 2010; Harveson et al. 2015). In Brazil, barley, black oat, white oat, canola, ryegrass and wheat have been reported as potential hosts of Cff, while wheat, maize, sunflower and alfalfa have been associated with an increased incidence of bacterial wilt in the United States (Harveson et al. 2015; Gonçalves et al. 2017). In addition, eggplant, pepper and tomatoes have already been reported as hosts of Cff (Osdaghi et al. 2018).

Although it is known that crops can serve as hosts for Cff, there is no information about Cff survival on pearl millet, forage turnip, velvet bean and sorghum. In addition, there are no studies on Cff ability to survive in the crops rhizosphere. Our study evaluated the population dynamics and the survival periods of Cff in the phyllosphere and rhizosphere of crops used in crop rotation with the common bean. This information is fundamental for more efficient bacterial wilt management.

2.2. Materials and methods

Bacterial strains, culture conditions and preservation

In all experiments, the strain Feij. 2628A of Cff, resistant to 100 µg mL^{-1} rifampicin and pathogenic to common bean, from the Plant Bacteriology Laboratory (FCA/UNESP) was used. The parental strain was isolated from bean cv. Pérola in Paranapanema, São Paulo, Brazil, in 1996. This strain was cultivated in nutrient sucrose agar culture medium (Schaad et al. 2001) supplement with rifampicin (NSAR), consisting of 20 g L^{-1} nutrient agar (NA; Merck), 5 g L^{-1} sucrose (Synth) and 100 ug mL-1 rifampicin (Rifaldin), in order to select naturally occurring rifampicin resistant strains. The strain was incubated at 28 °C for 48 h. For long-term preservation, the strain was maintained at 30% glycerol at -80 °C.

Crops cultivation

Thirteen crops used in crop rotation with common bean in Brazil (Posse et al. 2010) (Table 1), were selected for Cff survival experiments. Seeds were commercially purchased and sowed on 2 L pots (17.8 cm in diameter) containing organo-mineral substrate (Tropstrato HT, 1:1:1), supplemented with ammonium sulfate (0.6 kg m-3), simple superphosphate (1.7 kg m-3), potassium chloride (0.6 kg m-3) and dolomitic limestone (0.8 kg m-3). The plants were kept in a plastic tunnel, watered when needed and periodically monitored for the appearance of disease symptoms after inoculation.

Table 1 Crops used in survival experiments of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* in the phyllosphere and rhizosphere

Botanical family	Scientific name	Common name	Cultivars	Company	Experiment
Asteraceae	*Helianthus annus*	Sunflower	IAC[a] Iarama	IAC	1, 2
Brassicaceae	*Raphanus sativus*	Forage turnip	IPR 116	IAPAR[b]	1, 2
	Brassica napus	Canola	Hyola 61	Embrapa	3, 4
Fabaceae	*Glycine max*	Soybean	M6410 IPRO	Monsoy	1, 2
	Mucuna pruriens	Velvet bean	-	BRSEEDS	3, 4
	Phaseolus vulgaris	Common bean	IPR Campos Gerais	IAPAR	1, 2, 3, 4
Poaceae	*Avena strigosa*	Black oat	Embrapa 29	Embrapa	1, 2
	Avena sativa	White oat	IPR 126	IAPAR	3, 4
	Hordeum vulgare	Barley	BRS Cauê	Embrapa	3, 4
	Lolium multiflorum	Ryegrass	Barjumbo	Barenbrug	3, 4
	Pennisetum glaucum	Pearl millet	BRS 1501	Embrapa	1, 2
	Sorghum bicolor	Sorghum	Al Precioso	BRSEEDS	3, 4
	Triticum aestivum	Wheat	BRS 374	Embrapa	3, 4
	Zea mays	Maize	IAC 8390	IAC	1, 2

[a]Instituto Agronômico de Campinas; [b]Instituto Agronômico do Paraná.

Under high temperatures, the soil was irrigated everyday. However, on days with mild temperatures, it was irrigated every other day. All irrigations were carried out preserving the aerial part of the plants from spraying

Inoculation experiments and sampling

Four experiments were installed on 17 October 2017 (Experiment 1), 28 February 2018 (Experiment 2), 18 June 2019 (Experiment 3) and 10 October 2019 (Experiment 4), in a plastic tunnel. Crops were inoculated 30 days after sowing (DAS). The aerial parts of the plants were inoculated with a bacterial suspension (107 CFU mL-1) by sprinkling with the aid of a manual sprayer (Brudden Practical, Brudden Equipamentos) until the point of run-off. The soil of each pot was infested with 200 mL of the same bacterial suspension. Samplings were carried out every seven days, for up to 70 days, starting right after inoculation, to determine the initial Cff population in

the phyllosphere and rhizosphere. For sampling, three plants per species were removed from the pots at each evaluation, and the leaves and rhizosphere soil were transferred separately to plastic bags for homogenisation. Rhizosphere soil samples were collected with a PVC pipe (6 mm), up to 5 cm deep (Silva Júnior et al., 2020).

Sample processing

To determine the Cff population in the phyllosphere and rhizosphere, 5 g of leaves, sampled at random, and 10 g of rhizosphere soil were used. Samples were transferred to Duran flasks containing 100 mL of autoclaved water. The flasks were shaken for 30 min at 300 rpm, and then, rhizosphere samples remained at rest for another 30 min, for sedimentation. The suspensions were serial diluted (10^0 to 10^{-4}) and 100 µL of each were plated in duplicate on NSAR medium supplement with chlorothalonil (50 µg mL-1) and thiophanate methyl (50 µg mL-1), followed by incubation (28 °C for 96 h). The colonies that were morphologically identical to Cff were quantified.

Strains characterisation

For survival period confirmation, colonies morphologically identical to Cff were selected from all treatments and purified to NSA with 7% sodium chloride, followed by incubation (28 °C for 96 h) (Dye and Kemp, 1997; Maringoni and Camara, 2006). Isolates were identified by PCR, using the specific primers for Cff: CffFOR2 (5′- GTT ATG ACT GAA CTT CAC TCC -3′) and CffREV4 (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^{-1} and heated to 95 °C (15 min).

Each PCR reaction was carried out in a total volume of 25 µL, containing 12.5 µL GoTaq Green Master Mix (Promega), 0.5 µL of each primer, 8.5 µL of MilliQ water and 3 µL of DNA. The PCR was performed in a Mastercycler Gradient model thermocycler (Eppendorf) using the following PCR program: 94 °C (3 min), followed by 30 cycles of 94°C (1 min), 60°C (45 s) and 72°C (30 s). The reactions were incubated for 10 min at 72 °C. The amplified samples were run in a horizontal electrophoresis an agarose gel (1%) with 1X TBE buffer, using ethidium bromide (Sigma) (4 µL/120 mL). The gels were visualised and recorded on the BioDoc-It Imaging System for gel documentation (UVP).

Climate data

The climatic data of the experiments were collected daily, with a digital thermo-hygrometer (PD003, Tomate). Soil moisture was measured in all evaluations, and in all treatments, on a scale with an infrared moisture analyser (Gehaka, Model IV 2500). From the data, weekly averages were established, according to the Cff survival evaluated periods. The average weather conditions during the study period were: average temperature 19.6-29.4 °C and relative humidity 33-37% in Experiment 1; average temperature 16.7-29.6 °C and relative humidity 27-55% in Experiment 2; average temperature 19.7-27.5 °C and relative humidity 25-46 in Experiment 3 and average temperature 26.6-38.9 °C and relative humidity 10-42% in Experiment 4. (Supplemental Table 1).

Supplemental Table 1 Average temperature, relative humidity and soil moisture occurring during the survival experiments of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* in the phyllosphere and rhizosphere

Period	Average temp. (°C)	Relative humidity (%)	Soil moisture (%)	Period	Average temp. (°C)	Relative humidity (%)	Soil moisture (%)
Experiment 1				**Experiment 3**			
0	29.4	37.0	15.5	0	29.6	27.0	23.1
0-7	24.3	36.0	14.2	0-7	23.6	35.7	25.7
8-14	21.6	36.4	15.8	8-14	20.9	28.3	25.8
15-21	19.6	33.0	16.2	15-21	20.9	51.4	16.1
22-28	24.6	35.3	15.5	22-28	27.5	39.7	21.6
29-35	25.3	33.1	17.9	29-35	16.9	37.7	16.5
36-42	24.1	35.1	14.1	36-42	16.7	50.7	22.5
43-49	24.1	35.1	16.5	43-49	21.6	52.1	19.9
50-56	23.4	34.9	17.0	50-56	27.9	35.0	23.7
57-63	23.2	34.7	17.0	57-63	26.2	30.7	12.9
64-70	23.5	34.5	16.4	64-70	24.1	55.9	19.5
Average	23.9	35.0	16.0	**Average**	23.2	40.38	20.6
Experiment 2				**Experiment 4**			
0	24.0	37.0	18.9	0	38.9	10.0	23.3
0-7	27.3	44.4	18.9	0-7	32.3	23.7	21.4
8-14	27.5	46.9	10.8	8-14	27.7	41.0	17.6
15-21	27.1	38.1	11.7	15-21	35.6	23.6	21.3
22-28	23.5	34.9	15.1	22-28	31.4	33.9	17.8
29-35	22.8	36.4	16.4	29-35	31.0	42.7	21.9
36-42	21.9	33.7	19.3	36-42	26.6	33.1	11.9
43-49	20.8	35.3	16.8	**Average**	31.9	29.7	19.3
50-56	19.7	25.9	19.3				
57-63	22.1	29.9	17.3				
64-70	21.4	31.9	19.4				
Average	23.4	35.0	16.7				

Experimental design and statistical analysis

The experimental design was a randomised block, with the treatment number varying between experiments: 15 treatments in experiments 1 and 2 (7 crops × 2 survival niches, phyllosphere and rhizosphere, with pots containing soil without plants as negative control—NC) and 17 treatments in experiments 3 and 4 (8 crops × 2 survival niches + NC). For phyllosphere survival, common bean was used as a positive control. Pots containing only soil with bacterial infestation were used as a negative control for the rhizosphere. Each experimental plot was represented by one pot, containing three plants, in a total of eight replicates.

