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Survival analyses of *Diaphorina citri* immatures on young citrus orchard in São Paulo, Brazil

Análises de sobrevivência de imaturos de **Diaphorina citri** *em pomar jovem de citros em São Paulo, Brasil*

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ABSTRACT: The survival of eggs and nymphs of *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) was estimated on young plants (< 1 year) of *Citrus sinensis* (cultivar Valencia) grafted on *Citrus limonia* in 3 seasons (Winter and Spring 2013; and Autumn 2014) in Ribeirão Preto, São Paulo State, Brazil. Young shoots with eggs were selected from natural or artificial infestations. Eggs, small nymphs (1st to 3rd instar), and large nymphs (4th and 5th instar) were counted every 3 days. Shoots with large nymphs were caged until the emergence of the adults. The lowest eggs and nymph survival were observed during Winter (0.0%), and Autumn (0.4%); the highest survival rates were recorded during Spring (12.2%). The biotic factor of mortality identified was the parasitism of large nymphs by *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae), observed in all seasons. Air temperatures (minimum and maximum), and air relative humidity (minimum) could not explain the immature survival differences between Spring (12.2%) and Autumn (0.4%). Rainfall during Spring (165 mm) was higher than during Autumn (48 mm) and may have favored the survival of *D. citri* in the Spring. Adverse abiotic factors seem to be more important on immature *D. citri* survival than the biotic factor (parasitism).

KEYWORDS: Asian citrus psyllid; HLB; natural mortality; ecological life table; *Tamarixia radiata*.

RESUMO: A sobrevivência de ovos e ninfas de *Diaphorina citri* Kuwayama (Hemiptera: Liviidae) foi estimada em plantas jovens (< 1 ano) de *Citrus sinensis* (cultivar Valencia) enxertadas em *Citrus limonia* em 3 estações (inverno e primavera de 2013, e outono de 2014) em Ribeirão Preto, estado de São Paulo, Brasil. Ramos novos com ovos foram selecionados a partir de infestações naturais ou artificiais. Ovos, ninfas pequenas (ínstar 1 ao 3) e ninfas grandes (ínstar 4 e 5) foram contados a cada 3 dias. Ramos com ninfas grandes foram colocados em gaiolas até a emergência dos adultos. As menores sobrevivências de ovos e ninfas foram observadas no inverno (0,0%) e no outono (0,4%), e a maior sobrevivência ocorreu na primavera (12,2%). O principal fator biótico de mortalidade identificado foi o parasitismo de ninfas grandes por *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae), observado em todas as estações. As temperaturas (mínima e máxima) e umidade relativa (mínima) do ar não explicaram as diferenças nas sobrevivências de imaturos entre a primavera (12,2%) e o outono (0,4%). As chuvas na primavera (165 mm) foram maiores que no outono (48 mm) e podem ter favorecido a sobrevivência de *D. citri* na primavera. Fatores abióticos adversos parecem ser mais importantes na sobrevivência de imaturos de *D. citri* que o fator biótico (parasitismo).

PALAVRAS-CHAVE: psilídeo asiático dos citros; HLB; mortalidade natural; tabela de vida ecológica; *Tamarixia radiata*.

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INTRODUCTION

The Asian citrus psyllid, *Diaphorina citri* Kuwayama (Hemiptera: Liviidae), was reported in Brazil in the early 1940s (LIMA, 1942); however, it only became a citrus major pest after Huanglongbing (HLB) was reported, in 2004. This psyllid is the only known insect vector of the bacteria associated to plants with the disease symptoms, "*Candidatus* Liberibacter asiaticus", and "*Ca.* L. americanus" (BOVÉ, 2014), which causes the early fall and reduction of fruit size (BASSANEZI et al., 2011). In addition to the severe loss in fruit production, it leads to an increase in production costs with the adoption of recommended mitigation measures (STANSLY et al., 2014).

