CIÊNCIAS AGRÁRIAS, INDICADORES E SISTEMAS DE PRODUÇÃO SUSTENTÁVEIS

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

Atena Editora
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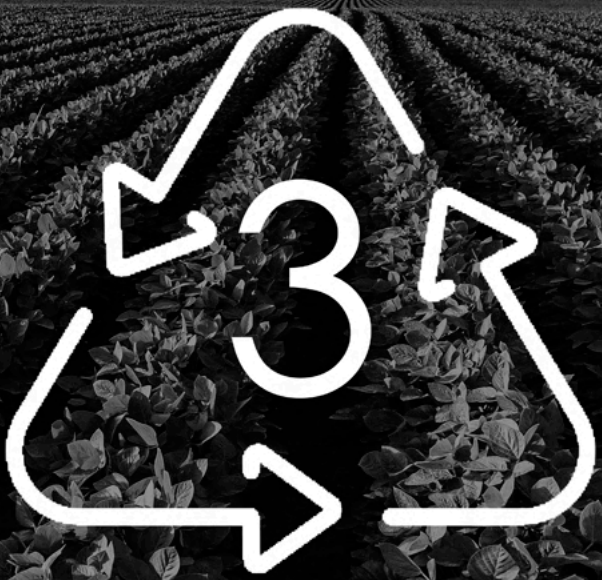

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Ano 2021

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Ciências agrárias, indicadores e sistemas de produção sustentáveis 3

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Dados Internacionais de Catalogação na Publicação (CIP)

C569 Ciências agrárias, indicadores e sistemas de produção sustentáveis 3 / Organizadores Pedro Henrique Abreu Moura, Vanessa da Fontoura Custódio Monteiro. – Ponta Grossa - PR: Atena, 2021.

Formato: PDF
Requisitos de sistema: Adobe Acrobat Reader
Modo de acesso: World Wide Web
Inclui bibliografia
ISBN 978-65-5983-702-1
DOI: https://doi.org/10.22533/at.ed.021212911

1. Ciências agrárias. I. Moura, Pedro Henrique Abreu (Organizador). II. Monteiro, Vanessa da Fontoura Custódio. III. Título.

CDD 630

Elaborado por Bibliotecária Janaina Ramos – CRB-8/9166

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Ponta Grossa – Paraná – Brasil
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APRESENTAÇÃO

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

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

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

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

Pedro Henrique Abreu Moura
Vanessa da Fontoura Custódio Monteiro

SUMÁRIO

CAPÍTULO 5

DISSEMINATION OF *Xanthomonas campestris* PV. *campestris* BY *Bemisia tabaci* AND *Myzus persicae*

Data de aceite: 01/11/2021
Data de submissão: 05/10/2021

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ABSTRACT: This study evaluated the dissemination of *Xanthomonas campestris* pv. *campestris* (Xcc) by *Bemisia tabaci* and *Myzus persicae*, important pests in brassicas crops. To evaluate Xcc adherence in the insects' bodies, three experiments were installed in B.O.D. Two experiments carried out at different times evaluated the bacterium adherence to insects exposed to kale discs inoculated with Xcc, for 96 h, and the evaluations were carried out every 24 h. In another experiment, Xcc adherence to insects exposed to inoculated kale plants was evaluated, being conducted for 15 days, and the evaluations carried out every 7 days. Xcc dissemination by insects was also evaluated in greenhouse experiments, with and without choice. The experiments were conducted for 15 days and Xcc adherence to insects was evaluated every 7 days. Xcc was recovered from *B. tabaci and M. persicae* from the kale discs for up to 48 h. In kale plants, Xcc was recovered from *M. persicae* at 7 days, but not from *B. tabaci*. In greenhouse, the insects did not disseminate Xcc from symptomatic kale plants to healthy plants. Xcc populations were not recovered from insects in all dissemination experiments. The results showed that apparently *B. tabaci* and *M. persicae* are not involved in the Xcc dissemination.
KEYWORDS: Bacterium, whitefly, aphid, black rot, brassicas.

