Natural parasitism of *Diaphorina citri* Kuwayama (Hemiptera, Psyllidae) nymphs by *Tamarixia radiata* Waterston (Hymenoptera, Eulophidae) in São Paulo orange groves

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ABSTRACT. Natural parasitism of *Diaphorina citri* Kuwayama (Hemiptera, Psyllidae) nymphs by *Tamarixia radiata* Waterston (Hymenoptera, Eulophidae) in São Paulo orange groves. The psyllid *Diaphorina citri* Kuwayama 1908 has become the main citrus pest species in the state of São Paulo, Brazil, after the introduction of the huanglongbing or citrus greening. This study evaluated the parasitism of 3rd, 4th and 5th instar *D. citri* nymphs by *Tamarixia radiata* (Waterston, 1922) in citrus groves under a regimen of regular insecticide applications in ten producing regions: Araraquara, Barretos, Bauru, Botucatu, Franca, Itapetininga, Jaú, Limeira, Lins and São João da Boa Vista. Sixty-nine samples of new branches infested with nymphs of *D. citri* were collected from 2005 to 2008 in orange groves ranging from 1 to 20 years old, of the varieties Hamlin, Pera, Valencia and Natal. The parasitoid *T. radiata* is widely distributed in São Paulo orange groves, and was identified in 50 (72%) of the samples, showing a mean parasitism rate of 12.4%. The highest parasitism rate was observed in the "summer" (from January through March), with a mean of 25.7%. Nymphal parasitism was above 90% in two samples. The probable causes of the variations in parasitism of *D. citri* by *T. radiata* are discussed.

KEYWORDS. Biological control; psyllid; *Citrus*, ectoparasitoid.

RESUMO. Parasitismo natural de ninfas de *Diaphorina citri* Kuwayama (Hemiptera, Psyllidae) por *Tamarixia radiata* Waterston (Hymenoptera, Eulophidae) em pomares de laranja em São Paulo. O psilídeo *Diaphorina citri* Kuwayama 1908 tornou-se a principal praga dos citros no estado de São Paulo após a introdução do *huanglongbing* ou *grenning* dos citros. Este estudo avaliou as proporções de ninfas de terceiro a quinto ínstares de *D. citri* parasitadas por *Tamarixia radiata* (Waterston, 1922) em pomares de laranja submetidos a pulverizações constantes de inseticidas em dez regiões produtoras, Araraquara, Barretos, Bauru, Botucatu, Franca, Itapetininga, Jaú, Limeira, Lins e São João da Boa Vista. Foram coletadas 69 amostras de ramos jovens infestados com ninfas de *D. citri* obtidas entre 2005 e 2008 em pomares de 1 a 20 anos de idade, das cultivares Hamilin, Pera, Valencia e Natal. O parasitóide *T. radiata* encontra-se amplamente distribuído em pomares de São Paulo, tendo sido observado em 50 amostras (72%), com taxa média de parasitismo de 12.4%. O maior parasitismo foi verificado no "verão" (janeiro a março) com média de 25.7%. O parasitismo de ninfas superou 90% em duas amostras. São discutidas as causas prováveis das variações entre o parasitismo de *D. citri* por *T. radiata*.

PALAVRAS CHAVE. Controle biológico; psilídeo; *Citrus*, ectoparasitóide.

The citrus psyllid, *Diaphorina citri* Kuwayama 1908 (Hemiptera, Psyllidae), has become the main citrus pest in the state of São Paulo in the past few years, after the introduction of the bacteria associated with *huanglongbing* (HLB, citrus greening), *Candidatus* Liberibacter asiaticus and *Candidatus* Liberibacter americanus (Coletta-Filho *et al.* 2004; Teixeira *et al.* 2005), both transmitted by this insect (Aubert 1987b; Bové 2006). Aggressive chemical control of the vector with the aim of excluding it from citrus groves has been recommended as part of programs to reduce HLB damage. However, frequent and intensive use of insecticides has already resulted in a lower population susceptibility to the main insecticides (Tiwari *et al.* 2011), as well as an increase in non-target pests, especially scales, and also a significant increase in citrus production costs (Fukuda *et al.* 2010).