Cff survival periods for each experiment were determined and the average survival periods calculated. The population data for each evaluation period were quantified and transformed into the log^{10} of the number of CFU g^{-1} of plant tissue (phyllosphere) and CFU g^{-1} of dry soil (rhizosphere).

Based on these data, the area under the curve (AUC) was calculated by Minitab 17 Statistical Software. A second polynomial regression model degree was adjusted for the Cff population in each crop, by SigmaPlot Software 14.0. The survival period, AUC and average temperature was correlated through principal component analysis (PCA), performed using Minitab 17 Statistical Software.

2.3. Results

The Cff population curves in the phyllosphere and rhizosphere of crops are shown in Figures 1 and 2. Except for ryegrass, barley and sorghum, the initial population of Cff in the phyllosphere was higher than that of the rhizosphere in the other crops (Figure 1 and 2).

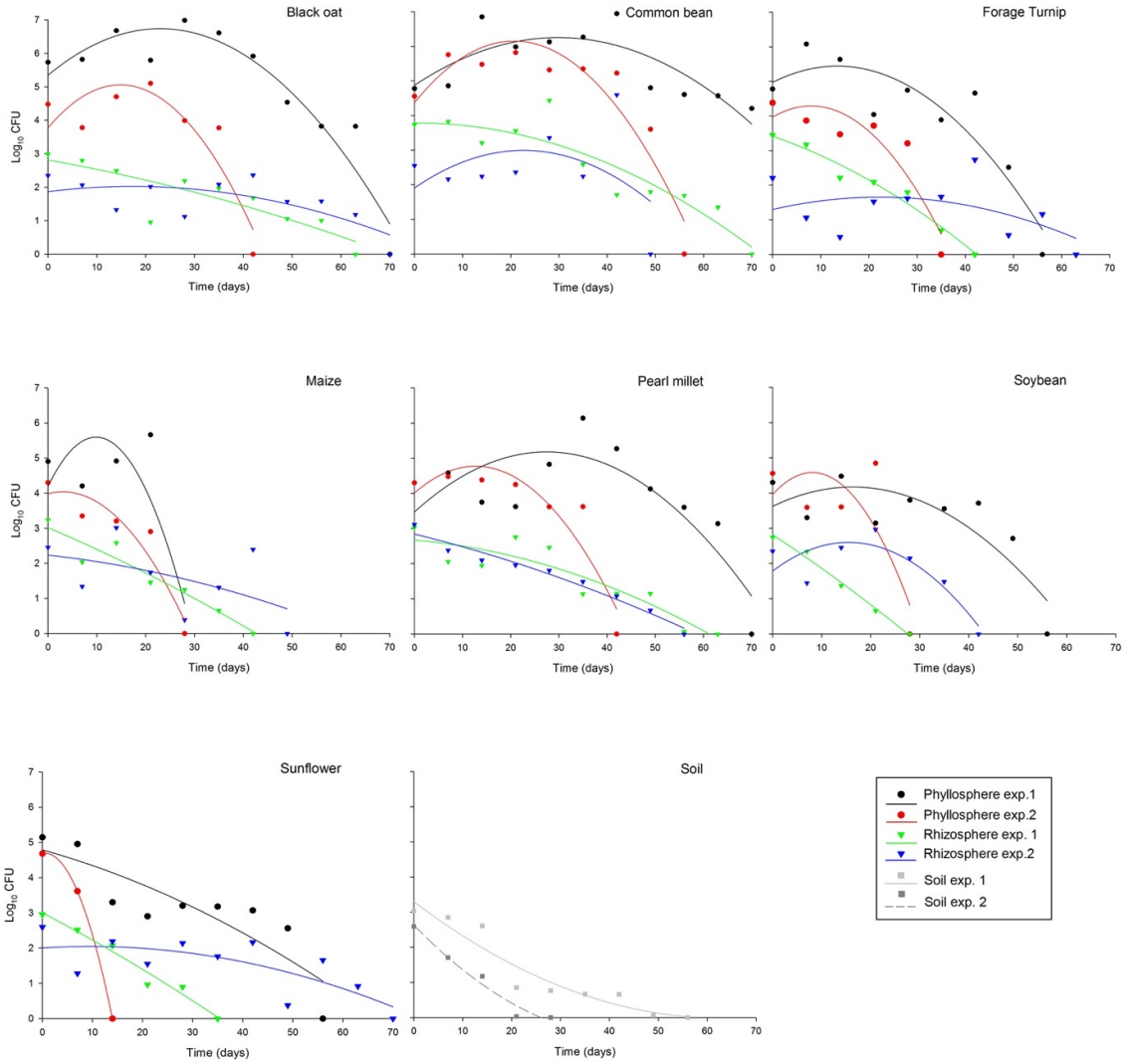

Fig. 1 Survival of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* in the phyllosphere and rhizosphere of seven crops and in the soil as a function of time, in experiments 1 and 2

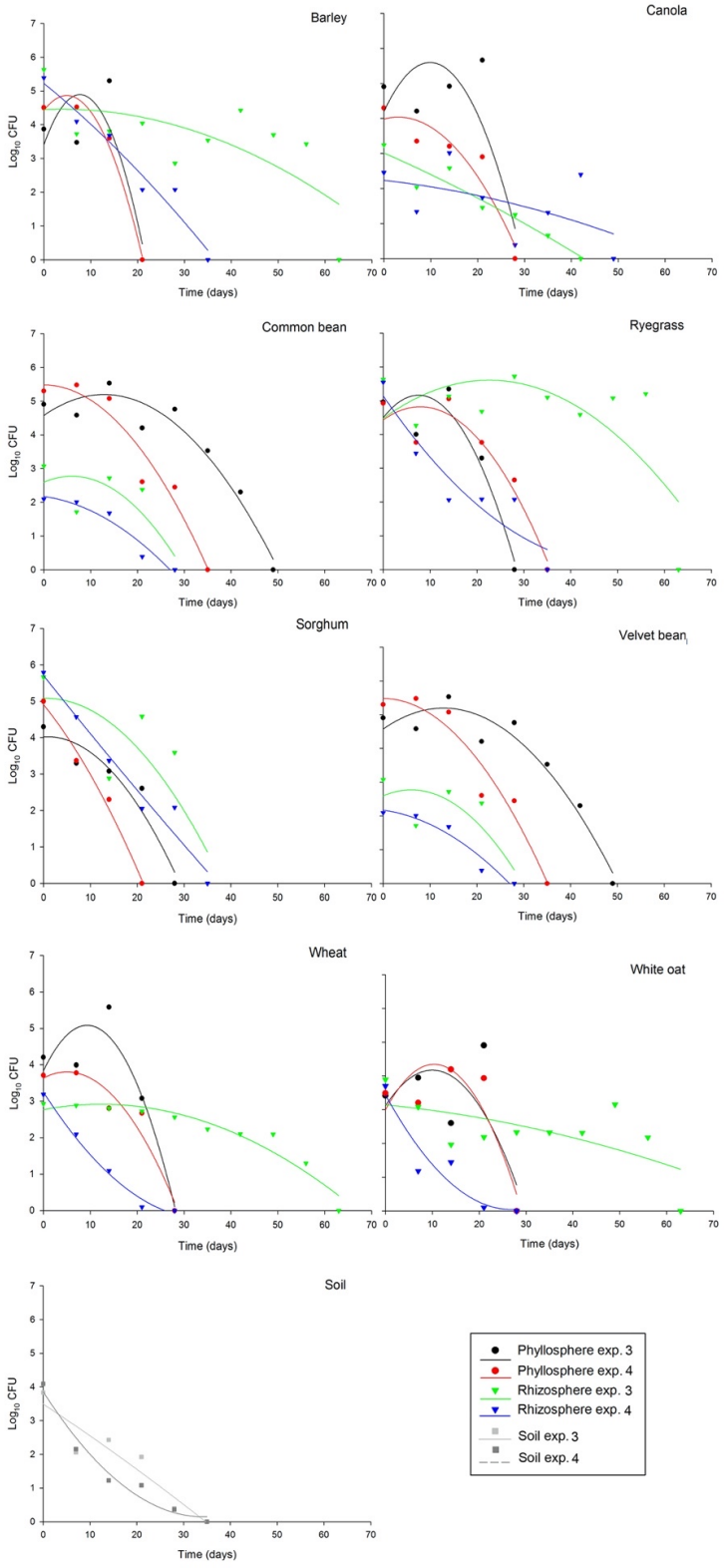

Fig. 2 Survival of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* in the phyllosphere and rhizosphere of eight crops and in the soil as a function of time, in experiments 3 and 4

Cff survival in the crops phyllosphere

In the phyllosphere, the highest survival period was observed in common bean (70 days), followed by black oat and pearl millet (63 days) (Table 2). In forage turnip, soybean and sunflower, Cff survived a maximum for 49 days, 42 days in velvet bean and 28 days in ryegrass (Table 2). In the other crops, barley, canola, maize, sorghum, wheat and white oat, the survival period varied between 14 to 21 days (Table 2). In common bean, the main host of Cff, the survival period varied between 28 to 70 days. Regarding the area under the curve (AUC) of Cff in the phyllosphere, the highest values occurred in common bean (388), followed by black oat (356) and pearl millet (277) (Table 2).

