







ALEYRODID (HEMIPTERA) DIVERSITY IN HOP VARIETIES  
GROWN IN BRAZIL

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**Abstract**

Whiteflies (Hemiptera: Aleyrodidae) are a diverse group of sap-sucking insect species that can cause severe damage in crops with global economic importance. Hops (*Humulus lupulus* L.) are a crop with considerable value for the brewing and pharmaceutical industries worldwide. The aim of the current study is to register whitefly species associated with this plant in Brazil. Specimens were collected from *H. lupulus* leaves in four different counties in Rio de Janeiro state (Cachoeiras de Macacu, Cordeiro, Nova Friburgo and Seropédica). Whiteflies belonging to nine genera and distributed in two subfamilies (Aleurodicinae and Aleyrodinae) were identified. Nine hop varieties (i.e., Brazylinsk, Cascade, Chinook, Columbus, Hallertau, Nugget, Saaz, Spalt, and Victoria) are used as host plants by these insects in Brazil. Except for *Trialeurodes vaporariorum* (Westwood), the other identified species were recorded in hop crops for the first time.

**Keywords:** *Humulus lupulus*. Aleyrodidae. Host Plant. Plant-Insect Interaction.

**1. Introduction**

Whiteflies (Hemiptera, Aleyrodidae) are sap-sucking insects represented by at least 1,560 species distributed in 161 genera (Martin and Mound 2007; Evans 2008; Ouvrard and Martin 2019). Some of these species are pests that are capable of affecting economically important agricultural crops in different countries; they can cause a greater than 50% yield decrease in infested crops (Kairo et al. 2001; Cassino et al. 2004; Kumar et al. 2016; Wosula et al. 2018; Rincon et al. 2019). Direct levels of economic losses are associated with nymphs and adult individuals who continuously feed on host plants by sucking their phloem sap (Borowiec et al. 2010; Carabalí et al. 2010; Murgianto and Hidayat 2017). Indirect damage can result from honeydew excretion, which functions as a substrate for the growth and proliferation of the fungus species *Capnodium* spp. This species causes sooty mold disease, which can reduce plants' photosynthetic and respiratory capacity, as well as the quality or market value of the harvested product (Hilje and Morales 2008). In addition, certain whitefly species, primarily those belonging to complex *Bemisia tabaci*, can lead to severe economic losses due to virus transmission (Navas-Castillo et al. 2011; Gilbertson et al. 2015).

*Humulus lupulus* L. (Cannabaceae), commonly known as hops, is an herbaceous, climbing, and perennial plant that spontaneously grows in hedges and roadsides in temperate climate regions in North America, Asia, and Europe (Small 2016). However, hops are highly valued by the brewing industry, since the female inflorescences (cones) of this plant produce secondary metabolites that impart bitterness, aromas, and antiseptic properties in beer, which is the third most consumed beverage in the world with an origin dating back to 6,000 b.C. (Bocquet et al. 2018; Teixeira and Silva 2019). In addition, hops are also notable as a medicinal plant (Biendl 2009; Franco et al. 2012; Bocquet et al. 2018; Hrnčič et al. 2019).

Germany, China, Ethiopia, the United States of America, and the Czech Republic are the main countries growing hops commercially to serve the beer production market, and Argentina is the only South American country presenting significant hop production (Faostat 2020). Some insect species belonging to different orders are hop crop pests in these countries, although these species do not include whiteflies (O'Neal et al. 2015; Dodds 2017; Allan-Perkins et al. 2019). However, there are two whitefly species associated with hops. *Trialeurodes vaporariorum* (Westwood) (Hemiptera: Aleyrodidae) and the two-spotted spider mite species *Tetranychus urticae* Koch (Acari: Tetranychidae) are common pests that affect hop crops grown under greenhouse conditions in New Zealand, which is a reference in organic hop production (MPI 2010; Turner et al. 2011). *Asterobemisia atraphaxius* (Danzig) (Hemiptera: Aleyrodidae) is another whitefly species associated with hops (Evans 2008), although its incidence is only registered in Europe, Iran, Russia, Kazakhstan and Korea (Martin and Mound 2007; Evans 2008; Suh 2009).

