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Original Article

Wolbachia pipientis: first detection in populations of *Glycaspis brimblecombei* (Hemiptera: Aphalaridae) and *Psyllaephagus bliteus* (Hymenoptera: Encyrtidae) in Brazil

Wolbachia pipientis: primeira detecção em populações de *Glycaspis brimblecombei* (Hemiptera: Aphalaridae) e *Psyllaephagus bliteus* (Hymenoptera: Encyrtidae) no Brasil

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Abstract

The sucking insect, *Glycaspis brimblecombei* Moore (Hemiptera: Aphalaridae), is originally from Australia and reduces the productivity of *Eucalyptus* crops. The parasitoid *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae) is the main agent used in the integrated management of *G. brimblecombei*. Endosymbionts, in insects, are important in the adaptation and protection of their hosts to the environment. The intracellular symbionts *Wolbachia*, induces reproductive changes such as cytoplasmic incompatibility, feminization, male death and parthenogenesis. The objective of this study was to report the first record of *Wolbachia pipientis* in populations of *G. brimblecombei* and of its parasitoid *P. bliteus* in the field in Brazil. Branches with adults of *G. brimblecombei* and *P. bliteus* were collected from eucalyptus trees in commercial farms in six Brazilian states and, after emergence, the insects obtained were frozen at -20 °C. Polymerase chain reaction (PCR) was performed to detect the *Wolbachia* endosymbiont. *Wolbachia pipientis* was identified in individuals of *G. brimblecombei* and its parasitoid *P. bliteus* from populations of the counties of Agudos and Mogi-Guaçu (São Paulo State), Itamarandiba (Minas Gerais State) and São Jerônimo da Serra (Paraná State) in Brazil.

Keywords: biological control, endosymbionts, red gum lerp psyllid.

Resumo

O inseto sugador, *Glycaspis brimblecombei* Moore (Hemiptera: Aphalaridae), é de origem australiana e reduz a produtividade de cultivos do gênero *Eucalyptus*. O parasitoide *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae) é o principal agente utilizado no manejo integrado de *G. brimblecombei*. Endossimbiontes, em insetos, são importantes na adaptação e proteção de seus hospedeiros ao ambiente que habitam. *Wolbachia*, um simbionte intracelular, induz alterações reprodutivas, como feminização, incompatibilidade citoplasmática, morte de machos e partenogênese. O objetivo foi relatar o primeiro registro de *Wolbachia pipientis* em populações de *G. brimblecombei* e de seu parasitoide *P. bliteus* em campo no Brasil. Ramos com adultos de *G. brimblecombei* e *P. bliteus* foram coletados em árvores de eucalipto em plantios comerciais em seis estados do Brasil e, após a emergência, os insetos obtidos foram congelados -20 °C. A reação em cadeia da polimerase (PCR) foi realizada para detectar o endossimbionte *Wolbachia*. *Wolbachia pipientis* foi identificado em indivíduos de *G. brimblecombei* e de seu parasitoide *P. bliteus* de populações de Agudos e Mogi-Guaçu (São Paulo), Itamarandiba (Minas Gerais) e São Jerônimo da Serra (Paraná), Brasil.

Palavras-chave: controle biológico, endossimbiontes, psilídeo-de-concha.

1. Introduction

Glycaspis brimblecombei Moore (Hemiptera: Aphalaridae), of Australian origin, damages several species of the genus *Eucalyptus* (Dal-Pogetto et al., 2022). The easy adaptation, dispersion and the great extension of the areas planted with eucalyptus increase the importance of integrated pest management programs (Ndlela et al., 2018). The parasitoid *Psyllaephagus bliteus* Riek (Hymenoptera: Encyrtidae) is the main agent used in the integrated management of *G. brimblecombei* (Cuello et al., 2021).