Table 2 Survival periods and area under the curve (AUC) of Curtobacterium flaccumfaciens pv. flaccumfaciens in the phyllosphere of crops.

Species	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Black oat	63[a] (356)[b]	35 (151)	-	-
Pearl millet	63 (277)	35 (144)	-	-
Common bean	70 (388)	49 (259)	42 (176)	28 (134)
Forage turnip	49 (228)	28 (103)	-	-
Velvet bean	-	-	42 (189)	28 (119)
Soybean	49 (178)	21 (83)	-	-
Sunflower	49 (171)	7 (29)	-	-
Ryegrass	-	-	21 (94)	28 (114)
White oat	-	-	21 (74)	21 (77)
Maize	21 (100)	21 (71)	-	-
Wheat	-	-	21 (92)	21 (68)
Sorghum	-	-	21 (68)	14 (49)
Canola	-	-	14 (46)	14 (59)
Barley	-	-	14 (56)	14 (60)

[a]Survival period (days); [b]Area under the curve (AUC) of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*

The PCAs for the Cff survival in the phyllosphere (Figure 3a; Figure 4a) showed that the survival periods and AUC are positively correlated, but these vectors (lines) are not correlated with the average temperature.

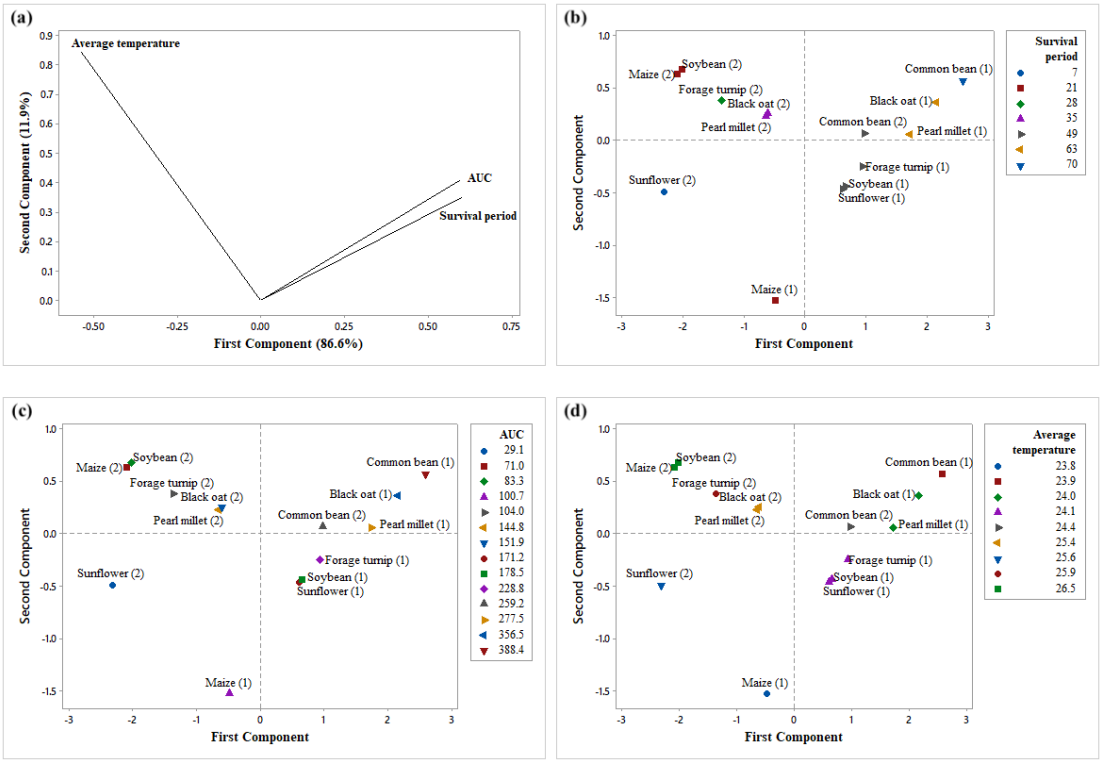

Fig. 3 Principal component analysis (PCA) of Curtobacterium flaccumfaciens pv. flaccumfaciens survival in the crops phyllosphere (Experiments 1 and 2). (a) Projection of vectors of traits: survival period, area under the curve (AUC) and average temperature (°C). Biplot graphics with dispersion of the crops according to the principal components, grouped by: (b) survival periods; (c) AUC; (d) average temperature (°C).

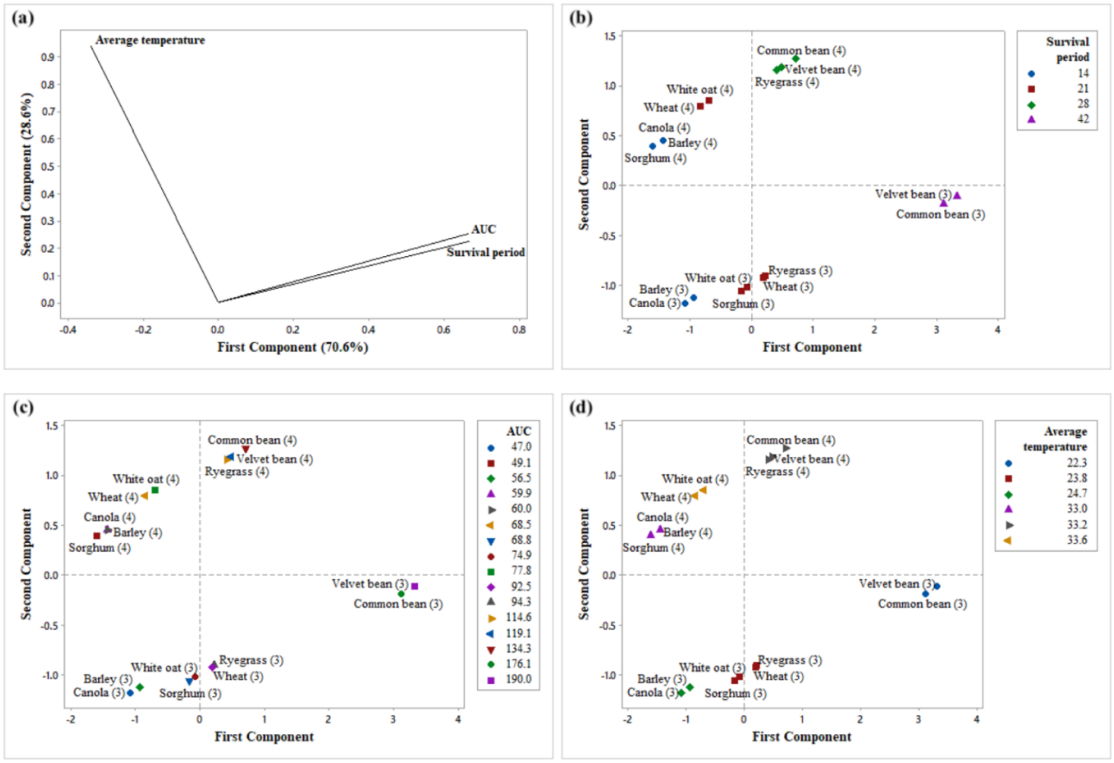

Fig. 4 Principal component analysis (PCA) of Curtobacterium flaccumfaciens pv. flaccumfaciens survival in the crops phyllosphere (Experiments 3 and 4). (a) Projection of vectors of traits: survival period, area under the curve (AUC) and average temperature (°C). Biplot graphics with dispersion of the crops according to the principal components, grouped by: (b) survival periods; (c) AUC; (d) average temperature (°C).