Although hops species are naturally grown in temperate regions worldwide, they are undergoing adaptation processes to be grown in other climates and present good yield under Brazil's edaphoclimatic conditions - hop crops have gained public prominence in the country less than five years ago, mainly due to small crops grown in the Mantiqueira Mountains. Currently, there are hop producers in such states as Minas Gerais, Paraná, Rio de Janeiro, Rio Grande do Sul, Santa Catarina, São Paulo and in the Federal District, although there are no estimates regarding the total hop production in Brazil (Fagherazzi et al. 2018; Teixeira and Silva 2019). The Rio de Janeiro state government has launched the first line of credit to encourage hop production to meet the growing demand for this product by artisanal breweries (Schuabb 2019).

Phytosanitary issues are expected to emerge due to the increased number of hop crop areas in Brazil. According to Teixeira and Silva (2019), such phytophagous arthropods as *Diabrotica speciosa* Germar (Coleoptera: Chrysomelidae) and the two-spotted spider mite species *T. urticae* infest hop crops grown in Cachoeiras de Macacu County, RJ. Spósito et al. (2019) have only reported leaf-cutting ants [*Atta sexdens* (L.), *Atta laevigata* (Smith) and *Acromyrmex* spp., Hymenoptera: Formicidae] as pests affecting commercial hop crops grown in Brazil, although these researchers did not specify regions or states. Nascimento et al. (2019) observed that phytosanitary issues associated with hop crops grown in the Horticulture Department field at UNESP (Botucatu County, SP) were caused by leaf-cutting ants and by two-spotted spider mites.

However, the technical and scientific literature lacks report on other insect species associated with hop crops grown in Brazilian states. The aim of this study was to report for the first time the use of *H. lupulus* as a host plant by whiteflies (Aleyrodidae) in Brazil.

## 2. Material and Methods

The study was conducted in four different counties in Rio de Janeiro state from 08/30<sup>th</sup>/2019 to 01/21<sup>st</sup>/2020: Cachoeiras de Macacu (latitude: 22°27'45" S, longitude: 42°39'11" W; altitude: 57 m), Cordeiro (latitude: 22°01'44" S, longitude: 42°21'39" W; altitude: 485 m), Nova Friburgo (latitude 22°16'55" S, longitude: 42°31'52" W; altitude: 846 m) and Seropédica (latitude 22°44'29" S, longitude: 43°42'19" W; altitude: 33 m).

Sampling in the first three counties was carried out in 1-year-old commercial hop crops; one property was studied per county. Sampling in Cachoeiras de Macacu County comprised a single hop variety (Cascade). Five hop varieties (Brazylinsk, Cascade, Hallertau, Saaz and Victoria) were sampled in Cordeiro County. Plants belonging to these same varieties, in addition to Nugget and Spalt, were sampled in a property in Nova Friburgo County. Sampling in Seropédica County was performed in two different locations: in the greenhouse of the Department of Soils (DS) of the Agronomy Institute of Federal Rural University of Rio de Janeiro (UFRRJ), where Cascade, Chinook, Columbus, Nugget and Saaz hop seedlings were inspected, and in the

Integrated System of Agroecological Production (SIPA - Sistema Integrado de Produção Agroecológica) (Neves et al. 2005), where 3 one-year-old Cascade hop plants were grown.

Leaves were randomly inspected with a 20x pocket magnifying glass; samples were collected when leaves were infested by nymphs belonging to different whitefly instars and packed in transparent 250-ml plastic pots to be transported to the laboratory of Ângelo Moreira da Costa Lima Entomological Collection (CECL) of the Entomology and Phytopathology Department (DEnF) of the Institute of Biological and Health Sciences (ICBS) at UFRRJ (Seropédica County, RJ), where they were identified by the research group's specialist, A.F. LIMA, based on several authors (Quaintance and Baker 1913, 1914, 1917; Baker and Moles 1921; Bondar 1923; Costa Lima 1928, 1942; Martin 1987, 1999, 2004, 2005, 2008; Hodges and Evans 2005; Dubey and Sundararaj 2015).