D. citri adults may occur in any period or season; however, eggs and nymphs are observed when there are new flushes and leaves in citrus (HALL et al., 2008). In São Paulo State, before it became the target of management programs, the largest adult populations of *D. citri* occurred during Spring and Summer (YAMAMOTO et al., 2001). The frequent presence of new flushes in young plants may favor the survival of *D. citri*, and an early HLB infection (HALBERT; MANJUNATH, 2004). Therefore, newly planted citrus trees have been protected with long acting systemic insecticides (QURESHI et al., 2014), and techniques that avoid the access of insects to citrus plants have been adopted (CROXTON; STANSLY, 2014; MIRANDA et al., 2015).

The populations of *D. citri* are regulated by ecological factors of biotic nature: competition, natural enemies and host plant, and abiotic nature: environmental conditions (YANG et al., 2006). Among the natural enemies of *D. citri*, the parasitoid *Tamarixia radiata* Waterston (Hymenoptera: Eulophidae) was reported as the most important biological control agent (PLUKE et al., 2008), and it was the only natural enemy observed in citrus areas of São Paulo (PAIVA; PARRA, 2012a). Although predators are important in the control of immature *D. citri* in other citrus areas (MICHAUD, 2004; QURESHI; STANSLY, 2009), there is no evidence that they are important in Brazil (São Paulo state).

In contrast to what occurs under artificial conditions, where immature individuals of *D. citri* have high survival rates (> 70%) (NAVA et al., 2007), these immatures have presented low survivals rates in citrus under natural conditions; less than 10% in Florida, USA, (MICHAUD, 2004), and less than 20% in São Paulo, Brazil (PAIVA; PARRA, 2012a). A high mortality of *D. citri* was observed under adverse artificial conditions by the combination of high temperatures and low air relative humidity (MCFARLAND; HOY, 2001), or in unsuitable host plants (BORGONI et al., 2014; HALL et al., 2015). Considering new planted citrus trees are highly attractive to *D. citri* adults due to the constant emission of new flushes, and are also susceptible to HLB infection, the survival of immature *D. citri* was estimated and compared in different seasons.

MATERIAL AND METHODS

Survival analyzes were carried out on orange trees younger than 1 year old (*Citrus sinensis*), cultivar Valencia, grafted on *Citrus limonia*. These trees were planted in June 2013, among orange trees (4 years old), cultivar Pera, in Ribeirão Preto, São Paulo state, Brazil (21°12'17"S, and 47°52'17"W). The citrus area was located at 630 m above sea level, in an eutrophic latosol with clay texture; and the climate, according to the classification by Köppen, was type Aw — tropical, with warm and humid Summers, and mild Winters with severe droughts.

Observations occurred in three periods: from July 1st to July 25th (Winter season); from October 18th to November 22nd (Spring season), 2013; and from March 23rd to April 18th (Autumn season), 2014. The trees were irrigated with 3 mm of water (drip system) in a daily basis, in dry periods. At the beginning of the experiments, the plants were pruned and fertilized (50 g of calcium nitrate per plant), and pesticides were not applied during the study.

In July 2013 (Winter), *D. citri* eggs were not observed, and new flushes were artificially infested. In each young flush, 5 adults of *D. citri* were caged for 2 days. The insects were reared in *Murraya paniculata*, at the facility of Escola Superior de Agricultura "Luiz de Queiroz" da Universidade de São Paulo (ESALQ-USP), in Piracicaba City, São Paulo. Fifty-seven flushes of 24 orange trees were infested during Winter. In October (Spring), in 13 orange trees, 52 flushes were naturally infested, and 30, artificially infested. In March of 2014 (Autumn), in 8 orange trees, 42 flushes with eggs were selected after a natural infestation.

Infested flushes were marked with a white line and a numbered label. Live eggs and nymphs were counted with a magnifying glass aid (10 ×) every 3 days. Small nymphs were considered from the 1st to 3rd instars, and large nymphs were those from the 4th and 5th instars. Branches with large nymphs received a cage, a voile tissue bag of 30 × 50 cm, until adult emergence. Emerged adults were withdrawn and counted. Nymphs parasitized by *T. radiata* were identified by characteristic mummification.