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Endosymbionts such as *Wolbachia* and *Rickettsia* have been reported in many insect species (Bryant and Newton, 2020; Milenovic et al., 2022). Endosymbionts are important in protecting hosts from environmental stress, such as natural enemies, heat and toxins (Liu and Guo, 2019). Wolbachia is an intracellular symbiont in reproductive tissues of arthropods and with maternal transmission. It can manipulate the reproduction of its hosts, inducing reproductive alterations, such as cytoplasmic incompatibility, feminization, male death and parthenogenesis to increase its transmission and persistence in the environment (Rocha et al., 2018; Bagheri et al., 2019; Mateos et al., 2020). Maternal transmission, *Wolbachia*'s reproductive advantage, can block the transmission of pathogens (Krafsur et al., 2020) and influence insect fitness (Cao et al., 2019), important variables in the rearing of natural enemies.

Relationships between endosymbionts, *G. brimblecombei* and its parasitoid *P. bliteus* are poorly studied and may improve the understanding of protecting and reproducing these insects. The objective was to report *Wolbachia pipientis* for the first time in populations of *Glycaspis brimblecombei* and of its parasitoid *Psyllaephagus bliteus* in the field in Brazil.

2. Material and Methods

2.1. Obtaining samples

Branches infested with *G. brimblecombei* and its parasitoid *P. bliteus* were collected from eucalyptus trees in commercial farms between May 2017 and March 2019 in six Brazilian states (Table 1). Individuals of the pest and its parasitoid, after emergence, were frozen at -20 °C.

2.2. Genomic DNA extraction

Genomic DNA was extracted from 20 adults from each population of *G. brimblecombei* and *P. bliteus*. These adults were macerated in a solution of 40 µl of 10% Chelex 100 resin (Bio-Rad Laboratories) and 4 µl of proteinase K (20 mg/mL) (Walsh et al., 1991). Samples of these adults were placed in a thermal block at 95 °C for 20 min. and the supernatant collected, after this period, for the polymerase chain reaction (PCR).

2.3. Polymerase chain reaction (PCR)

The *Wolbachia* endosymbiont was detected in PCR consisting of 12.5 µl of Gotaq, 7.5 µl of nuclease-free water, 1.0 µl of each primer (F: CGGGGGAAAAATTTATTGCT and R: AGCTGTAATACAGAAAGTAAA) (Heddi et al., 1999) and 3.0 µl of genomic DNA, totaling 25 µl per reaction. The PCR condition was 95 °C for (3'), followed by 30 cycles: 95 °C (30"), 55 °C (30") and 72 °C (30") and a final extension of 72 °C for 5' performed in an INFINIGEN thermal cycler (model TC-96CG). The expected length of the fragment is 900 bp. PCR products were visualized in 1% agarose gel with 100 bp molecular marker (Norgen) in ultraviolet light transilluminator (Major Science). DNA extraction procedures were performed at the Laboratory of Molecular Biology and Nematology of the Faculdade de Ciências

Table 1. Populations (Pop.), hosts, *Eucalyptus camaldulensis* (*E.c*), *Eucalyptus grandis* × *Eucalyptus camaldulensis* (*E.g* × *E.c*) and *E. grandis* × *Eucalyptus urophylla* (*E.g* × *E.u*), latitude, longitude, place and year of collections (Year) of *Glycaspis brimblecombei* (Hemiptera: Aphalaridae) (G.b.) and of its parasitoid *Psyllaephagus bliteus* (P.b.) (Hymenoptera: Encyrtidae) in the states of Goiás (GO), Maranhão (MA), Mato Grosso do Sul (MS), Minas Gerais (MG), Paraná (PR) and São Paulo (SP), Brazil between 2017 and 2019