It was possible to observe in the dispersion graph that, with the exception of common bean, the other crops evaluated in experiment 2, are in a temperature range between 25.4 °C to 26.5 °C, with a shorter Cff survival period, ranging from 7 to 35 days (Figure 3b; Figure 3d). In experiment 1, with the exception of maize, we verified that for the other crops, the temperature varied between 23.9 °C to 24.1 °C, and Cff survival period was longer, between 49 to 70 days (Figure 3b; Figure 3d). Therefore, for soybean, forage turnip, black oat, pearl millet and sunflower, it can be inferred that the Cff survival in the phyllosphere is negatively affected by the increase in temperature

(Figure 3b; Figure 3d). In the maize phyllosphere, Cff survived for 21 days in both experiments, at 23.8 °C and 26.5 °C.

The temperature range was more contrasting between experiments 3 and 4, varying between 22.3 °C to 24.7 °C in experiment 3, and between 33.0 °C to 33.6 °C in experiment 4 (Figure 4d). However, in most cultures evaluated, Cff survived for similar periods in the phyllosphere, regardless of temperature (Figure 4b; Figure 4d). In common bean and velvet bean, longer survival periods (42 days) were observed at a lower temperature (22.3 °C).

Among the four experiments, Cff survived in common bean, the main host, for a longer period in the phyllosphere (70 days) under an average temperature of 23.9 °C (Experiment 1), and a shorter survival period (28 days) was verified at 33.2 °C (Experiment 4).

Cff survival in the crops rhizosphere

Highest Cff survival period (63 days) was verified in the rhizosphere of black oat, sunflower and common bean, followed by the rhizosphere of pearl millet, forage turnip, ryegrass, barley, white oat and wheat (56 days) (Table 3). For the other crops (maize, velvet bean, sorghum, soybean and canola), the Cff survival period in the rhizosphere varied between 21 to 49 days. In the soil, Cff survived for a minimum of 21 days, and a maximum for 49 days. Only the black oat and pearl millet rhizosphere had a longer Cff survival period than in the soil, in both experiments. Regarding the AUC, highest value was verified in the common bean rhizosphere (294).

Table 3 Survival periods and area under the curve (AUC) of Curtobacterium flaccumfaciens pv. flaccumfaciens in the rhizosphere of crops and soil.

Species	Experiment 1	Experiment 2	Experiment 3	Experiment 4
Black oat	56[a] (218)[b]	63 (237)	-	-
Pearl millet	56 (211)	49 (186)	-	-
Sunflower	28 (108)	63 (230)	-	-
Forage turnip	35 (149)	56 (191)	-	-
Common bean	63 (294)	42 (194)	49 (192)	21 (79)
Ryegrass	-	-	56 (279)	28 (79)
Barley	-	-	28 (214)	56 (95)
White oat	-	-	56 (254)	21 (76)
Maize	35 (144)	42 (171)	-	-
Wheat	-	-	56 (250)	21 (79)
Velvet bean	-	-	49 (211)	21 (80)
Sorghum	-	-	28 (116)	28 (97)
Soybean	21 (81)	35 (154)	-	-
Canola	-	-	28 (120)	21 (69)
Soil	49 (167)	21 (71)	28 (115)	28 (102)

[a]Survival period (days); [b]Area under the curve (AUC) of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*

Survival period and AUC are again correlated each other, as in the phyllosphere experiment, but not correlated with the average temperature (Figure 5a). The dispersion graph showed that in the experiment 1, only in the pearl millet and common bean rhizosphere, Cff survived for longer period than experiment 2 (56 days and 63 days, respectively) (Figure 5b) In this case, the survival period decreased with increasing temperature (Figure 5d).

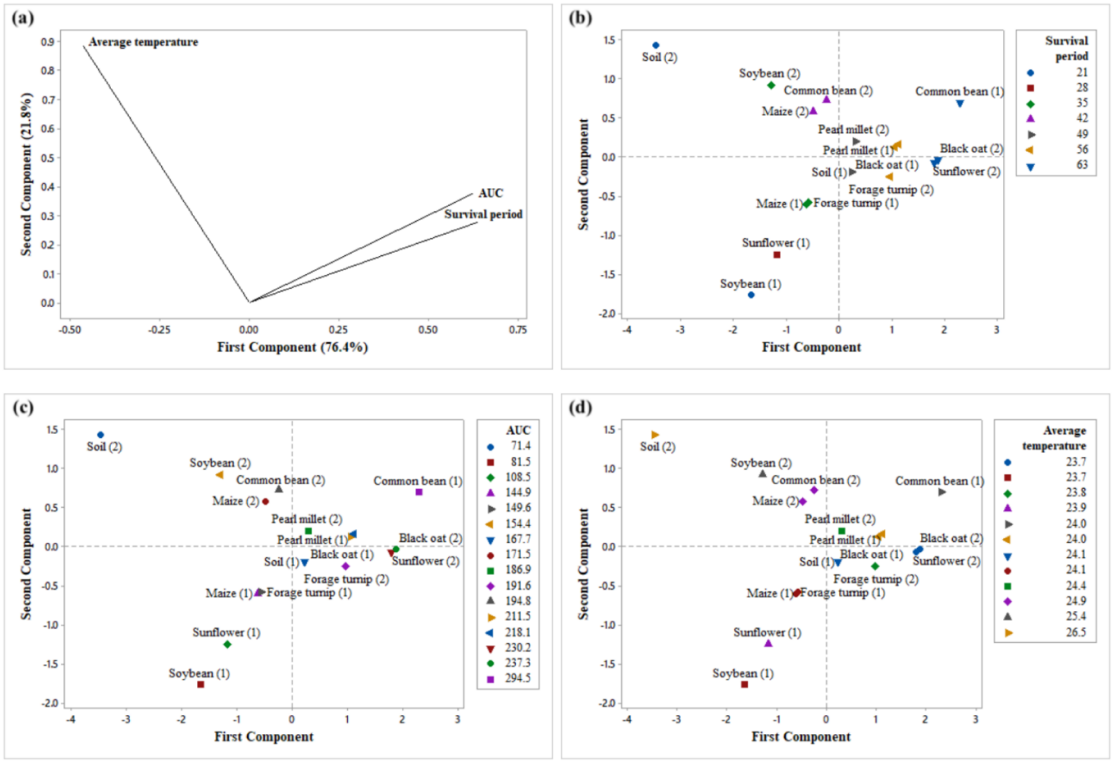

Fig. 5 Principal component analysis (PCA) of Curtobacterium flaccumfaciens pv. flaccumfaciens survival in the crops rhizosphere and soil (Experiments 1 and 2). (a) Projection of vectors of traits: survival period, area under the curve (AUC) and average temperature (°C). Biplot graphics with dispersion of the crops according to the principal components, grouped by: (b) survival periods; (c) AUC; (d) average temperature (°C).

For the other crops (black oat, sunflower, forage turnip, maize and soybean), highest survival period was observed in the experiment 2 (Figure 5b). Again, we observed that for black oat, sunflower and forage turnip, the increase in temperature negatively affected the Cff survival in the rhizosphere (Figure 5b; Figure 5d).

For the rhizosphere experiments 3 and 4, the PCA showed that the survival period, AUC and average temperature are not correlated with each other (Figure 6a).

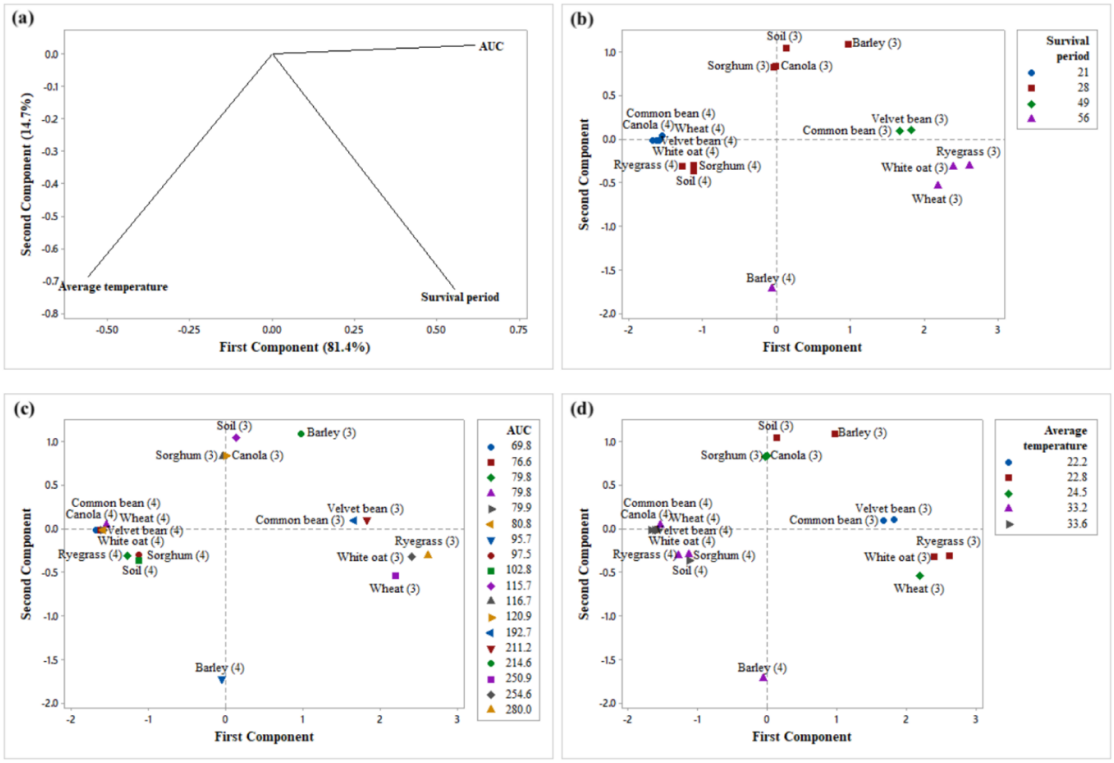

Fig. 6 Principal component analysis (PCA) of Curtobacterium flaccumfaciens pv. flaccumfaciens survival in the crops rhizosphere and soil (Experiments 3 and 4). (a) Projection of vectors of traits: survival period, area under the curve (AUC) and average temperature (°C). Biplot graphics with dispersion of the crops according to the principal components, grouped by: (b) survival periods; (c) AUC; (d) average temperature (°C).