Daily data of maximum and minimum temperatures, air relative humidity (ARH) at 3 p.m., and rainfall were obtained in an automatic meteorological station located at 200 m from the citrus area. For all seasons, the number of days with:

- minimum temperature below 13.5°C, a lower base temperature, estimated by NAVA et al. (2007);
- maximum temperature above 32.0°C, temperature unsuitable to development of *D. citri* immatures (NAVA et al., 2007); and
- ARH less than 30%, a condition in which the survival of nymphs is very low (PARRA et al., 2010) were calculated.

The egg-adult duration was calculated by the weighted mean, considering the number of emerged adults and the period (days) between egg stage and adult emergence. To build the life table, the model proposed by SOUTHWOOD (1978) was adopted. The number of dead insects (dx) at each stage was obtained by the difference between the number of live insects (lx) of the stage and the number of live insects in the next stage (lx1). Mortality was obtained through the relation between dead and living insects at each stage (dx / lx), and survival, through their difference (Sx = 1 - dx / lx). The parasitism of *D. citri* nymphs by *T. radiata* was calculated by the ratio (%) between mummified nymphs and large nymphs.

RESULTS

The survival of *D. citri* eggs was low in the 2013 Winter. After artificial infestations, 567 eggs were obtained, of which only 15 nymphs (2.6%) hatched (Table 1). Inviable eggs, initially of a light-yellow color, became dark in 2 or 3 days. The survival of small nymphs was 20%, and no large nymphs were observed in this season. Thus, with a mean minimum temperature of 12.7°C and maximum of 26.0°C, and 42.8% ARH at 3 p.m., no *D. citri* adults emerged during Winter (Table 2 and Fig. 1).

In the Spring of 2013, with minimum and maximum temperatures of 17.8 and 30.5°C, and minimum ARH of 41.6% (Table 2 and Fig. 1), the survival of *D. citri* immatures was higher than in the Winter of 2013, and in the Autumn of 2014 (Tables 1 and 2). From 705 eggs obtained in 82 branches (30 artificially and 52 naturally infested), 268 nymphs hatched (38.0%) (Table 1). From 705 eggs, 86 *D. citri* adults were obtained (12.2%) (Table 1). From oviposition, that occurred

Table 1. Life table for *Diaphorina citri* immatures on young trees (< 1 year old) of *Citrus sinensis* cultivar Valencia, during July 2013 and April 2014, in Ribeirão Preto City, São Paulo state, Brazil.

Stage x	Individuals lx	Deads dx	Mortality dx/lx	Survival rates Sx	Factor dxF
2013 Winter[1]					
eggs	567	552	0.974	0.026	weather and infertility
small nymphs	15	12	0.800	0.200	weather and host
large nymphs	3	1	0.333	0.667	parasitism
large nymphs	2	2	1.000	0.000	weather and host
adults	0				
2013 Spring					
eggs	705	437	0.620	0.380	weather and infertility
small nymphs	268	66	0.246	0.754	weather and host
large nymphs	202	20	0.099	0.901	parasitism
large nymphs	182	96	0.527	0.473	weather and host
adults	86				
2014 Autumn					
eggs	487	372	0.764	0.236	weather and infertility
small nymphs	115	91	0.791	0.209	weather and host
large nymphs	24	6	0.250	0.750	parasitism
large nymphs	18	16	0.889	0.111	weather and host
adults	2				

[1]Winter: July 1st to 25th, 2013; Spring: October 18th to November 22nd, 2013; Autumn: March 22nd to April 18th, 2014.

Table 2. Climatic variables of seasons, survival of *Diaphorina citri* immatures in young trees (< 1 year old) of *Citrus sinensis* cultivar Valencia, and parasitism of nymphs of *D. citri* by *Tamarixia radiata* in Ribeirão Preto, São Paulo state, Brazil.