Fourth instar aleyrodid nymphs ('pupae') were removed from the leaf blade with the aid of an entomological pin and mounted on Hoyer's and/or Canada Balm medium between slides and coverslips. Slides were labeled with information regarding hop variety, location, date, and collector; next, the slides were placed in an oven to dry at 37°C for at least 48 hours. Species identification was performed under stereoscopic (Wild M 5 and Olympus SZ 40) and optical (Wild M 20 and Olympus BX 41) microscopes. Identified specimens were deposited at the CECL of DEnF/ICBS/UFRRJ.

Whitefly pupae-infested leaves of Cascade and Nugget seedlings kept in the DS/UFRRJ greenhouses were also photographed, placed in transparent 500-ml plastic pots with lids and kept in the laboratory of the Integrated Pest Management Center (CIMP)/DEnF/ICBS/UFRRJ under controlled environmental conditions (25 ± 1°C, 60 ± 10% RH and 12-h photoperiod) to enable researchers to observe the likely emergence of adult individuals.

### 3. Results and Discussion

Identified whiteflies (Hemiptera: Aleyrodidae) belonged to nine different genera: two belonged to the subfamily Aleurodicinae (*Aleurodicus* Douglas and *Nealeurodicus* Hempel), whereas seven belonged to the subfamily Aleyrodinae (*Aleurothrixus* Quaintance & Baker, *Aleurotrachelus* Quaintance & Baker, *Aleurotulus* Quaintance & Baker, *Bemisia* Quaintance & Baker, *Parabemisia* Takahashi, *Tetraleurodes* Cockerell and *Trialeurodes* Cockerell) (Table 1). Eight species and seven morphospecies (i.e., new species to be described in the future) were identified.

**Table 1.** List of whitefly species (Hemiptera: Aleyrodidae) collected in hops grown in four counties in Rio de Janeiro state from 08/30<sup>th</sup>/2019 to 01/21<sup>st</sup>/2020 (x = detected; - = not detected).

Whitefly	Cachoeiras de Macacu	Cordeiro	Nova Friburgo	Seropédica	
				DS	SIPA
1. <i>Aleurodicus pulvinatus</i> (Maskell)	-	-	-	x	-
2. <i>Aleurothrixus trachoides</i> (Back)	-	-	x	-	-
3. <i>Aleurotrachelus</i> sp.1	-	-	x	-	-
4. <i>Aleurotulus mundururu</i> Bondar	-	-	-	x	-
5. <i>Aleurotulus</i> sp.1	-	-	x	-	-
6. <i>Bemisia tabaci</i> (Gennadius)	x	x	x	x	-
7. <i>Bemisia tuberculata</i> Bondar	x	-	-	x	-
8. <i>Bemisia</i> sp.1	-	x	x	x	-
9. <i>Bemisia</i> sp.2	-	-	x	-	-
10. <i>Bemisia</i> sp.3	-	-	x	-	-
11. <i>Nealeurodicus moreirai</i> (Costa Lima)	-	x	x	-	-
12. <i>Parabemisia myricae</i> (Kuwana)	-	x	x	-	-
13. <i>Tetraleurodes</i> sp.1	-	-	x	-	x
14. <i>Trialeurodes vaporariorum</i> (Westwood)	-	x	x	-	-
15. <i>Trialeurodes</i> sp.1	-	-	x	-	-
Total number of species	2	5	12	5	1

Except for *T. vaporariorum*, the other whitefly species identified in the present study were reported in association with hops for the first time (Evans 2008; MPI 2010). However, all of these species have already

been reported to associate with other host plants in Brazil (Martin and Mound 2007; Sottoriva et al. 2011; Alonso et al. 2012; Trindade et al. 2012; Gamarra et al. 2016; Kumar et al. 2016; Ouvrard and Martin 2019).

