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RESEARCH ARTICLE

Relative importance of natural enemies and abiotic factors as sources of regulation of mealybugs (Hemiptera: Pseudococcidae) in Brazilian coffee plantations

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Abstract

In the present study, we determined the critical stages and the key factors of mortality for *Planococcus citri* (Hemiptera: Pseudococcidae) in Brazilian coffee plantations using a life table to understand the role of natural biological control on its population. Predators, parasitoids, rainfall, sunlight, physiological disturbances and fungal diseases were collectively responsible for 98.79% in the total mortality of *P. citri*. Predators belonging to the Chrysopidae, Syrphidae, Dolichopodidae and Coccinellidae families were the most important mortality factors in the early developmental stages of *P. citri* (i.e. eggs and 1st and 2nd instar nymphs), whereas predators belonging to the Coccinellidae and Chrysopidae families were the most important mortality factors for the last instars (i.e. 3rd instars and adults) for *P. citri*. The generalist predators *Harmonia axyridis*, *Chrysoperla genanigra* and *Chrysoperla externa* were the key mortality factors for *P. citri*. The third nymph stage was considered the critical life stage (i.e. the life stage that most influences population size). Our results show that generalist predators and climatic factors are important sources of natural mortality of *P. citri* governing the population dynamics of this pest in the field.

Introduction

Understanding the factors that regulate insect pest populations is of fundamental importance in the development of suitable pest management systems. Among these factors, natural enemies, weather conditions and host-plant attributes are the most important factors affecting the population dynamics of pest species. Several studies have explored the roles played by both biotic and abiotic mortality factors, such as weather and natural enemies, on the regulation of arthropod pests in tropical regions (Pereira *et al.*, 2007*a*,*b*; Semeão *et al.*, 2012*a*,*b*; Rosado *et al.*, 2014). However, the magnitude of these mortality factors may be different for each insect species, such as those described in several studies of the ecological life tables for *Tuta absoluta* (Miranda *et al.*, 1998), *Diaphania hyalinata* (Gonring *et al.*, 2003*a*), *D. nitidalis* (Gonring *et al.*, 2003*b*), *Leucoptera coffeella* (Pereira *et al.*, 2007*b*), *Triozoida limbata* (Semeão *et al.*, 2012*b*) and *Coccus viridis* (Rosado *et al.*, 2014).

Among the main research instruments used to study the determining factors of pest attacks on crops are ecological life tables. They enable the qualitative and quantitative evaluation of the effects of natural mortality factors on populations. In addition, ecological life tables help to determine a pest's critical mortality stages and the key factor of mortality (Morris, 1963; Harcourt, 1969; Podoler & Rogers, 1975). The critical mortality stage is the life stage that most influences population size, while the key factor of mortality is the most important mortality factor responsible for changes in the population density between generations (Morris, 1959; Varley & Gradwell, 1960; Bellows *et al.*, 1992). The information obtained from life table studies can help in understanding the magnitude of the effects of these factors on insect pest population dynamics and in identifying natural biological control agents.

Planococcus citri (Risso) is a severe pest that occurs worldwide in several crops like citrus (CABI, 2016), grapes (CABI, 2016; Morandi Filho *et al.*, 2008), cotton (CABI, 2016; Astridge *et al.*, 2005), ornamental plants (CABI, 2016; Astridge *et al.*, 2005) and coffee (CABI, 2016; Williams, 1992). The damage caused by *P. citri* to coffee production can reach up to 100% when control measures are not taken (Santa-Cecília *et al.*, 2002; Correa *et al.*, 2005; Morandi Filho *et al.*, 2008). The life cycle details of *P. citri* have been described by several authors (Kriegler, 1954; Walton & Pringle, 2004; Correa *et al.*, 2005; Costa *et al.*, 2013). The developmental stages of *P. citri* are eggs, first, second and third nymphal instars, and adults. The bulk of *P. citri* dispersal occurs mainly in the early stages because at this stage the nymphs move intensively (Kerns *et al.*, 2004). In adulthood, females move from plant to plant and attach to branches, leaves or fruits before oviposition (Gallo *et al.*, 1988).

Despite the importance of *P. citri* as a pest, particularly in coffee plantations, very few studies have been performed to understand the role that natural biological control plays for this species. In addition, chemical control is still the main tactic used to control *P. citri* in coffee plantations in Brazil. However, *P. citri* individuals are protected by wax threads on their dorsal sides that reduce their exposure to and absorption of pesticides; therefore, chemicals often result in poor and ineffective control (Bartlett & Clancy, 1972; Wysoki *et al.*, 1981; Arshad *et al.*, 2015). Given this context, there is a need to investigate the life table parameters of *P. citri* and to identify the ecological mortality factors (including both biotic and abiotic factors, such as predators, parasitoids, rainfall and relative humidity) associated with this pest in the field. Therefore, in this study, we report the critical stage and key mortality factors for *P. citri* using an ecological life table with the goal of understanding the role of natural biological controls in regulating its population.