Season	Climatic variables							Survival (%)				Parasitism
	Rainfall[1]	Min. temp.[2]	Days < 13.5[3]	Max. temp.[4]	Days > 32.0[5]	ARH 3 pm[6]	Days < 30%[7]	Eggs	Small nynphs	Large nymphs	Egg-adult	Parasitized/ total (%)
Winter[8]	29	12.7	2/25	26.0	0/25	42.8	3/25	2.6	20.0	0.0	0.0	1/3 (33)
Spring	165	17.8	0/36	30.5	27/36	41.6	7/36	38.0	75.4	42.6	12.2	20/202 (10)
Autumn	48	18.5	0/27	29.9	21/27	45.9	1/27	23.6	20.9	8.3	0.4	6/24 (25)

[1]total rainfall (mm); [2]minimum temperature average (°C); [3]days with temperature below 13.5°C/period; [4]maximum temperature average (°C); [5]days with temperature above 32.0°C/period; [6]air relative humidity average (%) at 3 p.m.; [7]days with air relative humidity below 30%/period; [8]Winter: July 1st to 25th, 2013, Spring: October 18th to November 22nd, 2013, Autumn: March 22nd to April 18th, 2014.

for one week, there was an emergence of adults in two weeks. The estimated egg-adult duration was 18.8 days in the Spring of 2013. In this season, egg-adult survival rates varied among the 13 citrus trees, and in six of them there was no emergence of adults (Table 3). The presence of large nymphs did not guarantee *T. radiata* parasitism, observed in 4 out of 9 citrus trees. *D. citri* parasitism rates, by *T. radiata* during Spring, was 9.9% (20/202), and 25% (6/24) during Autumn, despite the lower large nymph density in this season (Tables 1 and 3).

In the Autumn of 2014, with temperatures similar to those of the Spring of 2013, and slightly higher ARH (45.9%) (Table 2), the survival of *D. citri* immatures was low (Table 1).