*Bemisia tabaci* (Gennadius) was the only species recorded in all four investigated counties (Table 1). Therefore, this whitefly species was capable of infesting seedling leaves grown under greenhouse conditions (Seropédica County), as well as leaves of adult plants grown in the field (Cachoeiras de Macacu, Cordeiro and Nova Friburgo counties); however, this species was not found in adult plants grown at SIPA. The aforementioned species is distributed worldwide in association with more than 250 host plant species belonging to different families (Evans 2008). It is acknowledged as a species complex that is capable of causing severe damage to crops with significant global economic importance, mainly due to its ability to transmit phytoviruses (Navas-Castillo et al. 2011; Gilbertson et al. 2015). Bing et al. (2014) conducted a study in China and reported that *B. tabaci* infests *Humulus scandens* (Lour.) Merr. (Cannabaceae), which is known as Japanese or wild hops, although it is not commercially grown.

*Aleurothrixus trachoides* (Back), *Aleurotrachelus* sp.1, *Bemisia* sp.2, *Bemisia* sp.3 and *Trialeurodes* sp.1 were only recorded in Nova Friburgo County (Table 1). *Aleurothrixus trachoides*, commonly known as solanaceae whitefly or pepper whitefly, is an important pest affecting pepper crops in the United States. This polyphagous species is native to the neotropical region and widely distributed in Central and South America (including the Caribbean) and in India. Although this species is associated with more than 70 host plant species distributed in 34 botanical families, it prefers plants belonging to families Solanaceae and Convolvulaceae and palm trees belonging to genus *Chamaedorea* Willd. (Arecaceae) (Dubey and Sundararaj 2015; Kumar et al. 2016; Wosula et al. 2018; Ouvrard and Martin 2019).

The other identified species were simultaneously recorded in two or three counties. *Bemisia* sp.1 was recorded in three counties (Cordeiro, Nova Friburgo and Seropédica). Species simultaneously recorded in two counties comprised *Bemisia tuberculata* Bondar (Cachoeiras de Macacu and Seropédica), *Nealeurodicus moreirai* (Costa Lima), *Parabemisia myricae* (Kuwana) and *T. vaporariorum* (Cordeiro and Nova Friburgo). Nova Friburgo County has also recorded the incidence of *Tetraleurodes* sp.1, which was also found in Seropédica County. The largest number of Aleyrodidae species was recorded in Nova Friburgo County.

Alonso et al. (2012) were the first researchers to record *B. tuberculata* infesting cassava leaves (*Manihot esculenta* Crantz, Euphorbiaceae) in Rio de Janeiro state. This whitefly species has other plant hosts belonging to the family Euphorbiaceae (*Euphorbia hypericifolia* L.) and it also infests plants belonging to the family Fabaceae (*Erythrina* sp.) and Malvaceae (*Gossypium hirsutum* L.) (Evans 2008). Families Annonaceae (*Annona squamosa* L.), Sapotaceae [*Manilkara zapota* (L.) P. Royen] and Arecaceae (unidentified palm species) are known in Brazil as host plants to *N. moreirai* (Howard 2001; Evans 2008). *Parabemisia myricae*, which is associated with approximately 80 host plant species distributed in 35 families, was first reported in Japan in 1924; this species caused severe damage to mulberry crops in the following year and has subsequently spread to all continents and reached approximately 20 countries, including Brazil (Franco et al. 1996; Evans 2008; Sottoriva et al. 2011; Ouvrard and Martin 2019).