Materials and methods

Study area

This study was carried out in a coffee plantation in Viçosa city (20°48′45″S; 42°56′15″W, altitude 600 m, and tropical climate), Minas Gerais state, Brazil. Life table data were collected from insecticide-free plots within an area that measured approximately 900 m^2 and had 12 rows of 3-year-old coffee trees with 30 coffee trees in each row (*Coffea arabica* L. variety Catuaí IAC 15). Coffee plants were planted in the field (spacing of 1.0 m × 1.5 m) and fertilised using dolomitic calcium and macro- and micronutrients following the recommendations for the region (Ribeiro & Guimarães, 1999; Martinez *et al.*, 2003). Climate data (e.g. rainfall, relative humidity, and temperature) were monitored by the Central Weather Station of the Federal University of Viçosa. In addition, we studied the abundance and diversity of natural enemies found in this study occurring naturally in this agroecosystem, which is traditionally grown with coffee plantations and surrounded by areas of native vegetation belong to the Atlantic Forest biome.

Insects

For the life table experiments, adults and nymphs of *P. citri* were collected from commercial crop plantings of Conilon coffee, *Coffea canephora*, located in São Mateus city (18°42′58″S; 39°51′21″W, altitude of 36 m, and humid climate), Espírito Santo state, Brazil. Approximately 800 mealybugs were collected, transferred to the laboratory and placed on coffee plants (*C. arabica*, variety Catuaí Vermelho) cultivated according to Ribeiro & Guimarães (1999) and Martinez *et al.* (2003). The mealybugs were reared on coffee plants inside wooden cages (1.0 × 0.5 × 1.0 m) covered with white organza cloth under controlled conditions (25 ± 1°C, 70 ± 10% relative humidity, and a 12 h photophase) in a greenhouse. Samples of adults and nymphs were collected, placed in glass vials containing a 70% alcohol solution and identified as *P. citri*.

Cohort establishment

Studies of the mortality factors for *P. citri* were evaluated in the field for eight sequential generations (from egg to adult) during a 2-year study. The studies were carried out during the following periods: October–November 2009 (spring 2009), January–February 2010 (summer 2010), April–May 2010 (fall 2010), August–September 2010 (winter 2010), November–December 2010 (spring 2010), January–February 2011 (summer 2011), April–May 2011 (fall 2011) and August–September 2011 (winter 2011). In each period, 11 plants of *C. arabica* were randomly chosen, and the first five apical branches and the first pair of fully expanded leaves from the shoot apex were chosen on each plant for cohort establishment. Eight plants were used for life table studies and three coffee plants were designated as controls. Before cohort establishment, the leaves and branches on the selected plants were inspected to remove with the aid of a fine camel's hair brush all eggs, nymphs, and adults of *P. citri* and all other arthropod species. Each plant was considered as one experimental plot, and each replicate consisted of a single coffee plant artificially infested with a cohort of *P. citri* eggs. We assume that each generation of *P. citri* is independent.

Table 1 Predator species observed in the ecological life table of *Planococcus citri* in *Coffea arabica*, Viçosa, MG, Brazil

Predators			
Order	Family	Subfamily	Species
Coleoptera	Coccinellidae	Coccinellinae	*Harmonia axyridis* (Pallas,1773)
			Cycloneda sanguinea (Linnaeus, 1763)
		Coccidulinae	*Azya luteipes* (Mulsant, 1850)
			Diomus seminulus (Mulsant, 1850)
			Diomus sennen (Gordon, 1999)
Coleoptera	Coccinellidae	Scymninae	*Cryptolaemus montrouzieri* (Mulsant, 1853)
			Cyra loricata (Mulsant, 1850)
			Hyperaspis festiva (Mulsant, 1850)
Neuroptera	Chrysopidae	Chrysopinae	*Chrysoperla genanigra* (Steinmann, 1964)
			Chrysoperla externa (Hagen, 1861)
Diptera	Syrphidae	Syrphinae	*Allograpta* sp.
			Ocyptamus sp.
Diptera	Dolichopodidae	Sciapodinae	*Condylostylus* sp.