In the rhizosphere of common bean, ryegrass, white oat, wheat, velvet bean and canola, the temperature increase negatively affected Cff survival. In experiment 3, where the rhizosphere of these cultures provided a longer survival period, compared to experiment 4, the temperature range remained between 22.2 °C to 24.5 °C (Figure 6b; Figure 6d). In experiment 4, the temperature range for these cultures was between 33.2 °C to 33.6 °C (Figure 6d). In the sorghum rhizosphere, the survival period was 28 days, regardless of temperature (24.5 °C or 33.2 °C). While in the barley rhizosphere, a longer survival period (56 days) was observed under a higher temperature (33.2 °C).

Longer Cff survival period (49 days) in the soil was verified in experiment 1 (24.1 °C), and shorter period (21 days) in experiment 2 (26.5 °C). However, as indicated by the PCAs, the temperature was not correlated with the survival of Cff in the soil. In experiment 4, the bacterium survived for 28 days, at 33.6 °C.

Characterisation of bacterial strains

All strains recovered from the phyllosphere and rhizosphere of the crops, in all experiments and evaluation periods, showed growth in NSA culture medium supplemented with sodium chloride and were confirmed by PCR, thus proving survival periods.

2.4. Discussion

Data obtained in this study showed that plants used in crop rotation with common bean have an important role as inoculum source of Cff. This bacterium survived for at least seven days in the phyllosphere and rhizosphere of barley, black oat, canola, forage turnip, maize, pearl millet, ryegrass, sorghum, soybean, sunflower, velvet bean, wheat and white oat.

Crop rotation is an efficient measure in the bacterial wilt management. Prior knowledge about the Cff survival in the crops used in rotation systems is necessary, preventing them from becoming a potential inoculum source in the field (Urrea and Harveson, 2014; Harveson et al. 2015).

In our study, longer Cff survival periods was observed in the phyllosphere of black oat and pearl millet, in addition to common bean, the main Cff host. Black oat

has already been reported as a host for Cff (Gonçalves et al. 2017). This is the first evidence that pearl millet may be a potential host of Cff, as well as forage turnip, velvet bean and sorghum. In barley, canola, maize, ryegrass, sunflower, wheat and white oat, Cff survival period varied from 14 to 49 days, but these crops have already been reported as alternative hosts (Silva Júnior et al. 2012a; Harveson et al. 2015; Gonçalves et al. 2017; Osdaghi et al. 2018). *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* no survived for more than 49 days in the soybean phyllosphere, and no symptoms were observed in the plant, although the bacterium has already been described as a causal agent of bacterial tan spot in the United States and in Brazil (Dunleavy, 1983; Soares et al. 2013).

Common bean developed necrosis on the leaves with a yellowish halo, but no symptoms of wilt. The other crops did not develop symptoms. Once as it was possible to recover Cff colonies from the phyllosphere of these crops, this indicates that epiphytic colonization had occurred. Cases in which bacterial asymptomatic dispersion occurs indicate that solely the lack of symptoms may not be enough to consider an area free of the pathogen (Marques and Samson, 2016). We identified potential asymptomatic hosts of Cff, that can be inoculum source for subsequent crops. White oat, pearl millet, forage turnip and maize have been previously described as asymptomatic hosts of Cff (Silva Júnior et al. 2012a).

Plants are able to select the microbial community in their leaves, where each species or genotype has particular leaf characteristics, making it possible to see distinct microbial communities in plant species that grow in the same place (Hunter et al. 2010; Vorholt, 2012; Vacher et al. 2016). Furthermore, the bacteria present in the phyllosphere need to adapt to the stresses to which they are subjected in this habitat. Exposures to ultraviolet radiation (UVR), high temperatures, low humidity and osmotic

stress that vary throughout the day can affect the survival of phytopathogenic bacteria (Vorholt, 2012).

High temperatures and rainfall reduced Cff survival in the weeds phyllosphere (Nascimento et al. 2020). We observed that for common bean, black oat, sunflower, pearl millet, forage turnip, soybean, velvet bean and sorghum, higher temperatures negatively affected Cff survival in the phyllosphere. This may be due to the harmful action of ultraviolet rays under the bacteria, which are exposed on the leaf surface (Vorholt, 2012). However, for maize, white oat, ryegrass, canola, barley and wheat, Cff survival in the phyllosphere is not negatively affected by temperature. The bacterium can even survive for up to 28 days at temperatures above 30 °C, in the phyllosphere of some crops.

High temperatures can also accelerate the growth of some plant species, which reach the end of their cycle more quickly, thus having less food availability for the bacteria. Therefore, it can be assumed that high temperatures not only affect bacteria directly, through sun exposure, but also indirectly.

Most studies on the ecology of phytopathogenic bacteria are dedicated only to the phyllosphere. In our work, we have shown that the rhizosphere of some crops may be a favourable niche for Cff survival. The exudates released by the roots stimulate the development of microorganisms in the rhizosphere region, in addition to being a more favourable environment for the survival of certain bacterial populations and for protecting cells from climatic fluctuations (Brencic and Winans, 2005; Wu et al. 2015). Bennet and Lynch (1981) found that *Curtobacterium* sp. survived in the rhizosphere of wheat and barley, but not in the maize rhizosphere, probably because the substrate

around the roots does not have a specific growth factor, required by the bacterium, or because some of the released compounds are inhibitor.

In this work we evaluated the temperature effect on Cff survival in the rhizosphere. In some weeds, high temperatures reduced the Cff survival in the rhizosphere (Nascimento et al., 2020). For black oat, sunflower, pearl millet, forage turnip, white oat, ryegrass, canola, velvet bean and wheat, shorter survival periods correspond to higher temperatures. However, for common bean, maize, soybean, barley and sorghum, the increase in temperature does not seem to negatively affect the Cff survival in the rhizosphere. The bacterium is even able to survive in temperatures above 30 °C, in the rhizosphere of white oat, ryegrass, canola, barley, velvet bean and sorghum.

Cff can survive for up to 70 days in the rhizosphere of weeds, demonstrating the potential for Cff survival in the rhizosphere when compared with soil (Nascimento et al., 2020). We observe that the rhizosphere of black oat and pearl millet showed a longer Cff survival period than in the soil, in both experiments. In other crops, it is not possible to confirm the effect of the rhizosphere on Cff survival, since in at least one of the experiments, survival periods similar or less than the soil were verified.

The survival period of Cff in the soil is variable and depends on the type of soil and climatic conditions; it is able to survive for prolonged periods under lower temperatures (20 °C) and soil with clayey texture (Silva Júnior et al. 2012b). At higher temperatures, the survivability of Cff is reduced (Silva Júnior et al., 2012b; Nascimento et al., 2020), but in our study the temperature didn’t affect the Cff survival in soil.

In conclusion, the ability of Cff to survive in bean crop debris (Silva Júnior et al. 2012b), some crops used in crop rotation with common beans (Harveson et al., 2015;

Gonçalves et al., 2017) and weeds (Nascimento et al., 2020) impairs bean bacterial wilt management.

By the methodology used in our work, the rhizosphere of most evaluated crops was favourable to Cff survival. However, there is still no information on the ability of Cff to survive associated with the rhizosphere of the crop in bean cultivation fields. Further studies should evaluate rhizosphere samples from plants grown in areas with the presence of Cff inoculum.

Based on the results obtained, it is not recommended to cultivate barley, black oat, canola, forage turnip, maize, pearl millet, ryegrass, sorghum, soybean, sunflower, velvet bean, wheat and white oat, in succession to common bean, in areas with a history of bacterial wilt occurrence.

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Compliance with ethical standards

Ethical statement

Authors declared that this manuscript has not published elsewhere. All authors read and approved the final version of this manuscript. The authors declare that the present work was developed without any potential conflict of interest, with no human or animal participants.

Conflict of interest

The authors declare that they have no conflict of interest.