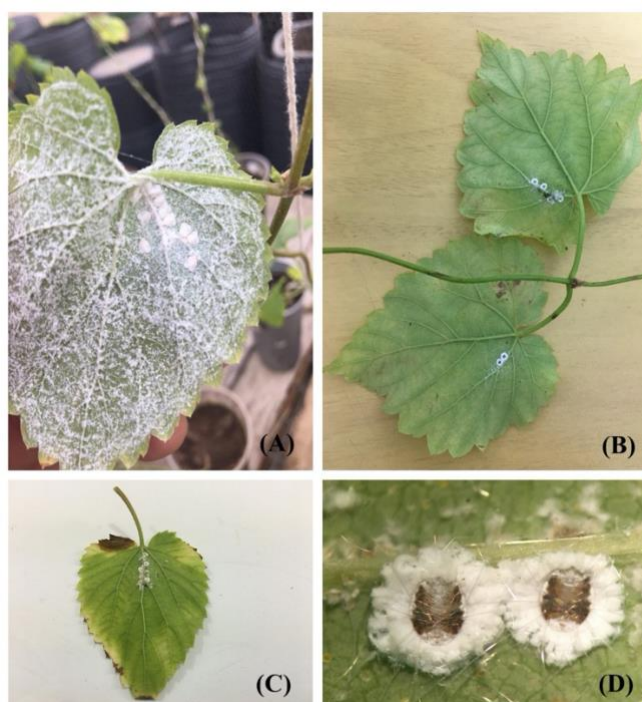
*Trialeurodes vaporariorum* is a common pest that affects hops grown under greenhouse conditions in New Zealand (MPI 2010). Based on the present study, this species was collected in the field in Cordeiro County in association with the hop varieties Saaz and Hallertau; the Hallertau hop variety was also infested by this aleyrodid insect in Nova Friburgo County (Table 2). This whitefly species is significantly distributed worldwide and is reported as a pest affecting vegetables and ornamental plants grown in more than 50 countries; however, this species has a wide list of host plants, which comprise approximately 270 species (Gamarra et al. 2016; Ouvrard and Martin 2019).

**Table 2.** Whiteflies associated with hop varieties grown in different counties in Rio de Janeiro state from 08/30<sup>th</sup>/2019 to 01/21<sup>st</sup>/2020.

Variety	Whitefly
Brazylnsk	<i>Bemisia tabaci</i> , <i>Bemisia</i> sp.2, <i>Parabemisia myricae</i> , <i>Trialeurodes</i> sp.1
Cascade	<i>Aleurodicus pulvinatus</i> , <i>Aleurotrachelus</i> sp.1, <i>Bemisia</i> sp.1, <i>Bemisia</i> sp.3, <i>Bemisia tabaci</i> , <i>Bemisia tuberculata</i> , <i>Nealeurodicus moreirai</i> , <i>Parabemisia myricae</i> , <i>Tetraleurodes</i> sp.1
Chinook	<i>Aleurodicus pulvinatus</i>
Columbus	<i>Bemisia tabaci</i> , <i>Bemisia tuberculata</i>
Hallertau	<i>Aleurothrixus trachoides</i> , <i>Bemisia tabaci</i> , <i>Parabemisia myricae</i> , <i>Trialeurodes vaporariorum</i>
Nugget	<i>Aleurodicus pulvinatus</i> , <i>Aleurotulus</i> sp1.
Saaz	<i>Aleurotulus mundururu</i> , <i>Bemisia</i> sp.2, <i>Bemisia tabaci</i> , <i>Parabemisia myricae</i> , <i>Tetraleurodes</i> sp.1, <i>Trialeurodes vaporariorum</i>
Spalt	<i>Bemisia</i> sp.2
Victoria	<i>Bemisia</i> sp.1, <i>Nealeurodicus moreirai</i> , <i>Parabemisia myricae</i>

The hop variety Cascade recorded the largest number of whitefly species ( $n = 9$ ) followed by Saaz ( $n = 6$ ), Brazylnsk and Hallertau ( $n = 4$ , each) (Table 2). Two whitefly species were collected in the hop varieties Columbus and Nugget. Chinook and Spalt were infested with a single whitefly species. Whiteflies belonging to the genus *Bemisia* Quaintance & Baker infested almost all hop varieties, except for Chinook and Nugget. *Aleurotulus mundururu* Bondar and *A. trachoides* infested one of the nine hop varieties, whereas *N. moreirai* infested two, and *P. myricae* infested five.