To standardise the age of the cohorts under study, one single *P. citri* female of 16-day-old and at a reproductive stage suitable for oviposition was transferred to each selected leaf according to Cloyd (2003). After the transfer of the female to the leaf, each branch was enclosed in an organza bag [35 cm (length) × 20 cm (width)] to protect her against the attack of natural enemies. Each female was allowed to oviposit for 72 h. On average, each female oviposited 10 eggs per day, and 10 females of *P. citri* were transferred to each coffee plant (for a total of 300 eggs per plant). Females of 16 days in age were chosen because the fertility peak of *P. citri* occurs between 16 and 20 days old (Costa *et al.*, 2016). After 72 h, both the adult females and the organza bag were removed, and the number of eggs was determined. A minimum of 300 eggs in 10 egg sacs (i.e. 30 eggs per egg sac) was kept in each plot for each coffee plant. Throughout the *P. citri* life cycle (from egg to adulthood), all natural mortality factors were monitored three times a day (7 am and 12 and 17 pm) after cohort establishment.

Assessment of natural mortality

Mortality factors for eggs

The number of eggs per cohort was difficult to determine in the field even using a 10× magnifying lens because *P. citri* eggs are covered by a waxy layer that interferes with accurate counting. To resolve this problem, during each period, the cohort established on control plants was removed and taken to the laboratory to evaluate the egg mass area and the number of eggs in each egg mass according to Cloyd (2003). Egg number in the field was estimated using the following equation: number of eggs = [(total egg mass area × no. eggs in control)/total egg mass area in control], where total egg mass area = egg mass high × egg mass diameter. All procedures used to evaluate the mortality caused by natural enemies (i.e. parasitoids, pathogens, predators) were based on methodologies discussed by Bellows *et al.* (1992).

Egg mortality was determined in the field using a 10× magnifying lens. Eggs that disappeared after a heavy rainfall were considered killed by rainfall. Mortality was attributed to predation when predators were observed preying on eggs or the remains of egg chorion were found (Pereira *et al.*, 2007*b*). From our preliminary study, it was confirmed that Chrysopidae (larvae) and Coccinellidae (adults and larvae) were easily observed feeding on the eggs in the field. Eggs attacked by Chrysopidae are easily distinguishable because after draining fluid from the egg, the larvae abandon them or transfer the chorion to their backs (Mantoanelli & Albuquerque, 2007). Coccinellidae leave only the remains of egg chorion after attack. Other predators were identified only by direct observation of predation (Table 1). Some predator species were collected from non-experimental coffee trees, packed in glass vials with 70% alcohol and identified.

The presence of fungal mycelium on the egg mass was considered mortality caused by fungal diseases. The egg masses with fungal mycelia were quantified, removed from the leaf with the aid of a brush, packed in Petri dishes (9 cm in diameter and 2 cm high) containing a piece of cotton moistened to maintain the humidity and identified.

Unhatched eggs, those not washed away by rain and that showed no evidence of fungal infection or predation, were collected in glass vials (10 cm long and 2 cm in diameter) containing a piece of cotton moistened to maintain the humidity and sent to the laboratory for observations of potential parasitoid emergence. The eggs in glass vials were assessed daily for 30 days.

All unhatched, unparasitized eggs were considered to be nonviable (Gonring *et al.*, 2003*a*; Pereira *et al.*, 2007*a*,*b*; Semeão *et al.*, 2012*a*,*b*). When it was not possible to identify the cause of egg death, mortality was attributed to unknown factors (other factors).

Mortality factors for nymphs and adults

Nymphs and adults that disappeared after heavy rainfall were considered killed. Mortality was attributed to predation when predators were observed preying on nymphs and adults or the remains of uneaten exuvia or dried exuvia were found (Rosado *et al.*, 2014). From our preliminary study, it was confirmed that Coccinellidae (adults and larvae) and Chrysopidae (larvae) were also easily observed feeding on the nymphs and adults of mealybugs in the field. Nymphs and adults attacked by Chrysopidae are easily distinguishable because the predators usually feed until the insect's exoskeleton fully collapses (Milbrath *et al.*, 1993), and then the larvae abandon them or transfer the insect's exoskeleton to their backs (Mantoanelli & Albuquerque, 2007). Early instar aphidophagous Coccinellidae larvae drain the fluid from the insect body and leave the empty exoskeleton. The last larval instar and adults suck the fluid from insect body and consume their exoskeleton (Hagen, 1962), leaving remains of the insect body as legs, exuvia pieces and the remaining leaf-bound waxes. Other predators were identified only by direct observation of predation in the field (Table 1).

