Natural Parasitism of the Citrus Leafminer (Lepidoptera: Gracillariidae) Over Eight Years in Seven Citrus Regions of São Paulo, Brazil

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Natural parasitism of the citrus leafminer (Lepidoptera: Gracillariidae) over eight years in seven citrus regions of São Paulo, Brazil

Paulo Eduardo Branco Paiva, and Pedro Takao Yamamoto*

Abstract

The citrus leafminer (CLM) *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) was first recorded in Brazil in 1996. In 1998, the parasitoid *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae) was introduced and established in many regions of the country. In this study, 130 one-hour-samplings of sweet orange leaves (*Citrus sinensis* [L.] Osbeck) hosting CLM pupal chambers were carried out to estimate the CLM parasitism rate (%) by its parasitoids in 7 regions of São Paulo State between 2000 and 2008. The sample sizes varied from 10 to 275 leaves (mean = 65). The most abundant parasitoid was the encyrtid *A. citricola* (found in 91.8% of the samplings). The highest level of CLM parasitism by *A. citricola* was recorded in the southern region (Botucatu), 70.2 ± 6.6 (mean ± SEM), and the lowest level was recorded in the northern region (Barretos), 12.8 ± 5.7%. CLM parasitism by *A. citricola* and by native parasitoids (*Galeopsomyia fausta* LaSalle, *Cirrospilus* spp. and *Elasmus* sp.) did not differ between seasons. The 6-fold increase in the use of insecticides in citrus groves, after 2004 when the Huanglongbing (HLB) disease was found in São Paulo State, did not reduce the level of CLM parasitism. The level of parasitism was 50.8 ± 4.2% before the advent of HLB (2000–2004) and 56.0 ± 4.4% thereafter (2005–2008), indicating adaptation of *A. citricola* in a disturbed agroecosystem.

Key Words: biological control; *Ageniaspis citricola*; *Phyllocnistis citrella*; insecticide; HLB management

Resumo

A minadora das folhas dos citros (MFC), *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae), foi encontrada pela primeira vez no Brasil em 1996. Em 1998, o parasitoide *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae) foi introduzido e se estabeleceu em várias regiões do país. Nesse estudo, foram feitas130 amostragens, de uma hora, de folhas de laranjeiras doces [*Citrus sinensis* (L.) Osbeck] com câmaras pupais da MFC, para se estimar o parasitismo da MFC em 7 regiões do estado de São Paulo, entre 2000 e 2008. O tamanho das amostras variou de 10 a 275 folhas (média = 65). O parasitoide mais abundante foi o encirtídeo *A. citricola* (encontrado em 91.8% das amostragens). O maior parasitismo da MFC por *A. citricola* foi observado na região sul do estado (Botucatu), 70,2 ± 6,6 (média ± EPM), e o menor parasitismo na região norte (Barretos), 12,8 ± 5,7%. O parasitismo da MFC por *A. citricola* e seus parasitoides nativos (*Galeopsomyia fausta* LaSalle, *Cirrospilus* spp. and *Elasmus* sp.) não diferiram entre as estações do ano. O aumento de seis vezes no uso de inseticidas nos pomares de citros, após 2004, quando o Huanglongbing (HLB) foi encontrado no estado de São Paulo, não reduziu o nível de parasitismo da MFC. O nível médio de parasitismo foi de 50,8 ± 4,2%, antes do HLB (2000-2004), e 56,0 ± 4,4%, após o HLB (2005-2008), indicando a adaptação de *A. citricola* a um agroecossistema perturbado.