RESUMO: Este trabalho avaliou a disseminação de *Xanthomonas campestris* pv. *campestris* (Xcc) por *Bemisia tabaci* e *Myzus persicae*, importantes pragas em cultivos de brássicas. Para avaliar a aderência de Xcc no corpo dos insetos, foram

instalados três experimentos em B.O.D. Dois experimentos realizados em épocas diferentes, avaliaram a aderência da bactéria nos insetos expostos a discos de couve inoculados com Xcc, por 94 h, e as avaliações realizadas a cada 24 h. Em outro experimento, foi avaliada a aderência de Xcc nos insetos expostos às plantas de couve inoculadas, sendo conduzido por 15 dias, e as avalições realizadas a cada 7 dias. A disseminação da bactéria pelos insetos também foi avaliada em experimentos em casa de vegetação, com e sem chance de escolha. Os experimentos foram conduzidos por 15 dias, e foi avaliada a aderência de Xcc nos insetos a cada 7 dias. Xcc foi recuperada de *B. tabaci e M. persicae* dos discos de couve por até 48 h. Em plantas de couve a bactéria foi recuperada de *M. persicae* aos 7 dias, mas não de B. tabaci. Em casa de vegetação, os insetos não disseminaram Xcc de plantas de couve sintomáticas para plantas sadias. Populações de Xcc não foram recuperadas dos insetos em todos os experimentos de disseminação. Os resultados demonstraram que aparentemente *B. tabaci* e *M. persicae* não estão envolvidos na disseminação de Xcc.
PALAVRAS-CHAVE: Bactéria, mosca-branca, afídeo, podridão negra, brássicas.

1 | INTRODUCTION

Black rot, caused by *Xanthomonas campestris* pv. *campestris* (Pammel) Downson (Xcc), is present in all countries where brassica are grown and is considered a problem in hot and humid regions (WILLIAMS, 2007; LEMA et al., 2012). The disease occurs in varieties of *Brassica oleracea*, radish, ornamental, weeds and *Arabidopsis thaliana*. Commonly observed symptoms include yellowing of leaves margins followed by necrosis and darkening of vascular tissue (VICENTE; HOLUB, 2013; MARINGONI; SILVA JÚNIOR, 2016). Damage caused by the pathogen in susceptible cultivars can cause plant death and consequently large economic losses (LEMA et al., 2012).

For the efficient management of black rot in areas with brassica cultivation, it is important to know the survival strategies and Xcc dissemination mechanisms (BROWN, 1997; WILLIAMS, 2007; MARINGONI; SILVA JÚNIOR, 2016). It is known that Xcc survives during the absence of the crop in seeds, soil, crop debris and weeds (SCHAAD; DIANESE, 1981; VICENTE; HOLUB, 2013; SILVA JÚNIOR et al., 2020), and is efficiently disseminated by seeds and rainwater splashes or irrigation (KOCKS; ZADOKS; RUISSEN, 1999; MARINGONI; SILVA JÚNIOR, 2016). However, Xcc dissemination by insects is little known and studied.

Insects are important agents for phytopathogens dissemination (AGRIOS, 2005; AMORIM; PASCHOLATI, 2018). Bacteria are accidentally disseminated from diseased plants to healthy plants, when they adhere externally to the insects' bodies, or through specific interactions with the insect, involving a period of acquisition and latency for transmission to occur (BEDENDO; BELASQUE, 2018). In addition to insects being involved in the dissemination of primary and secondary inoculum in the field, they can also cause injuries to plant tissues during feeding and favor of the bacteria penetration (ZANDJANAKOU-TACHIN et al., 2007).

In the literature it is possible to find some studies in which the ability of Xcc dissemination by insects was investigated. In the USA, it has been demonstrated that the beetle *Phyllotreta cruciferae* (Coleoptera: Chrysomelidae), an important brassica pest in the country, has limited potential to act as an agent of Xcc dissemination (SHELTON; HUNTER, 1985). In the Netherlands, *Calliphora vomitoria* (Diptera: Calliphoridae) flies inoculated with Xcc and kept in cages with flowering cauliflower plants disseminated the bacterium and were responsible for lots of seeds contaminated with Xcc (VAN DER WOLF; VAN DER ZOUWEN, 2010).