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CAPÍTULO 3

SOBREVIVÊNCIA DE *Curtobacterium flaccumfaciens* PV. *flaccumfaciens* NO SOLO

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Resumo

O conhecimento dos nichos ecológicos de sobrevivência de *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, agente causal da murcha de curtobacterium no feijão, e da mancha bacteriana marrom na soja, é essencial para seu manejo adequado. Este estudo objetivou avaliar a sobrevivência dos isolados Feij.2628A, do feijão, Cff1, Cff2 e Cff4, da soja, no solo sob condições controladas e, posteriormente, foram avaliadas a sobrevivência dos isolados Feij.2628A e Cff1 em três diferentes tipos de solo, sob condições de campo. Os solos foram infestados com suspensão bacteriana (10^7 UFC.mL^{-1}) de cada um dos isolados e, para os experimentos sob condições controladas, foram incubados em BOD a 20 °C. A sobrevivência dos isolados de Cff foi avaliada a cada sete dias, até a sua não detecção. A identidade da bactéria foi confirmada por PCR com os iniciadores específicos CffFOR2 e CffREV4, a partir de isolados recuperados de todas as amostras. Sob condições controladas, o período de sobrevivência do isolado Feij.2628A variou entre 140 a 154 dias e entre 77

a 119 dias para os isolados da soja, Cff1, Cff2 e Cff4. Em condições de campo, o período de sobrevivência dos isolados Feij.2628A e Cff1 variaram entre 14 a 91 dias, nos diferentes tipos de solo. Em alguns tratamentos, a sobrevivência de Cff foi influenciada negativamente por temperaturas altas, baixa umidade do solo e precipitação. Esses resultados demonstram o potencial de sobrevivência dos isolados de Cff do feijão e da soja no solo, tornando se fontes de inoculo para os cultivos posteriores.

Palavras-chave: Murcha de Curtobacterium. Mancha bacteriana marrom. Ecologia de fitobactérias. Feijão. Soja.

Abstract

Knowledge of the ecological survival niches of *Curtobacterium flaccumfaciens* pv. *flaccumfaciens*, causal agent of bacterial wilt in beans, and bacterial tan spot in soybeans, is essential for its proper management. This study aimed to evaluate the survival of isolates Feij.2628A, from beans, Cff1, Cff2 and Cff4, from soybeans, in the soil under controlled conditions and, subsequently, the survival of isolates Feij.2628A and Cff1 in three different types of soil were evaluated, under field conditions. The soils were infested with bacterial suspension (^{177} CFU.mL^{-1}) from each of the isolates and, for experiments under controlled conditions, were incubated in BOD at 20 °C. The survival of Cff isolates was assessed every seven days, until they were not detected. The identity of the bacterium was confirmed by PCR with the specific primers CffFOR2 and CffREV4, from isolates recovered from all samples. Under controlled conditions, the survival period of isolate Feij.2628A varied between 140 to 154 days and between 77 to 119 days for soybean isolates, Cff1, Cff2 and Cff4. In field conditions, the survival

period of Feij.2628A and Cff1 isolates ranged from 14 to 91 days, in different soil types. In some treatments, Cff survival was negatively influenced by high temperatures, low soil moisture and precipitation. These results demonstrate the potential for survival of Cff isolates from beans and soybeans in the soil, becoming sources of inoculum for later crops.

Keywords: Bacterial wilt. Bacterial tan spot. Bean. Phytobacteria ecology. Soybean.

3.1. INTRODUÇÃO

Curtobacterium flaccumfaciens pv. *flaccumfaciens* (Cff) é um dos principais patógenos do feijoeiro, causando a murcha de curtobacterium e, também relatado ocorrendo em soja, sendo a doença denominada nessa cultura como mancha bacteriana marrom (DUNLEAVY, 1983; HARVESON et al. 2015). A sobrevivência de Cff na entressafra pode ocorrer em restos culturais de feijoeiro, hospedeiros alternativos e poucos estudos demonstraram seu potencial de sobrevivência no solo (SILVA JÚNIOR et al. 2012; GONÇALVES et al. 2017; GONÇALVES et al. 2018).

Um dos principais modos de introdução e disseminação de Cff em lavouras de feijoeiro é através do uso de sementes infectadas (SAETTLER; PERRY, 1972; CAMARA et al., 2009). Contudo, relatos sugerem que Cff possa sobreviver no solo, sendo este uma possível fonte de inóculo em áreas que empregaram sementes certificadas (BURKE, 1957). No caso da soja, a taxa de transmissão de Cff da planta para a semente é baixa e, até então, as safras anteriores, são as mais prováveis fontes de inóculo para esta cultura (SOARES et al. 2018). É possível que, assim como verificado para isolados do feijão (SILVA JÚNIOR et al. 2012; GONÇALVES et al. 2018), o isolado da soja também possa sobreviver no solo.

O conhecimento dos nichos de sobrevivência de Cff pode auxiliar no manejo da doença no feijoeiro e na soja. Devido a escassez dessas informações, principalmente relacionadas ao isolado da soja, o presente trabalho objetivou: a) avaliar a sobrevivência de isolados de feijão e soja no solo sob condições controladas; b) avaliar a sobrevivência de um isolado de cada cultura, em três diferentes tipos de solo, sob condições de campo, correlacionando com os dados climáticos coletados durante os experimentos.

3.2. MATERIAL E MÉTODOS

Isolados bacterianos, condições de cultivo e preservação

Os isolados bacterianos utilizados foram Feij. 2628A, proveniente do feijoeiro e obtido da coleção pertencente ao Laboratório de Bacteriologia Vegetal da Faculdade de Ciências Agronômicas – FCA/UNESP, e Cff1, Cff2 e Cff4, fornecidos pela Embrapa Soja. Os isolados bacterianos foram cultivados em meio NSAR (nutriente sacarose ágar) acrescido de rifampicina (Rifaldin). Para a preservação durante longos períodos, os isolados foram mantidos em 30% de glicerol (v/v) a -80 °C.

Instalação dos experimentos de sobrevivência de *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* em solo sob condições controladas

Foram conduzidos dois experimentos (Experimentos 1 e 2) em condições controladas, avaliando-se a sobrevivência dos quatro isolados no solo. O solo foi peneirado em peneira de malha 8 (2,8 mm de abertura) e seco em casa de vegetação durante sete dias. Após a secagem, copos de polietileno de 50 mL foram preenchidos com 50 g de solo. Posteriormente, o solo de cada copo foi infestado com 15 mL de suspensão bacteriana de 10^7 UFC mL^1 dos respectivos isolados, elevando-se a umidade para aproximadamente 50% da capacidade de campo. Os copos foram vedados com papel alumínio e incubados em BOD a 20 °C.

As avaliações foram realizadas a cada sete dias, até a não detecção de Cff e, na ocasião da instalação dos experimentos, para determinação da população inicial. Para a amostragem, foram coletados os solos de três copos por tratamento e homogeneizados em uma única amostra.

Instalação dos experimentos de sobrevivência de *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* em solo sob condições de campo

Os experimentos foram instalados em 05/08/2019 (Experimento 3), 11/10/2019 (Experimento 4) e 20/12/2019 (Experimento 5), empregando se dois isolados, Feij. 2628A e Cff1, e três diferentes tipos de solo. Os solos foram coletados em áreas agrícolas na Fazenda Lageado da FCA/UNESP, Botucatu/SP, peneirados em peneira de malha 8 (2,8 mm e abertura) e secos em casa de vegetação durante sete dias. Posteriormente, foram determinadas as propriedades físico-químicas, conforme

análises conduzidas pelo Departamento de Solos e Recursos Ambientais da FCA/UNESP (Tabela 1).

Tabela 1 - Propriedades físicas e químicas dos solos utilizados nos experimentos 3, 4 e 5

Solo	Local de coleta	Propriedades do solo					
		Matéria orgânica (g/dm^3)	pH ($CaCl_2$)	Textura	Argila (g/kg)	Silte (g/kg)	Areia total (g/kg)
A	Botucatu, SP	28	5	Argilosa	536	301	163
B	Botucatu, SP	18	5,9	Argilosa	583	330	87
C	Botucatu, SP	25	5,4	Média	161	57	782

Vasos de 1 L foram preenchidos com cada um dos solos, infestados com 180 mL de suspensão bacteriana de 10^7 UFC mL^1 dos respectivos isolados, elevando-se a umidade para aproximadamente 50% da capacidade de campo, e mantidos em condições de campo. As avaliações foram realizadas a cada sete dias, até a não detecção de Cff e, na ocasião da instalação dos experimentos, para determinação da população inicial. Para a amostragem, os solos de três vasos por tratamento foram processados separadamente.

Os dados climáticos foram coletados durante o transcorrer dos experimentos (temperatura mínima, máxima e média, precipitação e umidade relativa), obtidos em uma estação metereológica localizada a 300 m da área experimental.

Processamento das amostras

Para todos os experimentos, foram pesados 10 g de cada amostra de solo em fracos Duran (250 mL), adicionando-se 100 mL de água autoclavada. Os frascos foram agitados em mesa agitadora (300 rpm) por 30 minutos, e mais 30 min para

sedimentação. As suspensões obtidas foram diluídas em serie (10^0 a 10^4) e 100 µL plaqueados em duplicata em meio NSAR suplementado com rifampicina (100 µg/mL) e fungicidas (50 $\mu g.mL^{-1}$ de clorotalonil e 50 $\mu g.mL^{-1}$ de tiofanato metílico). As placas foram incubadas a 28 °C por 96 h e colônias com características morfológicas idênticas a Cff quantificadas.