Palavras chaves: controle biológico; *Ageniaspis citricola*; *Phyllocnistis citrella*; inseticida; manejo de HLB

The citrus leafminer (CLM) *Phyllocnistis citrella* Stainton (Lepidoptera: Gracillariidae) was first recorded in Brazil in March 1996 in São Paulo State (Prates et al. 1996). Larvae of *P. citrella* may reduce the foliar area by compromising new leaf growth and by reduced photosynthesis in the damaged leaves (Schaffer et al. 1997). Necrosis and defoliation also are induced by the larvae, thus hindering plant development, particularly in younger plants. In addition to the direct damage, the presence of the CLM may facilitate the incidence and increase the severity of citrus canker, a disease caused by the bacterium *Xanthomonas citri* subsp. *citri* (Xanthomonadales: Xanthomonadaceae) (Chagas et al. 2001; Jesus Jr. et al. 2006; Hall et al. 2010).

Hymenoptera of the genera *Galeopsomyia*, *Cirrospilus* (Eulophidae), *Elasmus* (Elasmidae), *Pachyneuron* (Pteromalidae), and *Telenomus* (Scelionidae) were the first CLM parasitoids identified in citrus groves in Brazil (Penteado-Dias et al. 1997). Other parasitoids were subsequently reported, including *Horismenus* spp. (Eulophidae), *Eupelmus* sp. (Eupelmidae), and *Conura* sp. (Chalcididae) (Costa et al. 1999). After the introduction of CLM in the Americas, 3 new species belonging to the Eulophidae family were described: *Cirrospilus floridensis* Evans, *Cirrospilus neotropicus* Diez and Fidalgo, and *Galeopsomyia fausta* LaSalle (LaSalle & Peña 1997; Evans 1999; Diez & Fidalgo 2003). All 3 species occur in Brazil and were found to be the most frequent parasitoids in São Paulo from 1997 to 1999, particularly the eulophid *G. fausta* (Paiva et al. 2000; Sá et al. 2000).

In 1998, the encyrtid *Ageniaspis citricola* Logvinovskaya (Hymenoptera: Encyrtidae) was introduced in Brazil, as part of a classical biologi-

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cal control program. This species was soon established in several Brazilian states (São Paulo, Paraná, Minas Gerais, Rio Grande do Sul, Santa Catarina, Goiás, Bahia, Rio de Janeiro, Piauí, and Rio Grande do Norte) (Chagas et al. 2002; Parra et al. 2004). It became the most common CLM parasitoid, with native parasitoids occurring at lower levels (Sá et al. 2000). The estimated *A. citricola* parasitism was approximately 30% in post-release samples (1998–2000) (Chagas et al. 2002).

The combination of the damage caused by CLM and the occurrence of citrus canker highlighted the importance of the management and control of this insect, which generally involves only chemical control in nurseries and young trees. The use of cupric bactericides to prevent the occurrence of canker and insecticides for leafminer control became usual in endemic areas. However, the use of insecticides for CLM control has been incompatible to its biological control efforts in many cases (Villanueva-Jiménez & Hoy 1998; Villanueva-Jiménez et al. 2000).

In Brazil, the use of insecticides in citrus groves increased dramatically after Huanglongbing (HLB) was found in 2004, as part of the HLB mitigation, aimed at controlling the insect vector *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), and,we hypothesized that this could strongly affect the CLM parasitism. From 2003 to 2008, the use of insecticides in citrus, mainly in São Paulo State, increased 6-fold (Neves et al. 2010). In this scenario, the aims of this study were (i) to estimate the CLM parasitism by *A. citricola* and native parasitoids in citrus-growing regions of São Paulo State (northern, northeastern, central, western, and southern) between 2000 and 2008, and (ii) to compare how 2 periods, i.e., prior to HLB with lower number of insecticide sprays and after HLB with higher number of sprays, affected CLM parasitism.

Materials and Methods

A total of 130 samplings were carried out in sweet orange (*Citrus sinensis* [L.] Osbeck) groves (Hamilin, Pera, and Valencia cultivars) located in different regions of São Paulo State, Brazil (Fig. 1). Samples were taken from Dec 2000 to Nov 2008 in northern (Barretos, 1 grove), northeastern (Franca, 6 groves), central (Bauru, 3 groves; Casa Branca, 2 groves; São Carlos, 4 groves), western (Lins, 4 groves), and southern (Botucatu, 4 groves) regions of the state (Tables 1 and 2). The leaves with intact CLM pupal chambers were collected in a random sampling during 60 min in 4 ha orange groves. According to the availability of infested leaves, we obtained samples with an average of 65 (10 to 275) pupal chambers.