Although *D. citri* has been recorded in Brazil for more than 70 years (Costa Lima 1942), it was considered a minor pest because of the direct damage that it caused, and has been relatively little studied. Until 2004, there were no records of the occurrence of parasitoids or predators of this species (Aubert 1987a; Paiva 1996), until nymphs parasitized by the eulophid *Tamarixia radiata* (Waterston, 1922) (Hymenoptera, Eulophidae) were found in several regions of São Paulo state (Gómez Torres *et al.* 2006). Biological control of *D. citri* has been considered insufficient for disease control (Halbert & Manjunath 2004), although there are cases of successful classical biological control (Aubert 1987b; Etienne *et al.* 2001). The parasitoid *T. radiata* was recently introduced into the Americas, where its efficiency on *D. citri* has varied considerably, with high parasitism rates in Puerto Rico, with a mean of 70%, reaching 100% in some locations (Pluke *et al.* 2008), and very low rates in the U.S.A. (Florida), between 1 and 3% (Michaud 2004; Qureshi & Stansly 2009). In São Paulo, early estimates of parasitism rates varied from 27.5 to 80% (Gómez Torres *et al.* 2006; Parra *et al.* 2010).

The idiobiont ectoparasitoid *T. radiata* is native to northern India, where it develops on *D. citri* nymphs, preferentially those in the 3rd to 5th instars (Etienne *et al.* 2001), and is considered a specialist parasitoid (Zuparko *et al.* 2011). The female *T. radiata* also kills its host by feeding, and one female can cause the death of up to 500 nymphs from feeding and parasitism (Hoy & Nguyen 2001). The life cycle of *T. radiata* in *D. citri* is slightly more than 10 days at 25°C, a temperature which results in the highest reported parasitism rate, a mean of 167 psyllid nymphs per female (Parra *et al.* 2010).

Since *T. radiata* was first recorded in Brazil, samples of citrus psyllid nymphs have been collected from various areas to estimate its abundance and efficiency. The objective of this study was to estimate the parasitism rates of large nymphs of *D. citri* by *T. radiata* in orange groves under a regime of frequent insecticide applications, mainly to control *D. citri*, in the main producing regions of São Paulo, during a four- year period.

MATERIAL AND METHODS

Groves of the four main varieties of sweet oranges *Citrus sinensis* Osbeck grown in São Paulo: Hamlin, Pera, Valencia and Natal, were sampled from the beginning of 2005 until mid-2008 to obtain estimates of nymphal parasitism in the citrus psyllid *D. citri* by the eulophid *T. radiata*. Nineteen groves, managed according to current horticultural practices, were chosen, distributed in ten regions: Araraquara, Barretos, Bauru, Botucatu, Franca, Itapetininga, Jaú, Limeira, Lins and São João da Boa Vista.

The samples consisted of new orange-tree branches with large nymphs of *D. citri*, preferably 4th and 5th instars, which were removed and placed in paper bags. Sampling was carried out randomly in the plot where the psyllid occurred, for about one hour. Samples with more insects were collected from those plots with the highest nymphal infestations; and where infestations were low, the samples contained fewer insects. Sixty-nine samples were collected, with a mean sample size of 195 nymphs, ranging from 19 to 972 insects.

The groves were between 1 and 20 years old, with the age being the difference between the planting year and the year when the trees were sampled. To study the effect of the time of year on parasitism, the January-March period was adopted as austral "summer", with 22 samples; from April through June as "autumn", with 21 samples; July through September as "winter", with 8 samples; and October through December as "spring", with 18 samples. The municipalities where the samples were taken were grouped according to the classification of agricultural regions by the state Department of Agriculture.

Parasitism was evaluated the same day as the samples were taken, using a stereoscopic microscope. The parasitized nymphs contained eggs or young larvae of the parasitoid on their bodies, or they were mummified. The percentage parasitism rates were calculated from the ratio between the number of parasitized nymphs and the total number of parasitized and non-parasitized nymphs.

In some areas, in addition to collecting samples to quantify parasitism, psyllid infestation was estimated by sampling 60 new branches and calculating the ratio of new branches with nymphs to all instars and adult psyllids, with the aim of relating parasitism to host infestation.

The decimal data for the number of parasitized nymphs and the total number of nymphs were transformed, $y = \text{arc sine } x^{1/2}$, and submitted to analyses of variance with the F test, comparing the effect of the year, the time of year (season), the region, the grove age and the orange variety, as well as the dependence of parasitism on estimated host density. The software SAS (2000) was used. The seven samples collected from groves with more than one orange variety were excluded from the analysis of the effect of this variable.

RESULTS

Sixty-nine samples were collected from 2005 to 2008. A total of 13,474 *D. citri* nymphs of the 3rd to the 5th instars were observed during the study, of which 1,672 nymphs (12.4%) were parasitized by *T. radiata*. The distribution of samples in the regions and between years is given in Table I. Of the 69 samples, 19 had no parasitized *D. citri* that is slightly more than one-quarter of the samples. A larger group of samples, 36.2%, showed parasitism rates varying between 1 and 10%, and 10.1% of the samples showed parasitism percentages between 11 and 20%. Thus, almost 75% of the samples had no *T. radiata* or had a parasitism rate below 20%.