In Brazil, several insects are described as pests in the brassica's cultivation, such as the whitefly *Bemisia tabaci* (Hemiptera: Aleyrodidae) and the aphid *Myzus persicae* (Hemiptera: Aphididae), but there is no information about the importance of these insects in the dissemination of Xcc in the country (HOLTZ et al., 2015). Within this context, this study evaluated the potential of *B. tabaci* and *M. persicae* in the Xcc dissemination.

2 | MATERIAL AND METHODS

2.1 Experiments location

The experiments were carried out at the Departamento de Proteção Vegetal, Faculdade de Ciências Agronômicas (FCA), Universidade Estadual Paulista 'Júlio de Mesquita Filho' (UNESP), Botucatu, São Paulo.

2.2 Bacterial strain, cultivation and preservation conditions

In all experiments, the Xcc strain 3098C was used. This strain is resistant to 100 $\mu g.mL^{-1}$ rifampicin, and pathogenic to brassica plants, and was previously cultivated in nutrient sucrose agar plus rifampicin - NSAR [20 $g.L^{-1}$ of nutrient agar (Merck, Germany), 5 $g.L^{-1}$ sucrose (Synth, Brazil) and 100 $\mu g.mL^{-1}$ rifampicin (Rifaldin)] and incubated at 28 °C for 48 h. The strain was preserved in glycerol 30 % (v/v) and kept at - 80 °C.

2.3 Obtaining and maintaining *B. tabaci* and *M. persicae* populations

Populations of *B. tabaci and M. persicae* were obtained from creations of the Departamento de Proteção Vegetal. The creations were kept in healthy kale plants (cv. Georgia) grown in a greenhouse (temperature 22 - 28 °C and relative humidity 60 - 90 %).

2.4 Confirmation of Xcc absence in *B. tabaci* and *M. persicae*

The protocol adapted from Van Der Wolf and Van Der Zouwen (2010) was used, in which 100 insects of each species were collected and distributed in microcentrifuge tubes (10 insects of each species per tube) containing 300 µL of phosphate-saline buffer (PBS) 10 mM (8 g of NaCl, 0.2 g KCl, 2.9 g Na_2HPO_4, 0.2 g KH_2PO_4, 1 L autoclaved distilled water) pH 7.2. Subsequently, the insects were crushed, the tubes shaken (200 rpm/ 1 min.), and

100 µL of the suspensions plated in NSA. The plates were incubated (28 °C/ 48 h) and the presence of Xcc colonies analyzed. After incubation, the presence of Xcc in NSA was not observed, confirming the absence of the bacterium in the insects.

2.5 Xcc adhesion in *B. tabaci* and *M. persicae*

2.5.1 Exposure of B. tabaci and M. persicae to kale discs inoculated with Xcc

Two experiments (called experiments 1 and 2) were carried out at different times to evaluate the ability of Xcc to adhere to the body of *B. tabaci* and *M. persicae,* via exposure of insects to kale discs (cv. Georgia) inoculated with Xcc.

In each experiment, 128 kale discs with 38 mm^2 were used, obtained with a cork borer of 22 mm in diameter, being 64 discs immersed in bacterial suspension 10^8 CFU mL^{-1} (OD_{600} = 0.1), and 64 discs immersed in distilled water for 5 min. After removing excess of water and inoculum, the discs were transferred to Petri dishes (90 x 15 mm) containing filter paper sheets moistened (4 discs per dish) and kept in B.O.D (26 °C and 12 h photoperiod) for 24 h. The transfer of insects (20 insects per dish) was performed with a manual aspirator, by means of a circular hole in the lids of Petri dishes, subsequently sealed with Parafilm M®. The dishes were kept for 96 h in B.O.D and the evaluations were performed every 24 h, recovering Xcc cells adhered to the insects.