Caracterização dos isolados bacterianos

Em cada período de avaliação, colônias com características semelhantes as de Cff foram selecionadas e purificadas em meio NSA com 7% de cloreto de sódio (MARINGONI; CAMARA, 2006) e caracterizadas por PCR empregando-se iniciadores específicos para Cff, CffFOR2 (5′- GTT ATG ACT GAA CTT CAC TCC -3′) e CffREV4 (5′- GAT GTT CCC GGT GTT CAG -3′) (TEGLI; SERENI; SURICO, 2002). O DNA total de cada isolado bacteriano foi extraído, por aquecimento a 90°C, por 15min ajustando-se uma suspensão a 10^8 CFU/mL, e rapidamente resfriada a 4 °C.

A reação de PCR foi realizada para um volume total de 12,5 µL, contendo 6,25 µL GoTaq Green Master Mix (Promega, USA), 0,25 µL de primer, 4,25 µL de água MilliQ e 1,5 µL de DNA. A desnaturação inicial foi realizada a 94 °C por 3 min., seguida de 30 ciclos de desnaturação a 94°C por 1min., anelamento a 60°C por 45s e extensão a 72°C por 30s; e polimerização final por 10 min a 72°C. Os amplicons obtidos foram submetidos a eletroforese horizontal e visualizados em gel de agarose a 1% corado com brometo de etídeo, sob transluminador com luz UV.

Delineamento experimental e análise estatística

O delineamento experimental foi inteiramente casualizado, com o número de tratamentos variando entre os experimentos: 4 tratamentos nos experimentos 1 e 2 (quatro isolados e um tipo de solo) e 6 tratamentos nos experimentos 3, 4 e 5 (dois isolados x três tipos de solo). Cada repetição foi composta por um vaso/copo.

Os dados de população bacteriana para cada período de avaliação foram quantificados e transformados em $\log_{10}$ do número de UFC.g^{-1} de $solo^{-1}$, e calculados, através do programa estatístico MiniTab 17, os coeficientes de correlação de Spearman entre a população bacteriana e umidade do solo, para os experimentos 1 e 2, sob condições controladas, e entre a população bacteriana e variáveis climáticas (temperatura máxima, mínima, média, precipitação e umidade do solo) para os experimentos 3, 4 e 5, sob condições de campo.

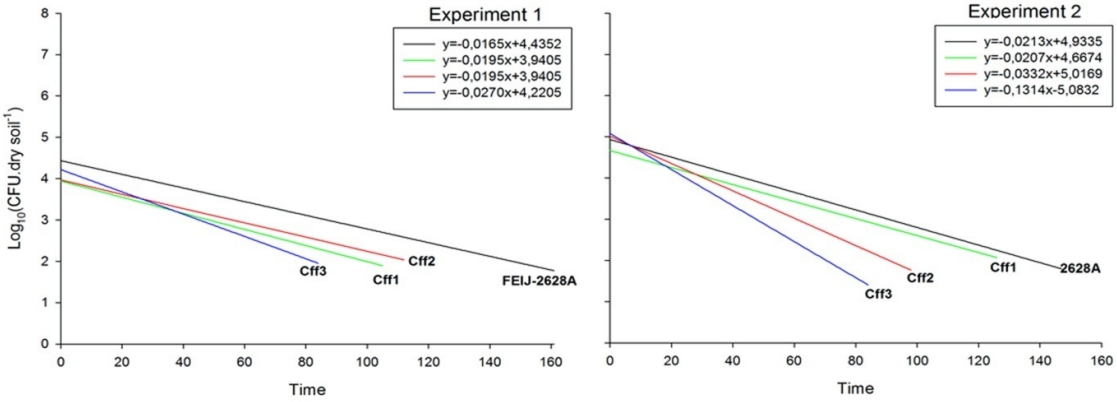
Um modelo de regressão polinomial de segundo grau foi ajustado para a população de Cff em função do tempo, para cada isolado, em todos os experimentos, pelo software SigmaPlot 14.0.

3.3. RESULTADOS

Sobrevivência de *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* em solo sob condições controladas

Maiores períodos de sobrevivência foram verificados para o isolado Feij. 2628A, que variaram entre 140 a 154 dias (Figura 1). Para os isolados Cff1, Cff2 e Cff4, esse período variou entre 77 a 119 dias (Figura 1).

Figura 1 - Sobrevivência dos isolados Feij. 2628A, Cff1, Cff2 e Cff4 em solo sob condições controladas, em função do tempo, nos experimentos 1 e 2



A análise de correlação mostrou que, com exceção do isolado Cff4 no experimento 1, maiores umidades de solo estão positivamente correlacionadas com a maior sobrevivência de Cff (Tabela 3). No entanto, Cff foi detectada no solo com um teor mínimo de 1,8% de umidade (dados não apresentados).

Tabela 2 - Análise de correlação (Spearman) entre a população de *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* e a umidade do solo, nos experimentos 1 e 2, sob condições controladas

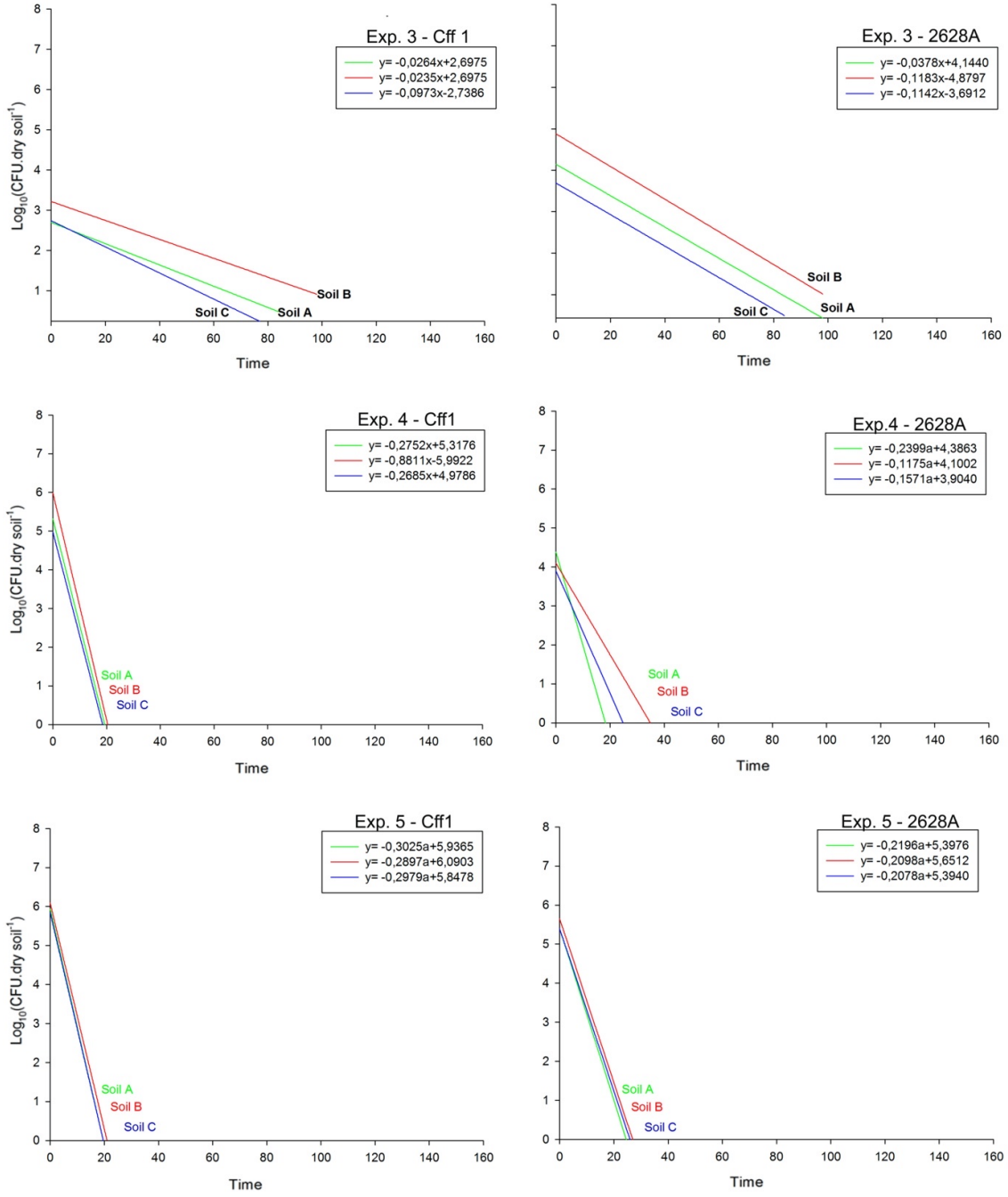
Isolado	Umidade do solo	
	Experimento 1	Experimento 2
2628A	0,630	0,836
Cff1	0,699	0,647
Cff2	0,609	0,611
Cff4	NS*	0,039

*p<0,05.; **NS: não significativo

Sobrevivência de *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* em solo sob condições de campo

Em condições de campo, o período de sobrevivência dos isolados de Cff variou entre os experimentos (Figura 2). No experimento 3, Feij.2628A e Cff1 sobreviveram entre 77 a 91 dias e, 70 a 91 dias, respectivamente. Nos experimentos 4 e 5, os períodos de sobrevivência variaram entre 14 a 28 dias, para ambos os isolados (Figura 2).