The parasitism rate was obtained from each sample, despite the variable sample size. The leaves were collected and stored in plastic bags with 4 holes (DAC® 25 ´ 36 cm) containing wet cotton to prevent the desiccation of the leaves and pupae. The leaves were then transferred to the laboratory on the same day of sampling for parasitoid identification and parasitism rate estimation. The pupal chambers were opened, and the pupae found were separated for identification. The identification of *P. citrella*, *A. citricola*, and *G. fausta* was possible from the pupal morphology. The pupae of other species were transferred to properly closed Petri dishes with moist filter paper until adult emergence. The emerged parasitoids were identified by comparison with reference collections and with keys proposed by Penteado-Dias et al. (1997) and Schauff et al. (1998). The parasitism rate was calculated as the proportion (percentage) of pupal chambers with the presence of parasitoid related to the pupal chamber inspected.

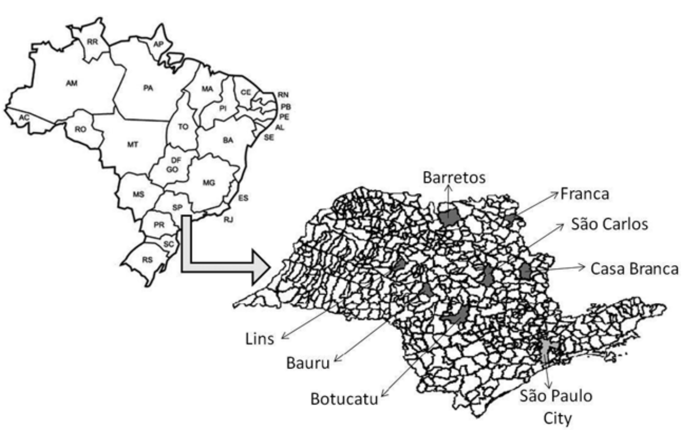

Fig. 1. Location of São Paulo State in Brazil and the citrus regions sampled.

These orange groves were rarely sprayed with insecticides before 2004—if needed, only once a year with petroleum oil or dimethoate. After 2005, insecticide use was intensified, from 12 to 24 times a year, to suppress HLB vector *D. citri*, using different materials (i.e., beta-cyfluthrin, bifenthrin, chlorpyrifos, cypermethrin, dimethoate, fenpropathrin, phosmet, imidacloprid, pyriproxifen, thiamethoxam).

The data were organized by region and season: Jan to Mar (summer), Apr to Jun (fall), Jul to Sep (winter), and Oct to Dec (spring). Data (parasitism rates) were transformed by arcsine (sqrt (x+100) and compared using analysis of variance with the F test ($P < 0.05$).

Results

Of the 130 samplings, *A. citricola* was found in 119 (91.5%), and native parasitoids were recorded in 45 (34.6%). The most frequent native parasitoid was the eulophid *G. fausta*, which was present in 44 samples (33.8%). *Cirrospilus* sp., another eulophid, occurred in 17 samples (13.1%). The parasitoid *Elasmus* sp. was the rarest and was present in only 2 samples (1.5%).