The experiments consisted of 4 treatments (treatment 1, exposure of *B. tabaci* to discs immersed in Xcc suspension; treatment 2, exposure of *M. persicae* to discs immersed in Xcc suspension; treatment 3, exposure of *B. tabaci* to discs immersed in distilled water; treatment 4, exposure of *M. persicae* to discs immersed in distilled water) and 8 replications.

2.5.2 Exposure of B. tabaci and M. persicae to kale plants inoculated with Xcc

For this experiment (experiment 3), kale plants (cv. Georgia) with four expanded leaves were inoculated with bacterial suspension 10^8 UFC.mL^{-1} of Xcc strain 3098C and kept in B.O.D (26 °C and 12 h photoperiod). The plants were protected by individual cages, and the insects were transferred (60 insects of each species per plant), 24 h after inoculation, with the aid of a manual aspirator. The plants were kept in B.O.D for 15 days and the evaluations were performed every 7 days, recovering Xcc adhered to the insects.

The experiment consisted of 4 treatments (treatment 1, exposure of *B. tabaci* to plants inoculated with Xcc; treatment 2, exposure of *M. persicae* to plants inoculated with Xcc; treatment 3, exposure of *B. tabaci* to non-noculated plants; treatment 4, exposure of *M. persicae* to non-inoculated plants) and 8 replications.

2.6 Xcc dissemination by *B. tabaci* and *M. persicae*

Three experiments (experiments 4, 5 and 6) were carried out in a greenhouse to evaluate the dissemination of Xcc by *B. tabaci* and *M. persicae*. Kale plants (cv. Georgia, four expanded leaves) with symptoms of black rot were obtained from small incisions with scissors on the leaves margins, followed inoculation by spraying the bacterial suspension 10^8 UFC.mL^{-1}, to the runoff point.

In experiments 4 and 5, with no choice, 1200 insects of each species were exposed to kale plants infected with Xcc and expressing disease symptoms for 48 h; later, the insects were transferred to cages with anti-aphid mesh (1.0 m long, by 0.80 m wide and 1.0 m high) containing healthy kale plants, being kept for 15 days in these plants. In experiment 6, with a choice, 600 insects of each species were exposed to symptomatic and healthy plants in the same cage and kept for 15 days to verify the dissemination of Xcc. Evaluations consisted of the observation of symptoms in plants and recovery of Xcc adhered to insects every 7 days.

2.7 Determination of the Xcc population in the phyllosphere

To recover Xcc from the surface of the leaf discs in experiments 1 and 2 (recovery periods: 24 h, 49 h, 72 h and 96 h), four discs of treatments 1 and 2 (two discs per treatment) and four discs of treatments 3 and 4 (two discs per treatment) were randomly selected. The discs were individually distributed in Falcon tubes containing 15 mL of PBS, shaken (200 rpm/ 10 min), serially diluted (10^0 to 10^{-4}) and 100 µL plated in triplicate in NSAR medium, plus 50 µg.mL^{-1} chlorothalonil and 50 µg.mL^{-1} methyl thiophanate (NSARF). Plates were incubated (28 °C/ 72 h), the presence of Xcc evaluated, and the data transformed into cfu/ cm^2 of tissue.

For Xcc recovery in experiments 3, 4, 5 and 6, initially kale leaves were randomly selected from some plants, in each treatment, and detached from the stem. Subsequently, four 38 mm^2 discs were obtained with a 22 mm cork borer and submitted to Xcc recovery as described above.

2.8 Xcc processing from *B. tabaci* and *M. persicae*

For all experiments, populations of *B. tabaci* and *M. persicae* were collected and distributed in microcentrifuge tubes (2 mL) containing 300 µL of PBS. Ten insects were deposited per tube for *B. tabaci* (total of 30 insects) and 5 insects for *M. persicae* (total of 15 insects), followed by crushing and shaken (200 rpm/ 1 min). Plating was performed in triplicate in NSARF medium. Plates were incubated (28 °C/ 72 h), the presence of Xcc confirmed, and the data transformed into cfu/ insect.