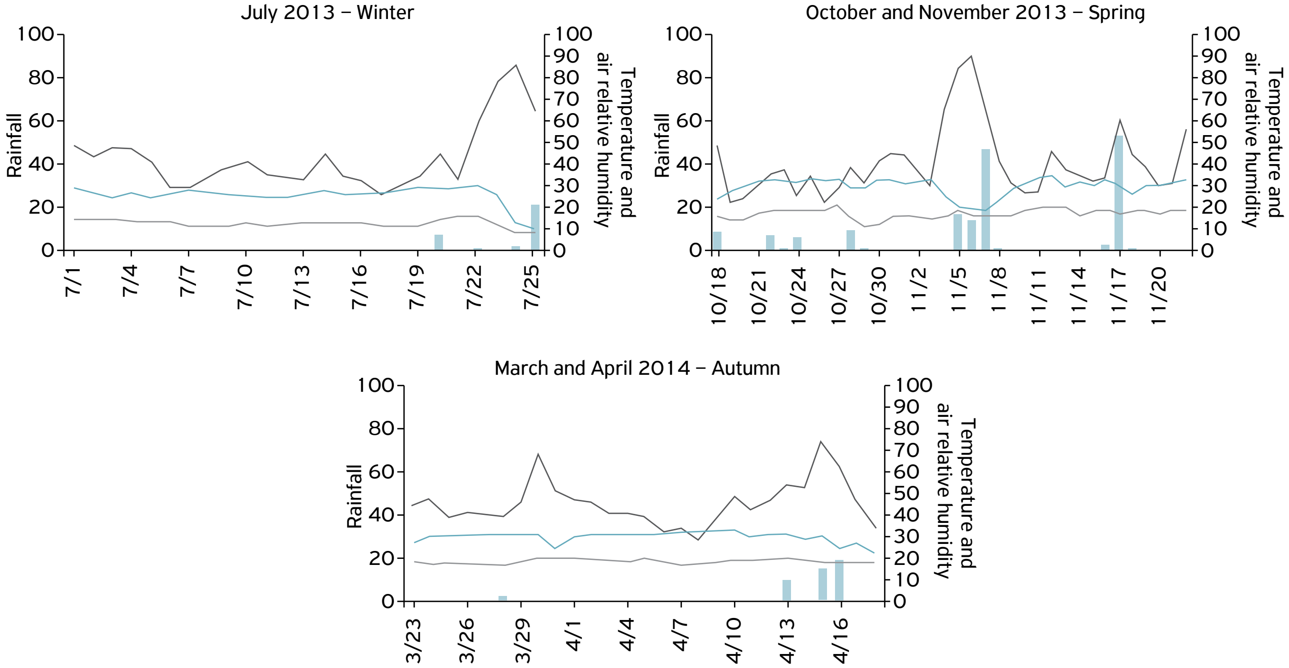

Figure 1. Daily data: rainfall (mm) (blue bar), maximum temperature (°C) (gray line), minimum temperature (°C) (purple line), air relative humidity (%) at 3 p.m. (blue line), during all seasons in Ribeirão Preto City, São Paulo state, Brazil.

Table 3. Number and survival of *Diaphorina citri* immatures during spring of 2013, and parasitism by *Tamarixia radiata* in 13 individual young trees (< 1 year old) of *Citrus sinensis* cultivar Valencia in Ribeirão Preto, São Paulo state, Brazil.

Tree	Number of *D. citri*					Survival (%)				Parasitism
	Eggs	Small nymphs	Large nymphs	Parasitized nymphs	Adults	Eggs	Small nymphs	Large nymphs	Egg-adult	(Parasitized/total)
1	115	58	49	10	23	50.4	84.5	46.9	20.0	10/49
2	66	10	4	0	3	15.2	40.0	75.0	4.5	0/4
3	27	20	17	0	11	74.1	85.0	64.7	40.7	0/17
4	36	2	1	0	0	5.6	50.0	-	0.0	0/1
5	76	28	10	4	2	36.8	35.7	20.0	2.6	4/10
6	82	16	10	1	4	19.5	62.5	40.0	4.9	1/10
7	7	0	0	0	0	0.0	-	-	0.0	-
8	24	14	12	0	0	58.3	85.7	-	0.0	0/12
9	113	95	88	0	38	84.1	92.6	43.2	33.6	0/88
10	51	2	0	0	0	3.9	0.0	-	0.0	-
11	12	4	0	0	0	33.3	0.0	-	0.0	-
12	72	19	11	5	5	26.4	57.9	45.5	6.9	5/11
13	24	0	0	0	0	0.0	-	-	0.0	-
	705	268	202	20	86	38.0	75.4	42.6	12.2	20/202

From 487 eggs, only 2 adults (0.4%) emerged. Despite similar temperatures and ARHs in Spring and Autumn, rainfall was higher and more frequent in the Spring of 2013 (165 mm) than in the Autumnof 2014 (48 mm) (Table 2 and Fig. 1). *D. citri* eggs survival was higher during Spring than Winter and Autumn (Tables 1 and 2). The survival of small nymphs was similar during Winter (20%) and Autumn (21%) (Table 1), and the survival of large nymphs was higher during Spring (47.3%) than Winter (0.0%) and Autumn (11.1%) (Table 1). Only during 2 days of Winter, the minimum temperature was below the base temperature (13.5°C) estimated for this specie. In Spring and Autumn, during most of the study period, maximum temperatures were above 32.0°C (Table 2), a thermal condition that would limit immature development.

DISCUSSION

Under natural conditions with thermal and hydric variations, which directly influence the insect and the host plant, the survival of *D. citri* eggs and nymphs was low in this study. In a 4 years old Valencia sweet orange trees, PAIVA; PARRA (2012a) reported survival variation of *D. citri* immatures from 10% in Spring (2006) and Autumn (2007) to 20% in Summer and late Autumn (2007). The mortality of eggs and small nymphs was the key phase for the population growth of this insect.