Because only 1 sample was obtained in 2002, this rate was not included in Fig. 2. The lowest CLM parasitism rates by *A. citricola* were estimated for 2000 and 2007, when few samples were obtained. In 2000, the parasitism rate was 21.0 ± 16.7% (mean ± SEM; $n = 4$), and in 2007 it was 24.9 ± 10.0% ($n = 6$) (Fig. 2). In the other 6 years, the annual CLM parasitism rates ranged from 49.7 ± 7.7% in 2003 to 63.5 ± 5.7% in 2005 (Fig. 2). Differences in CLM parasitism by *A. citricola* ($F = 4.44$; df = 6, 123; $P = 0.0004$) and native hymenopterans ($F = 5.12$; df = 6, 123; $P = 0.0001$) were detected when the sampled regions were compared (Table 3). The parasitism rate by *A. citricola* was lower in the Barretos (northern) than in the other regions (Barretos ´ other regions: $F = 19.29$; df = 1, 128; $P < 0.0001$). In other regions, CLM parasitism rates by introduced parasitoids did not differ (Bauru ´ Botucatu ´ Casa Branca ´ Franca ´ Lins ´ São Carlos: $F = 1.34$; df = 5, 112; $P = 0.25$). The parasitism by native species was significantly higher in Franca (northeast) with 9.4 ± 2.3% (Franca ´ other regions: $F = 25.45$; df = 1, 128; $P < 0.001$). In all other regions, the parasitism rates by native species were lower (< 5%) (Table 3) and similar among them (Barretos ´ Bauru ´ Botucatu ´ Casa Branca ´ Lins ´ São Carlos: $F = 1.31$; df = 5, 103; $P = 0.27$).

CLM parasitism by *A. citricola* was similar between seasons (Table 3). The estimated rates were 60.0 ± 4.2% in summer, 43.8 ± 5.3% in spring, 43.0 ± 7.8% in winter, and 54.4 ± 13.5% in fall. The largest samples were collected during spring and summer ($n = 45$ and 60, respectively), most likely due to the greater availability of new citrus flush, resulting in higher numbers of hosts. The occurrence of native CLM parasitoids was low during all seasons, approximately 4.0%, and did not differ among the seasons ($F = 1.20$; df = 3, 126; $P = 0.31$) (Table 3).

The estimated CLM parasitism by *A. citricola* before HLB (from 2000 to 2004, $n = 77$) did not differ significantly from the parasitism after HLB (from 2005 to 2008, $n = 53$) ($F = 0.71$; df = 1, 128; $P = 0.40$). The parasitism rates ranged from 0% to 100% in both periods, indicating a high variability of CLM parasitism by *A. citricola* in the sampled areas.

Table 1. Month, year, and number of samplings for citrus leafminer parasitism in São Paulo orange groves.

Region	Month.year (samplings)	Sample size (*n*)	No. of samplings
Barretos	I.2001(4), II.2001(3), X.2001(1), XII.2003(3), I.2007(1)	16 to 25	12
Bauru	XII.2000(1), I.2001(4), II.2001(4), XII.2001(1), XI.2002(1), XII.2003(1), X.2005(1), II.2006(2), XI.2006(1), II.2008(1)	11 to 179	17
Botucatu	XII.2000(1), II.2001(4), I.2003(2), V.2004(1), XII.2005(2), II.2005(5), XII.2006(1), II.2008(2), III.2008(2)	22 to 118	20
Casa Branca	XII.2000(1), I.2001(3), II.2001(1), I.2003(2), XI.2004(3), I.2005(1), VIII.2005(1), XI.2006(2)	10 to 200	14
Franca	II.2004(1), XII.2004(4), IX.2005(2), XI.2005(4), XII.2006(1), III.2007(1), IV.2007(2), II.2008(1), IV.2008(1), VI.2008(1), VII.2008(1), IX.2008(1), XI.2008(1)	29 to 275	21
Lins	XII.2000(1), I.2001(2), II.2001(8), XII.2003(2), IX.2005(1), XI.2005(3), V.2008(1), VI.2008(1), XI.2008(1)	22 to 130	20
São Carlos	I.2001(2), II.2001(2), XII.2001(1), I.2003(8), III.2003(2), XI.2003(1), XII.2003(1), IX.2005(1), X.2005(2), IX.2006(1), XI.2006(1), X.2007(1), XI.2007(1), III.2008(1), IX.2008(1)	42 to 120	26

The increased use of insecticides in citrus groves, after 2004, when HLB was reported, did not reduce the level of CLM parasitism in Bauru, Botucatu, Casa Branca, Franca, Lins, and São Carlos (Table 4).