3 | RESULTS

3.1 Xcc population in the phyllosphere

Average populations of Xcc varied in the kale phyllosphere during the evaluation periods. In the experiments with discs kept in B.O.D, average populations of Xcc ranged from 4.1 x 10^5 cfu/ cm^2 of tissue to 1.1 x 10^7 cfu/ cm^2 of tissue in experiment 1, and from 1.6 x 10^5 cfu/ cm^2 of tissue to 3.3 x 10^8 cfu/ cm^2 in experiment 2. Xcc populations were not recovered from the immersed discs in distilled water (Table 1).

Experiments	Treatments	Evaluation periods				
		0 hours	24 hours	48 hours	72 hours	96 hours
1	1 - 2	*4.1 x 10^5	8.8 x 10^6	2.7 x 10^7	9.2 x 10^6	1.1 x 10^7
	3 - 4	UNP	UNP	UNP	UNP	UNP
2	1 - 2	1.6 x 10^5	3.7 x 10^6	6.6 x 10^7	5.4 x 10^7	3.3 x 10^8
	3 - 4	UNP	UNP	UNP	UNP	UNP

*Population in cfu/ cm^2 of tissue; UNP = unrecovered population.

Table 1 - Average population of *Xanthomonas campestris* pv. *campestris* in the phyllosphere of disks in B.O.D.

In experiments with kale plants, the average populations ranged from 1.0 x 10^4 cfu/ cm^2 of tissue to 6.7 x 10^4 cfu/ cm^2 of tissue in experiment 3; from 1.5 x 10^3 cfu/ cm^2 of tissue to 9.5 x 10^5 cfu/ cm^2 of tissue in experiment 4; from 1.9 x 10^4 cfu/ cm^2 of tissue to 8.3 x 10^2 cfu/ cm^2 of tissue in experiment 5; and from 9.5 x 10^4 cfu/ cm^2 of tissue to 3.7 x 10^5 cfu/ cm^2 of tissue in experiment 6 (Table 2). Xcc populations were not recovered from non-inoculated plants.

Experiments	Treatments	Evaluation periods		
		0 days	7 days	14 days
3	Inoculated plants	*1.0 x 10^4	1.9 x10^6	6.7 x 10^4
	Non-inoculated plants	UNP	UNP	UNP
4	Plants with symptoms	1.5 x 10^3	9.9 x 10^4	9.5 x 10^5
	Non-inoculated plants	UNP	UNP	UNP
5	Plants with symptoms	1.9 x 10^4	2.2 x 10^5	8.3 x 10^2
	Non-inoculated plants	UNP	UNP	UNP
6	Plants with symptoms	9.5 x 10^3	6.3 x 10^4	3.7 x 10^5
	Non-inoculated plants	UNP	UNP	UNP

*Population in cfu/ cm^2 of tissue; UNP = unrecovered population.

Table 2 - Average population of *Xanthomonas campestris* pv. *campestris* in the phyllosphere of kale plants kept in B.O.D and greenhouse.

3.2 Xcc adherence to *B. tabaci* and *M. persicae*

Xcc was recovered for up to 48 h from *B. tabaci* and *M. persicae* exposed to kale discs immersed in Xcc suspension in experiments 1 and 2 (Table 3). After this period, the populations on both insects died, and the evaluations were no longer carried out. Xcc was not recovered from insects when exposed to kale discs immersed in distilled water (Table 3).

Experiments	Treatments	Evaluation periods	
		24 hours	48 hours
1	1 – *B. tabaci* exposed to discs with Xcc	*7.7 x 10^0	2.7 x 10^1
	2 – *M. persicae* exposed to discs with Xcc	4.2 x 10^4	8.0 x 10^2
	3 – *B. tabaci* exposed to discs without Xcc	UNP	UNP
	4 – *M. persicae* exposed to discs without Xcc	UNP	UNP
2	1 – *B. tabaci* exposed to discs with Xcc	4.3 x 10^2	9.9 x10^2
	2 – *M. persicae* exposed to discs with Xcc	3.3 x10^2	1.2 x 10^5
	3 – *B. tabaci* exposed discs without Xcc	UNP	UNP
	4 – *M. persicae* exposed to discs without Xcc	UNP	UNP

*Population in cfu/ insect; UNP = unrecovered population.