Table I. Number of samples of new branches of orange trees with *Diaphorina citri* nymphs, in orange groves in 10 regions of the state of São Paulo, taken during each year of the study.

	Region	2005	2006	2007	2008
North	Barretos	5	1		1
	Franca		1	4	2
Center	Araraquara	6	2	3	
	Bauru	3		4	
	Jaú	1			1
South	Botucatu	1	1	3	
	Itapetininga			1	1
	Limeira	5	3	2	
	S J Boa Vista	2			
West	Lins	11		4	1

On the other hand, parasitism rates between 31 and 50% were observed in nine samples, (13% of the total), and seven (10%) of the samples had more than 50% parasitism. In two samples, more than 90% of the nymphs were parasitized.

There was a negative relationship between the parasitism rate (% between parasitized and total nymphs) and the percentage of branches with *D. citri* nymphs ($F = 4.7024$, $df = 1,27$, $p = 0.0390$), but there was no relationship between the nymphal parasitism rate and the percentage of branches infested with adult *D. citri* ($F = 0.1223$, $df = 1,25$, $p = 0.7295$). Therefore, for lower nymph infestations, parasitism rates were the highest for this stage, suggesting that even in low *D. citri* infestations there may be *T. radiata* parasitism under field conditions.

The highest parasitism rate during the four-year study period was in 2008, with a mean of 35.5% of nymphs parasitized; and the lowest rate was in 2007, with 5.4% (Table II). In 2008, one of the samples collected in the Jaú region, municipality of Itajú, in February, had 91.7% parasitism. On the other hand, in 2007, the highest percentage, 32.4%, was observed in the Limeira region, municipality of Analândia, in September. In 2005 and 2006, intermediate parasitism rates were recorded.

The season with the highest parasitism rate of *D. citri* nymphs was the summer (January through March), when temperatures are high and rainfall is abundant in São Paulo, resulting in many new branches being available for citrus psyllid feeding and oviposition. The mean rate observed during this period was 25.7%, followed by autumn (April through June) with 14.6% of nymphs with *T. radiata,* although one sample in each season showed more than 90% parasitism.

There was no statistical difference in the parasitism rates between regions, suggesting that *T. radiata* is well adapted to the climatic conditions where oranges are grown in São Paulo (Table II). The two samples with more than 90% parasitism were found in the central region, one in Itajú and the other in Pederneiras municipality.

Two characteristics of the orange groves where samples were taken were compared: grove age and the orange variety. There were no statistically significant differences in the observed parasitism rate between the grove age ranges compared, even though the mean rate observed in 4 to 6-year-old groves was 24.6%, almost four times higher than the estimated 6.6% rate in the younger 1 to 3-year-old groves. However, the orange variety significantly affected parasitism, with high parasitism levels observed in nymphs on the early-maturing Hamlin variety compared to the later-maturing Valencia and Natal varieties.

DISCUSSION

The ectoparasitoid *Tamarixia radiata* is the best studied natural enemy of *Diaphorina citri* in the world, and has been introduced into several countries for classical biological control, including Réunion, China, Indonesia, Malaysia and, more recently, the U.S.A., in 1998, and into Guadeloupe in 1999 (Chien *et al.* 1989; Etienne *et al.* 2001; Hoy & Nguyen 2001). In other countries of the Americas (Puerto Rico, Mexico, Brazil and Argentina), *T. radiata* was only recorded after its introduction into the U.S.A. and Guadeloupe.

In the 1980s, Aubert (1987a) found no parasitoids in the *D. citri* populations of three Brazilian states, Bahia, São Paulo and Sergipe. The same author found that these populations were highly parasitized by *T. radiata* in the laboratory, and recommended its introduction into Brazil.