*Aleurodicus pulvinatus* (Maskell) and *A. mundururu* specimens were only collected from Cascade, Chinook, and Nugget seedlings in Seropédica County (Table 2). All the *A. pulvinatus* developmental stages (eggs, nymphs from different instars and adults, both male and female) were recorded in the leaves of these hop varieties (Figure 1). Fourth instar nymphs ('pupae') kept in Nugget and Cascade leaves were capable of developing into adult individuals in the laboratory. These results indicate that *H. lupulus* is a suitable plant species for *A. pulvinatus* development. However, further research is warranted to determine the potential of this whitefly species as a pest in hop plants based on its biology.



**Figure 1.** A - abaxial face of *Humulus lupulus* leaf (Chinook variety) infested by *Aleurodicus pulvinatus* adults on 9/27<sup>th</sup>/2019; B - abaxial face of *H. lupulus* leaves (Nugget variety) infested by *A. pulvinatus* nymphs on 10/21<sup>st</sup>/2019; C - abaxial face of *H. lupulus* leaf (Cascade variety) infested by *A. pulvinatus* nymphs near the petiole on 10/21<sup>st</sup>/2019; D - approximate view of 4<sup>th</sup> instar *A. pulvinatus* nymphs ('pupae') on the abaxial face of *H. lupulus* leaf (Nugget variety).

*Aleurotulus mundururu* was first identified in Brazil in the botanical family Melastomataceae (*Miconia* sp.) in 1923. Subsequent records also included species *Strychnos* sp. (Loganiaceae) as a host, as well as its incidence in Belize, Costa Rica, Ecuador, Guyana, and Trinidad (Evans 2008; Ouvrard and Martin 2019). *Aleurodicus pulvinatus*, which is native to Central America, South America and several Caribbean islands, is a pest species affecting coconut trees (*Cocos nucifera* L., Arecaceae) and many ornamental plants, although it is associated with approximately 19 botanical families (Kairo et al. 2001; Evans 2008; Ouvrard and Martin 2019).

#### 4. Conclusions

Whiteflies (Hemiptera: Aleyrodidae) belonging to nine genera and fifteen species (*Aleurodicus pulvinatus*, *Aleurothrixus trachoides*, *Aleurotrachelus* sp.1, *Aleurotulus mundururu*, *Aleurotulus* sp.1, *Bemisia tabaci*, *Bemisia tuberculata*, *Bemisia* sp.1, *Bemisia* sp.2, *Bemisia* sp.3, *Nealeurodicus moreirai*, *Parabemisia myricae*, *Tetraleurodes* sp.1, *Trialeurodes vaporariorum* and *Trialeurodes* sp.1) are associated with nine hop varieties (Brazylinsk, Cascade, Chinook, Columbus, Hallertau, Nugget, Saaz, Spalt and Victoria) distributed in Cachoeiras de Macacu, Cordeiro, Nova Friburgo and Seropédica counties (RJ). This study presents the first record of aleyrodids in *Humulus lupulus* in Brazil.

**Authors' Contributions:** FERNANDEZ, P.S.C.: conception and design, acquisition of data, analysis and interpretation of data, writing the original draft; AGUIAR-MENEZES, E.L.: conception and design, acquisition of data, analysis and interpretation of data, critical review of important intellectual content; LIMA, A.F.: acquisition of data, analysis and interpretation of data, identifying the insect species, critical review of important intellectual content; FRADE, P.M.R.: conception and design, acquisition of data; TEIXEIRA, A.J.: conception and design, critical review of important intellectual content; BUCHER, C.A.: conception and design, critical review of important intellectual content. All authors have read and approved the final version of the manuscript.

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**Ethics Approval:** Not applicable.

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