Table 3 - Average population of *Xanthomonas campestris* pv. *campestris* adhered to *Bemisia tabaci* and *Myzus persicae*.

In experiment 3, Xcc was recovered from *M. persicae* with an average population of 2 cfu/ insect, 7 days after the insects were released in inoculated kale plants but was not recovered after 15 days. The bacterium was not recovered from *B. tabaci* during the evaluations and from *B. tabaci* and *M. persicae* when exposed to non-inoculated plants.

3.3 Xcc dissemination by *B. tabaci and M. persicae* in kale plants

B. tabaci and *M. persicae* were not able to disseminate Xcc from kale plants with symptoms of black rot to health kale plants in experiments 4, 5 and 6. Xcc populations were not recovered from the insects sampled in these experiments.

4 I DISCUSSION

Insects play an important role in diseases epidemiology, as they act as agents for the dissemination of the primary and secondary inoculum of pathogens in the field (AGRIOS, 2005; AMORIM; PASCHOLATI, 2018). However, for several pathosystems the role of insects in dissemination is little known and studied, which can impact the control strategies used in disease management (BROWN, 1997; AGRIOS, 2005). For Xcc, the results obtained here demonstrated that *B. tabaci* and *M. persicae* may not be involved in the dissemination of Xcc, considering the conditions under which the experiments were conducted.

Xcc is adapted to survival in the host plants phyllosphere (ARIAS; NELSON; ALVAREZ, 2000), which explains the behavior of populations on the surface of leaf discs kept in B.O.D and in the phyllosphere of inoculated kale plants. Xcc was recovered from *B. tabaci* and *M. persicae* exposed to kale discs during the experiments. The location of Xcc in the body of insects, however, was not investigated, but it is assumed that they were externally adhered to the body. For Xcc to be found in the internal tissues of *B. tabaci* and *M. persicae,* it would be necessary for the bacterium to be acquired by the insects during feeding in the phloem vessels, a process that is unlikely, since Xcc was present in a high population on the surface of the discs. Bacterial populations can adhere to different insects organs. For example, *X. axonopodis* pv. *manihotis* and *X. axonopodis* pv. *vignicola* were found in legs and jaws of *Zonocerus variegatus* (Orthoptera: Pyrgomorphidae) (ZANDJANAKOU-TACHIN et al., 2007). *Erwinia amylovora* was present in the antennae, legs and proboscis of *Aphis pomi* (Hemiptera: Aphididae) (HILDEBRAND; DICKLER; GEIDER, 2000).

Despite being recovered from insects when kept in leaf discs, Xcc populations were not recovered from *B. tabaci* exposed to kale plants kept in B.O.D. In *M. persicae,* a low Xcc population was recovered after 7 days and was no longer recovered from insects after 15 days. These results demonstrated that Xcc had a limited survival capacity in insects, even when kept in direct contact with the inoculated plant. Xcc populations can vary on leaf surface depending on nutrients availability, water and antagonist microflora action. These populations are not found evenly distributed on the leaf surface, but rather in small aggregates or biofilms located in depressions formed in the junctions of epidermal cells, along the ribs and in the bases of the trichomes (VORHOLT, 2012; SCHLECHTER; MIEBACH; REMUS-EMSERMANN, 2019). Thus, depending on the location of populations in plant tissue, insects may not come into contact with the bacterium. In experiments with kale discs, however, this phenomenon may not have occurred, due to tissue size and Xcc population.

The dissemination of Xcc by *B. tabaci* and *M. persicae* did not occur from plants with symptoms of black rot to healthy plants in greenhouse experiments, and the bacterium was also not recovered from the insects collected during the experiments. These results demonstrated that these insects may not be able to disseminate Xcc among brassica plants. The beetle *P. cruciferae* was not considered an efficient vector of Xcc, as it was not able to readily acquire the bacterium during leaf feeding. In addition, environmental conditions may have contributed to the bad acquisition of Xcc by the insect (SHELTON; HUNTER, 1985). The pollinating fly *C. vomitoria,* on the other hand, proved to be efficient in dissemination of Xcc. Although the bacterium survived for only 5 days in the insect's body, Xcc was efficiently transmitted to the seeds, after contaminated flies were kept with the plants during the flowering period (VAN DER WOLF; VAN DER ZOUWEN, 2010).