The most successful case of biological control of *D. citri* by *T. radiata* has been described in Reunion Island, and attributed to the local absence of hyperparasitoids (Aubert 1987b; Etienne *et al.* 2001). In the U.S.A., low levels of parasitism of *D. citri* were attributed to the high predation of parasitized nymphs (Michaud 2004; Qureshi & Stansly 2009). Since no hyperparasitoids or even predators of *D. citri* nymphs have

Table II. Percentage of parasitism of 4th and 5th instar nymphs of *Diaphorina citri* by *Tamarixia radiata*, between 2005 and 2008, in orange groves of different ages and varieties in São Paulo.

		n	mean	min	max	value F[1]	df (treat,res)	p
Year	2005	34	20.0	0.0	91.6	3.5445	3,65	0.0192
	2006	8	12.8	0.0	67.7			
	2007	21	5.4	0.0	32.4			
	2008	6	35.5	0.0	91.7			
"Season"	"spring" (Oct-Dec)	18	10.7	0.0	67.7	3.0722	3,65	0.0338
	"summer" (Jan-Mar)	22	25.7	0.0	91.7			
	"autumn" (Apr-Jun)	21	14.6	0.0	91.6			
	"winter" (Jul-Sep)	8	5.6	0.0	32.7			
Region	North	14	13.3	0.0	66.7	0.4388	3,65	0.7259
	Center	20	20.9	0.0	91.7			
	West	16	11.4	0.0	43.7			
	South	19	16.9	0.0	67.7			
Grove age (yr)	1-3	15	6.6	0.0	67.7	2.3331	3,65	0.0821
	4-6	20	24.6	0.0	91.6			
	7-9	17	12.6	0.0	66.7			
	10 +	13	18.6	0.0	91.7			
Orange variety	Hamlin	11	35.6	1.0	91.7	4.3048	3,58	0.0082
	Pera	15	20.1	0.0	67.7			
	Valencia	31	9.1	0.0	43.7			
	Natal	5	7.3	0.0	30.4			

[1] Analyses of variance, by the F test, with the ratios of the number of parasitized nymphs/total nymphs transformed to arc sine $x^{1/2}$.

been observed in Brazil, the low parasitism rates observed in this study could be due to the absence of nymphs for feeding and parasitism by *T. radiata.* Intensive insecticide use for psyllid control, including aerial applications, may kill the parasitoids (Hall & Nguyen 2010) and limit parasitoid action. Genetic differences between the *T. radiata* populations in Central and North America have recently been found (Barr *et al.* 2009), which may be another cause for the differences in efficiency.

Estimates of parasitism rates based on the ratio of parasitized and total nymphs or on the ratio of emerged *T. radiata* adults to *D. citri* represent an attempt to quantify the efficiency of the natural enemy in field conditions. However, these rates may not represent the true importance of the natural enemy as a mortality factor and regulator of the insect-pest population. In the case of *T. radiata,* the nymphal mortality caused by female feeding is greater than the parasitism itself (Skelley & Hoy 2004), and this factor was not evaluated in the present study.

However, parasitism rates in the field are easily obtained and, as in the present study, can be estimated in different locations over time. Although samples with no parasitism or with rates of less than 20% predominated, *T. radiata* is widely distributed throughout the orange-producing regions of São Paulo, and should be protected in integrated pest and disease management programs in citrus.

The highest percentages of parasitism were observed in São Paulo in the summer and autumn, and the lowest percentage in the winter. However, in Florida, U.S.A., parasitism rates were lower than 20% in spring-summer and increased to 39 to 56% in the autumn (Qureshi *et al.* 2009). In China, a 36% parasitism rate was observed on new branches in the summer, and 46% on branches in the autumn (Yang *et al.* 2006). The results of the present study also showed that there were no differences in parasitism between the northern, central, western and southern regions of São Paulo, confirming the wide distribution of *T. radiata*. Considering that this insect was probably introduced by the end of the 1990s, there has already been wide natural dispersal.

In younger groves (1–3 years old), parasitism rates were one-fourth of the rates found in mature groves (4–6 years old), although the differences were not statistically significant. Young trees may be less suitable for *T. radiata* parasitism, or these groves may have been under more aggressive psyllid control regimens, since the damage caused by HLB is more severe in young plants.

The psyllid *D. citri* develops differently on different species of *Citrus* and other genera of Rutaceae, such as *Murraya* (Nava *et al.* 2007). However, there was no difference in *D. citri* development between the Pera and Natal varieties of *C. sinensis* (Nava *et al.* 2010). The parasitoid can use the host plant of its prey to locate it, and therefore, the Hamlin variety may be more attractive than the Valencia and Natal varieties, although this hypothesis must be validated by further research.

The occurrence of *T. radiata* in São Paulo orange groves depends on the presence of host nymphs, preferably those in the 4th and 5th instars. However, the persistence of the parasitoid in the environment appears to be incompatible with the current situation of HLB in São Paulo, where some growers have not eliminated diseased trees, thus increasing the disease reservoir and requiring those growers who have eliminated the inoculum to use insecticides even more aggressively to control the vector.

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