The results obtained under field conditions differ from those obtained under ideal conditions for insect development (25°C, and 60 − 80% ARH, constants). Under controlled conditions, the host plant did not affect the survival of *D. citri* eggs and nymphs; 75% of the immature *D. citri* survived in *M. paniculata* plants, which originated from seeds (LIU; TSAI, 2000). As to *C. limonia*, the immatures survival was of 72% (NAVA et al., 2007). Similarly, in Valencia sweet orange grafted on *C. limonia*, the survival rate was 66% (ALVES et al., 2014), and greater than 60% in Pera and Natal oranges (NAVA et al*.*, 2010; BORGONI et al*.*, 2014). In our study, in the field, for *C. sinensis* trees younger than 1 year old , grafted on *C. limonia*, there was no immature survival of *D. citri* in the Winter of 2013, and only 0.4% of them reached their adult life in the Autumn of 2014.

The higher survival of eggs and nymphs may explain the higher population density and population peaks of *D. citri* during Spring and Summer (YANG et al., 2006; YAMAMOTO et al*.*, 2001). During Autumn and Winter, the population was reduced, probably due to ecological factors, such as competition, natural enemies, host plants, and abiotic nature (YANG et al., 2006). Although *D. citri* adults occur at any period and season, eggs and nymphs were only observed when there were citrus flushes. Moreover, population outbreaks can occur if environmental conditions are favorable and new shoots are available (HALL et al., 2008).

The frequent presence of flushes in young plants may favor the survival of *D. citri* and an early HLB infection (HALBERT; MANJUNATH, 2004). Therefore, in new plantations, young trees are rigorously protected with systemic insecticides of long residual action (QURESHI et al., 2014), or foliar insecticide sprays, based on a fixed schedule (BELASQUE JUNIOR et al., 2010). Additionally, methods that prevent insect access to plants may be adopted (CROXTON; STANSLY, 2014; MIRANDA et al., 2015).

Adults of *D. citri* exhibit a high tolerance to extreme temperatures, and can survive in low-temperature conditions (0 or 5°C for 1 day) (EL-SHESHENY et al., 2016), as well as oviposit in warm environments (35°C), depositing about 30 eggs within 48 hours (HALL et al., 2011). Similarly, *D. citri* adults can survive in very dry environments (MCFARLAND; HOY, 2001). This fact may explain the peak population of *D. citri* during late Winter and early Spring, as observed by YAMAMOTO et al. (2001) for the conditions of São Paulo.

D. citri parasitism by *T. radiata* occurred in all seasons of the study, even in very low densities from the third to the fifth instar nymphs (2013 Winter, and 2014 Autumn). In previous research, the highest rates of parasitism were observed during Summer (25%) and Autumn (15%), and the lowest, during Spring (11%) and Winter (6%) (PAIVA; PARRA, 2012b). The data obtained in this study for Spring were similar to those obtained by these authors, indicating that this season is the most favorable to the development of *D. citri*, and that the lower parasitism by *T. radiata* may be one key factor for the greater population observed during Spring.

In addition to the parasitoids, the role of predators in the regulation of the *D. citri* population should be considered, mainly of nymphs, a stage with low mobility and restricted to new shoots. MICHAUD (2002) considered the contribution of predators to the biological control of *D. citri* greater than that of *T. radiata* in Florida (USA), and suggested that parasitism could be overestimated, because mummified (parasitized) nymphs are not predated and remain longer in citrus leaves. The *T. radiata* parasitoid, as far as it is concerned, is the only natural enemy associated with *D. citri* in São Paulo (PAIVA; PARRA, 2012a).

The low survival of eggs and small nymphs, and additional parasitism of the fourth and fifth instars by *T. radiata* may limit the natural growth of *D. citri* population in citrus. Although abiotic factors are not enough to prevent the spreading of HLB pathogens, they play an important role in the natural mortality of eggs and small nymphs, preventing the insect from reaching high populations in São Paulo citrus orchards.

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