Figura 2 - Sobrevivência dos isolados Feij. 2628A e Cff1 em solo sob condições de campo, em função do tempo, nos experimentos 3, 4 e 5



No experimento 3, maiores temperaturas média, mínima e máxima foram negativamente correlacionadas a sobrevivência do isolado Feij.2628A nos solos A, B e C, enquanto que maiores temperaturas máximas afetaram negativamente a sobrevivência de Cff1 nos solos A e B (Tabela 3). A precipitação foi correlacionada negativamente com a sobrevivência de Cff1 e Feij.2628A no solo B e maior teor de umidade correlacionou positivamente com a sobrevivência de Feij.2628A no solo A (Tabela 3).

Tabela 3 - Análise de correlação (Spearman) entre a população de *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* e temperatura média, máxima e mínima, precipitação e umidade do solo, nos experimentos 3, 4 e 5, sob condições de campo

Isolado	Solo	Temp. média	Temp. máxima	Temp. mínima	Precipitação	Umidade do solo
Experimento 3						
Cff1	A	NS*	-0,571	NS	NS	NS
Cff1	B	NS	NS	NS	-0,572	NS
Cff1	C	NS	-0,597	NS	NS	NS
2628A	A	-0,795	-0,804	-0,768	NS	0,553
2628A	B	-0,687	-0,697	-0,708	-0,595	NS
2628A	C	-0,734	-0,741	-0,746	NS	NS
Experimento 4						
Cff1	A	NS	NS	NS	NS	NS
Cff1	B	NS	NS	NS	NS	NS
Cff1	C	NS	NS	NS	NS	NS
2628A	A	NS	NS	NS	NS	0,980
2628A	B	NS	NS	NS	NS	0,846
2628A	C	NS	NS	NS	NS	NS
Experimento 5						
Cff1	A	NS	NS	NS	-0,991	0,961**
Cff1	B	NS	NS	NS	-0,972	NS
Cff1	C	NS	NS	NS	-0,986	NS
2628A	A	NS	NS	NS	NS	0,899
2628A	B	NS	NS	NS	-0,902	NS
2628A	C	NS	NS	NS	NS	0,913

*p<0,05.; **NS: não significativo

No experimento 4, maior teor de umidade do solo influenciou a sobrevivência do isolado Feij.2628A nos solos A e B (Tabela 3). No experimento 5, a sobrevivência

do isolado Cff1 nos solos A, B e C, e 2628A no solo B, foi negativamente correlacionada com a precipitação (Tabela 3). O maior teor de umidade do solo favoreceu apenas a sobrevivência do isolado Cff1 no solo A e Feij.2628A nos solos A e C.

Todos os isolados obtidos de cada tratamento, nos experimentos e períodos de avaliação, apresentaram crescimento em meio de cultura NSA suplementado com cloreto de sódio e, após serem submetidos ao PCR, amplificaram uma banda de 306 pb.

3.4. DISCUSSÃO

Os dados obtidos neste estudo demonstraram que isolados de Cff do feijão e da soja podem sobreviver no solo por períodos variáveis, sendo este um importante nicho de sobrevivência da bactéria em lavouras. Além disso, esta é a primeira evidência de sobrevivência de isolados da soja no solo.

A sobrevivência do isolado de feijão Feij. 2628A no solo, sob condições controladas (20 °C), já foi relatada por períodos variando entre 30 a 94 dias, de acordo com o tipo de solo, contudo, a umidade do solo foi ajustada entre 15 a 22%, ao decorrer desses experimentos (GONÇALVES et al., 2018). Em nosso estudo, optamos por não realizar tais ajustes, e, como a sobrevivência de Cff no solo está positivamente correlacionada a umidade, verificamos que a população bacteriana decresceu conforme os teores de umidade diminuíram. Ainda assim, a bactéria foi recuperada do solo com um teor mínimo de 1,8% de umidade, evidenciando sua resistência a secagem.

Estudos de sobrevivência em solo foram realizados com outros isolados do feijoeiro sob condições controladas (SILVA JÚNIOR et al., 2012; GONÇALVES et al., 2018), indicando uma discrepância na capacidade de sobrevivência entre os diferentes isolados e tipo de solo, mas, para isolados da soja, este é o primeiro relato, podendo sobreviver por períodos entre 77 a 109 dias.

Nos experimentos de sobrevivência de isolados de Cff em condições de campo, a textura, pH e teor de matéria orgânica dos solos não influenciaram na sobrevivência da bactéria. Os solos A e B foram caracterizados como argilosos, enquanto o solo C, textura média. O teor de matéria orgânica variou entre 18 e 28 g dm^3 e o pH entre 5 e 5,9 nos diferentes solos.

A maioria das bactérias sobrevivem por mais tempo em solos argilosos, por se manterem dentro de agregados de argila, aumentando assim a disponibilidade de água e a proteção contra antagonistas (HATTORI, 1973). No entanto, apenas no experimento 1, ambos isolados sobreviveram por menor período no solo com textura média, se comparado aos solos argilosos, nos demais experimentos, a textura do solo não influenciou a sobrevivência de Cff.

Condições climáticas, propriedades físicas e químicas do solo, atividade antagonista e praticas culturais afetam a sobrevivência de bactérias fitopatogênicas no solo (SCHUSTER; COYNE, 1974). Maiores teores de matéria orgânica no solo favorecem os microrganismos antagônicos, que reduzem, por sua vez, as populações de bactérias fitopatogênicas (PATRICK, 1954). Como não houve maiores variações entre a matéria orgânica nos diferentes tipos de solo, não pudemos constatar o efeito da mesma na sobrevivência da bactéria. Estudos já comprovaram que a sobrevivência de Cff é favorecida por temperaturas mais amenas, em torno de 20 °C (SILVA JÚNIOR

et al., 2012) e, de fato, verificamos nesse estudo que altas temperaturas, assim como a maior ocorrência de precipitações, afetaram negativamente a sobrevivência de Cff.

Bactérias que habitam o xilema, como Cff, devem lidar com estresse abióticos presentes neste nicho, uma vez que a composição da seiva é pobre e a disponibilidade de oxigênio, limitada (FATIMA; SENTHIL-KUMAR, 2015).Técnicas de inoculação de Cff em sementes de feijão utilizando se meio com restrição hídrica, assim como o crescimento da bactéria em meio suplementado com cloreto de sódio, demonstram que Cff se adapta bem a ambientes com baixo potencial mátrico (MARINGONI; CAMARA, 2006; DEUNER et al., 2011). Os dados obtidos comprovam que Cff é capaz de sobreviver em períodos mais secos e em solos com baixo teor de umidade.

Este foi o primeiro relato de sobrevivência no solo, sob condições controladas e a campo, de isolados da soja. O solo é um importante nicho de sobrevivência de bactérias fitopatogênicas e, com base nos resultados obtidos, os isolados de feijão e soja sobrevivem por até 91 dias neste habitat, tornando-se potenciais fontes de inoculo para o cultivo subsequente.

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CONSIDERAÇÕES FINAIS

Curtobacterium flaccumfaciens pv. *flaccumfaciens* sobreviveu por pelo menos sete dias na filosfera e rizosfera de 13 espécies de plantas cultivadas em sucessão ao feijoeiro: aveia branca, aveia preta, azevém, cevada, canola, nabo forrageiro, milho, milheto, sorgo, soja, girassol, mucuna e trigo. Por serem potenciais hospedeiras, essas plantas devem ser evitadas em áreas com histórico de ocorrência da doença.

A sobrevivência de Cff em algumas plantas daninhas esteve correlacionada com as condições climáticas. Em épocas chuvosas e com temperaturas acima de 30 °C, a sobrevivência de Cff foi menor em algumas espécies. Devido a sobrevivência mínima de sete dias na filosfera e rizosfera das 21 plantas daninhas avaliadas, recomenda-se que todas sejam erradicadas das lavouras com histórico de ocorrência da doença, com destaque para *Amaranthus viridis, Conyza bonariensis, Emilia fosbergii, Galinsoga parviflora, Gnaphalium purpureum, Raphanus sativus, Lepidium virginicum, Commelina benghalensis, Ipomoea triloba, Cyperus rotundus, Senna obtusifolia, Digitaria insularis, Nicandra physalodes* e *Solanum americanum*.

Estas são as primeiras evidencias de sobrevivência de Cff em plantas daninhas e em milheto, nabo forrageiro, mucuna e sorgo.

O solo se mostrou um nicho favorável a sobrevivência de isolados de Cff do feijão e da soja, sendo a sobrevivência correlacionada positivamente com maiores teores de umidade do solo.

Os resultados obtidos com esse estudo podem auxiliar no manejo da murcha de curtobacterium no feijão e da mancha bacteriana marrom na soja.

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