Pop.	Hosts	Latitude	Longitude	Collection location	Year
1 G.b.	*E.c*	-22.82352	-48.43146	Botucatu, SP	2017
2 G.b.	*E.g* × *E.u*	-22.35065	-46.97842	Mogi-Guaçú, SP	2017
3 G.b.	*E.g* × *E.u*	-22.81269	-48.15861	Anhembi, SP	2018
4 G.b.	*E.g* × *E.u*	-22.4694	-48.59221	Agudos, SP	2019
5 G.b.	*E.g* × *E.u*	-22.77489	-48.35739	Botucatu, SP	2019
6 G.b.	*E.g* × *E.u*	-20.51734	-51.75398	Três Lagoas, MS	2017
7 G.b.	*E.g* × *E.u*	-17.8164	-42.90058	Itamarandiba, MG	2018
8 G.b.	*E.g* × *E.u*	-14.16676	-48.34232	Niquelândia, GO	2018
9 G.b.	*E.g* × *E.c*	-23.74529	-50.76339	São Jerônimo da Serra, PR	2019
10 G.b.	*E.g* × *E.u*	-5.19567	-47.5584	Trecho Seco, MA	2019
11 P.b.	*E.c*	-22.82352	-48.43146	Botucatu, SP	2017
12 P.b.	*E.g* × *E.u*	-22.35065	-46.97842	Mogi-Guaçú, SP	2017
13 P.b.	*E.g* × *E.u*	-22.45275	-48.92557	Agudos, SP	2019
14 P.b.	*E.g* × *E.u*	-20.51734	-51.75398	Três Lagoas, MS	2017
15 P.b.	*E.g* × *E.u*	-16.59597	-44.01011	Montes Claros, MG	2017
16 P.b.	*E.g* × *E.u*	-17.8164	-42.90058	Itamarandiba, MG	2018
17 P.b.	*E.g*× *E.c*	-23.74529	-50.76339	São Jerônimo da Serra, PR	2019

Agronômicas (FCA), Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), in Botucatu, São Paulo, Brazil.

2.4. DNA purification and Sanger sequencing

PCR products were purified using the PCR Purification Kit (Cellco, Cat.DPK-106S) following the manufacturer's recommendations. Amplifications were sequenced by an automated DNA Sanger sequencer (Model: ABI 3500-Applied Biosystems) at the Instituto de Biotecnologia (IBTEC/UNESP) in Botucatu, São Paulo, Brazil. The sequences obtained were compared and deposited in the GenBank database (NCBI, 2022) for the identification of genetic similarity of the researched endosymbionts.

3. Results

The facultative endosymbiont *W. pipientis* was sequenced with a 900 bp fragment and two sequences were obtained, one sequence identified in *G. brimblecombei* (GenBank accession MW079901), with sequencing coverage of 100% identity for *W. pipientis* (closest GenBank accession MN123211.1), and the other sequence in its parasitoids *P. bliteus* (GenBank accession MW086624.1), with sequencing coverage of 99% (closest GenBank accession MW727486.1), in the populations of Agudos and Mogi-Guaçu (São Paulo), Itamarandiba (Minas Gerais) and São Jerônimo da Serra (Paraná), Brazil.

4. Discussion

The identification of the endosymbiont *W. pipientis* in different populations of *G. brimblecombei* and of its parasitoid *P. bliteus* is similar to that reported for species of the order Hemiptera, such as *Laodelphax striatellus* Fallén (Delphacidae) (Bing et al., 2020) and *Oxycarenus laetus* Kirby (Lygaeidae) (Sureshan et al., 2022) and Hymenoptera, such as *Trichogramma dendrolimi* Matsumura (Trichogrammatidae) (Liu et al., 2018) and *Anagyrus vladimiri* Triapitsyn (Encyrtidae) (Izraeli et al., 2021). This is due to the co-evolution between *W. pipientis* and its hosts, with a variety of mutual adaptations (Shaikevich et al., 2019; Bi and Wang, 2020; Burdina and Gruntenko, 2022). The presence of *W. pipientis* can reduce the number of offspring, shorten the life cycle, kill males, cause parthenogenesis and feminization, and increase female reproduction and male fertility (Driscoll et al., 2020; Singh and Linksvayer, 2020), such as reported for *Empoasca onukii* Matsuda (Hemiptera: Cicadellidae) (Zhang et al., 2021). In addition, it may also increase host resistance to stress and viral infection, as reported for *Aedes fluviatilis* Lutz (Diptera: Culicidae) (Silva et al., 2022). The presence of *Wolbachia* can also cause cytoplasmic incompatibility, when an infected male fertilizes an uninfected female and causes the death of the embryos (Arai et al., 2019; Xiao et al., 2021). The presence of *Wolbachia* did not benefit *A. vladimiri* in mass rearing in the laboratory (Izraeli et al., 2021).

The presence of *W. pipientis* is the first report of this bacterium in different populations of *G. brimblecombei* and *P. bliteus* in Brazil, and may serve to develop tools to increase the efficiency of rearing this pest and of its natural enemy in integrated pest management programs.

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