Discussion

A shift from the native ectoparasitoids of CLM to the introduced endoparasitoid *A. citricola* was observed in São Paulo. Before this insect was introduced in Brazil, the niche was temporarily occupied by eulophid insects, particularly *G. fausta* and *Cirrospilus* sp., and less frequently by *Elasmus* and *Horismenus*. However, *A. citricola* has prevailed in São Paulo since 2001. The *G. fausta* occurrence, before the introduction of *A. citricola*, was reduced from 98% to 38%, and 60% parasitism was found for the introduced parasitoid (Sá et al. 2000). The replacement of CLM native parasitoids by *A. citricola* also occurred in Florida (USA) after its introduction in 1994. In Florida, *A. citricola* parasitism rates increased from 2% to 86%, and the parasitism rates by native species decreased from 30% to 2% (Pomerinke & Stansly. 1998).

The CLM parasitized by *A. citricola* in *C. sinensis* groves in São Paulo State was stable from 2001 to 2006 (excluding 2002), ranging from 55% to 65%. The contribution of native eulophid insects (*G. fausta* and *Cirrospilus* sp.) was low, with a parasitism rate of 4.0%. After the introduction of *A. citricola*, the parasitism rate of this introduced species was estimated to exceed 30% in the southern and central regions of São Paulo (Chagas et al. 2002). In fact, the parasitism rate reached 76%, even in areas with pesticide application (Parra et al. 2004).

The smallest CLM parasitism rate by *A. citricola* was recorded in Barretos, a northern region of São Paulo, where high temperatures and low relative humidity prevail throughout the year. Thus, the climate may have exerted a negative effect on the introduced parasitoid. Chagas et al. (2002) reported an average parasitism rate of only 4.4% (varying from 0 to 14%) for the same region, and it was suggested that this was most likely due to the high humidity requirements of *A. citricola* adults (Edwards & Hoy 1998). Thus, the less favorable northern climate did not hinder the establishment of the insect but may have limited its efficiency. According to the Köppen classification, the weather on the São Paulo plateau (north of São Paulo State) is Aw and Cw, with little or no rainfall in fall and winter. The annual precipitation ranges from 1,500 mm to 2,000 mm, in spring and summer. Additionally, the average temperatures in the north region are higher than those in the south region.

The increased incidence of citrus canker in São Paulo observed in 1999 was associated with the introduction of CLM in Brazil in 1996 (Belasque Jr. et al. 2010). A connection between insect damage and disease was later described and characterized by Chagas et al. (2001) and Jesus Jr. et al. (2006). It is assumed that the CLM introduction effectively completed the pathosystem, causing changes in the epidemiology and spatial distribution of citrus canker. During the decade 2000, the incidence of the disease dropped considerably (Belasque Jr. et al. 2010). While CLM contributed to the increased incidence of citrus canker, the parasitoid *A. citricola* was of paramount importance for (indirectly) reducing the disease during that decade.

The increased use of insecticides in citrus after the identification of HLB in Brazil (Neves et al. 2010) in 2004 did not seem to affect negatively the parasitism of CLM by *A. citricola*. The *A. citricola* parasitism rates were similar before and after HLB. CLM is abundant in São Paulo citrus groves and may occur when flush is available. The similar presence of *A. citricola* throughout the year indicates that flush is available

Table 2. Mean temperature (°C), annual precipitation (mm), altitude (meters above sea level), and the Köppen classification of São Paulo citrus-growing regions.

	Temperature (°C)				
Region	Minimum	Maximum	Precipitation (mm)	Altitude (m)	Köppen classification
Barretos	17.4	31.8	1,254	540	Aw
Bauru	16.0	29.1	1,331	530	Aw
Botucatu	15.3	26.1	1,359	840	Cwa
Casa Branca	15.1	28.0	1,310	679	Aw
Franca	15.8	27.0	1,594	1000	Aw
Lins	16.3	29.5	1,300	484	Aw
São Carlos	15.3	27.0	1,423	830	Cwa

Source: http://www.cpa.unicamp.br/outras-informacoes/clima-dos-municipios-paulistas.html (last accessed 16 Dec 2014).