Several factors can influence the ability of phytopathogenic bacteria to be disseminated by insects (NADARASAH; STAVRINIDES, 2011; SUGIO et al., 2014). Environmental factors

have a great influence on this process. Exposure to UV radiation can cause damage to bacterial cells and reduce the ability of insects to disseminate phytopathogenic bacteria (ZANDJANAKOU-TACHIN et al., 2007; LEVEAU, 2018). In addition to climatic factors, the insect's body and its defense mechanisms can prevent the adherence of bacteria, as well as interfere with survival and dissemination (NADARASAH; STAVRINIDES, 2011). Insects that are members of the Hemiptera order, such as *B. tabaci* and *M. persicae*, stand out for producing large amounts of waxes in the form of particles, which cover their bodies and act as a hydrophobic barrier, protecting them from environment variations and the attack of pathogens (BYRNE; HADLEY, 1988; BUCKNER, 2014. NELSON; MARDAUS, 1994). This characteristic may explain the low adherence capacity of Xcc in these insects in the experiments, especially in *B. tabaci*.

The presence of antagonistic microorganisms can contribute to the reduction of phytopathogenic bacterial populations in insects. The low survivability in *C. vomitoria* can be explained by the presence of antagonistic bacteria to Xcc (VAN DER WOLF; VAN DER ZOUWEN, 2010). The endosymbiont community can also influence in many aspects the ecology, behavior and physiology of insects, including responses to climate change, and protection against natural enemies, parasites and pathogens, and can also interfere with the dissemination of phytopathogens. In addition to these factors, the adaptive cost for the bacterium to associated with different hosts can influence survival and dissemination. Phytopathogenic bacteria disseminated by insects have been proven to have significant reduction of virulence in plants (NADARASAH; STAVRINIDES, 2011; SUGIO et al., 2014).

In Brazil, *B. tabaci* and *M. persicae* are important crop pests and are involved in the dissemination of several species of phytopathogens. However, in this study, they were not able to disseminate Xcc from plants with symptoms of black rot to healthy plants and, therefore, may not be involved in the dissemination of the bacterium.

ACKNOWLEDGEMENTS

The first author thanks the São Paulo State Research Foundation (FAPESP) for the granting of the doctoral scholarship and for the financial support (scholarship: 2017 / 13822-0). This study was financed in part by the Coordenação de Aperfeiçoamento de Pessoal de Nível Superior - Brasil (CAPES) - Finance Code 001.

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ÍNDICE REMISSIVO

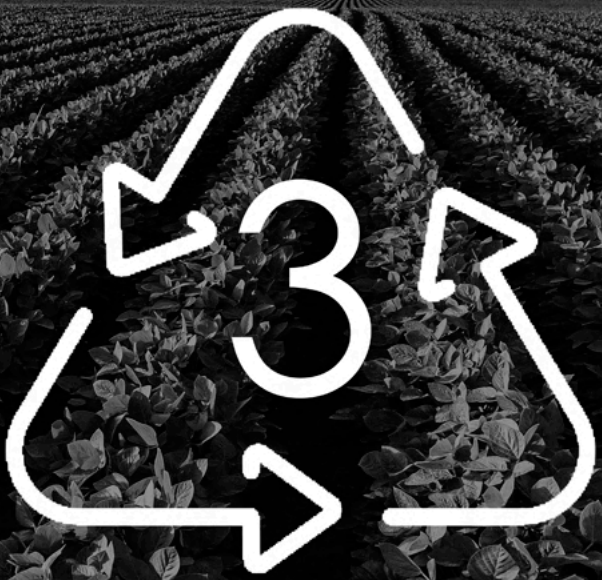
Q

R

S

T

CIÊNCIAS AGRÁRIAS, INDICADORES E SISTEMAS DE PRODUÇÃO SUSTENTÁVEIS



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