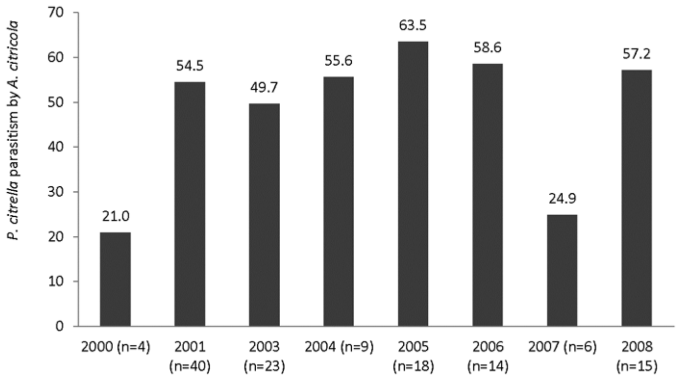

Fig. 2. Annual parasitism of *Phyllocnistis citrella* by *Ageniaspis citricola* in São Paulo orange groves (*n* = samplings).

for CLM and, consequently, for its parasitoids. Thus, an adequate availability of CLM hosts may lead to higher *A. citricola* parasitism rates (Jahnke et al. 2006).

Parasitism of early stages of CLM by *A. citricola,* i.e., of eggs and 1st instars (Edwards & Hoy 1998), may allow this parasitoid to occupy the niche before the native parasitoids, which parasitize only the final stages of the insect cycle (Foelkel et al. 2008). No studies regarding CLM parasitoid competition in Brazil have been reported. It remains unknown whether larvae parasitized by the specialist *A. citricola* may also be parasitized by generalist eulophids or if eulophids are able to recognize and avoid an already-parasitized host. Lioni & Cividanes (2004) previously reported hyperparasitism of *A. citricola* pupae by *G. fausta*. Additionally, the predation of CLM larvae by feeding *C. neotropicus* adults has been shown in the laboratory, contributing to increased mortality (Foelkel et al. 2008).

The *G. fausta* eulophid is a koinobiont ectoparasitoid. This is an unusual combination because the female does not paralyze or kill the host at oviposition, increasing the risk of mortality of the offspring (Llácer et al. 2005). This behavior can reduce the number of hosts killed by *G. fausta*, although it typically parasitizes the pupa. The native host of *G. fausta* is unknown. However, it was found very soon after *P. citrella* was introduced in America (LaSalle & Peña 1997).

The highest parasitism rate by native Hymenoptera was recorded in the northeastern region of São Paulo State (Franca). In this region, there are large areas of coffee cultivation, and citrus production was initiated recently. One of the Eulophidae species that occurs on the coffee leafminer, *Leucoptera coffeella* (Lepidoptera: Lyonetiidae), is *C. neotropicus* (Melo et al. 2007), which also occurs on *P. citrella* (Jahnke et al. 2005, 2007; Foelkel et al. 2008). However, *G. fausta*, which has not been reported to occur on *L. coffeella*, was the predominant species found in orange groves in the northeastern region. Therefore, the high parasitism rate by native species in this region does seem to be related not to the coffee cultivation but to the initial citrus groves.

The higher occurrence of native parasitoids may be related to the groves' age. In 2 samples from a 1-yr-old orange grove collected in Jan 2003 in São Carlos, the parasitism rates by *G. fausta* and *A. citricola* were 22% and 17%, respectively. In a 10-yr-old grove, at the same location and date, only parasitism by *A. citricola* was observed, 88.0%. This finding suggests that generalist parasitoids parasitize the first CLM infestations in young plants. As the orange trees grow, however, generalist parasitoids are replaced by *A. citricola*, the more competitive species.

The CLM mortality caused by arthropod predators is significant, and in some regions these predators are more important than parasitoids (Amalin et al. 2002; Xiao et al. 2007). Native biological control agents, predators, and generalist parasitoids should be evaluated, and possibly the introduction of specialist parasitoids should be avoided, even for exotic insect pests (Michaud 2002). In Brazil, native natural predators and parasitoids have not been adequately studied. Particularly in São Paulo, the role of predators and the efficiency of native parasitoids were limited. Lioni & Cividanes (2004) found that the key factors influencing CLM mortality were *A. citricola* parasitism and predation in 3rd instars. Thus, *A. citricola* parasitism (estimated to be between 55% and 65%) and the additional contribution of the native parasitoid species have helped control CLM, even in highly disturbed agricultural environments with intense insecticide use and other chemical inputs, indicating that the natural biological control (conservation) is not always disrupted by chemical control.

Table 3. Parasitism rates (mean ± standard error of mean) of *Phyllocnistis citrella* by *Ageniaspis citricola* and native parasitoids in 7 São Paulo citrus-growing regions and in different seasons of the year.

		A. citricola		Natives	
Region	*n*[1]	Mean (± SEM)	Range	Mean (± SEM)	Range
Barretos (northern)	12	12.8 (± 5.7)	0–70.6	0.9 (± 0.7)	0–8.8
Bauru (central)	17	59.2 (± 8.6)	0–100	2.5 (± 1.2)	0–17.6
Botucatu (southern)	20	70.2 (± 6.6)	0–100	3.2 (± 1.1)	0–18.0
Casa Branca (central)	14	62.3 (± 9.5)	10.2–100	1.5 (± 1.0)	0–12.0
Franca (northeastern)	21	56.5 (± 6.0)	0–92.3	9.4 (± 2.3)	0–39.0
Lins (western)	20	44.5 (± 7.7)	0–100	0.5 (± 0.3)	0–4.5
São Carlos (central)	26	52.6 (± 7.2)	0–100	3.1 (± 1.2)	0–22.0
F, *P* value (df = 6, 123)		$F = 4.44$, $P = 0.0004$		$F = 5.12$, $P = 0.0001$	
Season	*n*	Mean (± SEM)	Range	Mean (± SEM)	Range
Spring	45	43.8 (± 5.3)	0–95.0	3.6 (± 1.1)	0–39.0
Summer	69	60.0 (± 4.2)	0–100	2.5 (± 0.6)	0–22.0
Fall	7	54.4 (± 13.5)	13.6–100	6.7 (± 3.9)	0–24.0
Winter	9	43.0 (± 7.8)	0–80.0	5.5 (± 2.6)	0–20.7
F, *P* value (df = 3, 126)		$F = 2.96$; $P = 0.034$		$F = 1.20$; $P = 0.31$	

[1]: samplings

Table 4. Parasitism rates (mean ± standard error of mean) of *Phyllocnistis citrella* by *Ageniaspis citricola* in 7 São Paulo citrus-growing regions, before (2000–2004) and after (2005–2008) HLB detection.

Region	Low insecticide use 2000–2004		Intensive insecticide use 2005–2008		
	n[1]	Mean (± SEM)	*n*[1]	Mean (± SEM)	*F*, df res, *P* value
Barretos (northern)	11	7.8 (± 2.5)	1	70.6 (-)	—
Bauru (central)	12	56.6 (± 10.1)	5	47.9 (± 18.4)	0.08,15,0.87
Botucatu (southern)	9	65.2 (± 10.7)	11	64.3 (± 8.7)	0.00,18,0.98
Casa Branca (central)	10	58.1 (± 11.8)	4	49.5 (± 18.2)	0.05,12,0.81
Franca (northeastern)	5	61.7 (± 5.6)	16	49.3 (± 7.2)	2.29,19,0.15
Lins (western)	13	38.2 (± 9.5)	7	46.9 (± 13.7)	0.34,18,0.56
São Carlos (central)	17	53.4 (± 9.1)	9	43.2 (± 12.1)	0.68,24,0.41

[1]: samplings

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