Mealybugs exhibiting dark colours or microperforations on the external part of their bodies were considered killed by parasitoids. These characteristics of parasitism have been observed in some previous field studies (Rosado *et al.*, 2014). Furthermore, mealybugs with symptoms of parasitism were collected in glass vials [10 cm (length) × 2 cm (diameter)] and taken to the laboratory to confirm that parasitism had occurred. Subsequently, the parasitoids were collected, counted, labelled to morphospecies, packed in glass tubes with 70% alcohol and sent to Dr. Geoffrey B. White (Systematic Entomology Laboratory, Agricultural Research Service, US Department of Agriculture) for identification.

Dry bodies found on the tops of leaves were also removed from the plant and taken to the laboratory. These individuals were examined using a 40× stereoscopic microscope. When the bodies of these individuals had been torn apart by the action of predators, they were considered to have been killed by natural enemies (Miranda *et al.*, 1998; Gonring *et al.*, 2003*a*,*b*; Pereira *et al.*, 2007*a*,*b*). However, when no visible injuries were present, these individuals were considered killed by desiccation (solar radiation).

Individuals that died while adhered to exuviae were considered killed by physiological disorders during moulting (Pereira *et al.*, 2007*b*). Mealybugs that presented fungal mycelia on their bodies were considered killed by fungal diseases (Liu *et al.*, 2011; Rosado *et al.*, 2014). Nymphs and adults with symptoms of fungal diseases were collected and sent for identification. When it was not possible to identify the cause of death, mortality was attributed to unknown factors (other factors).

Construction and analysis of life tables

The data for life table construction were collected from both experiments of mortality factors for eggs and mortality factors for nymphs and adults. The mortality in each stage of the life cycle of *P. citri* was determined to conform to the total number of individuals that reached the beginning of each stage over the entire generation as well as their mortality factor. Therefore, we used different *P. citri* mortality data to determine the proportion of individuals that reached the adult stage to construct the life table.

Each table is composed of the columns x, L_x, d_x, d_xF, and $100q_x$, where x is the developmental stage of the mealybug, L_x is the number of individuals alive at the beginning of each stage, d_x is the number of individuals killed during each stage, d_xF is the mortality factor responsible for d_x, and $100q_x$ is the percentage of apparent mortality ($100q_x = 100^*d_x/L_x$). Partial mortalities were calculated at each stage and for each mortality factor [$k = \log(100q_x)$] and for all stages ($K = \sum$k) (Varley *et al.*, 1973; Southwood & Henderson, 2000). The mortality values were expressed as k-values for subsequent analyses. The k-values are additive across stages and mortality factors and are therefore useful for comparing the relative importance of factors (Pereira *et al.*, 2007*a*).

To identify the critical mortality stages and the key factors of mortality in *P. citri*, correlations and simple linear regression analyses were carried out using the partial mortality rates (k) and total mortality (K). The critical mortality stages or key factors of mortality were identified through correlation analyses between partial stage-specific mortality and the total mortality of all stages combined (Varley *et al.*, 1973). When more than one stage or mortality factor resulted in a significant correlation, a simple regression analysis of partial mortality to total mortality was regarded as a critical or key factor whose mortality regression curve presented the greatest angular coefficient ($P < 0.05$) (Pereira *et al.*, 2007*b*). The difference between the angular coefficient was verified at a 95% confidence interval (Podoler & Rogers, 1975). The statistical analysis for determination of mortality means and confidence intervals of the angular coefficient were done using the univariate procedures PROC UNIVARIATE

(SAS Institute, Cary, NC, USA, 2011), while, the correlation and regression analyses were done using PROC CORR and PROC REG (SAS, 2011).

Determination of marginal rates of mortality

To estimate each mortality factor, we determined the marginal rates of mortality of *P. citri* using the methods outlined by Buonaccorsi & Elkinton (1990), Elkinton *et al.* (1992) and Naranjo & Ellsworth (2005). The marginal rate is used to estimate the level of mortality arising from a single factor as if that was the only factor operating at the time. At least six mortality factors were observed to be operating during each developmental stage of *P. citri* and acted in a contemporaneous fashion. However, a mortality factor may be obscured by the action of another factor that can operate in a contemporaneous fashion with no obvious sequence of events. Rainfall is an example of a factor for which the apparent rate of mortality is equal to the marginal rate because it cannot be obscured by any other factor. To determine the marginal rate of mortality, M_B, we used the general equation (Eqn 1) derived from Naranjo and Ellsworth (2005):

$$M_B = d_B / \left(1 - d_A\right), \quad (1)$$

where A and B denote competing contemporaneous mortality factors, M_B is the apparent rate of mortality from B, and d_A is the sum of apparent mortalities from all other relevant, competing contemporaneous factors.

Irreplaceable mortality analysis

To verify the impact of the key factors of mortality in the population growth of *P. citri*, the increase in net reproductive rate (R_0) was calculated by considering that this mortality factor was suppressed, according to Eqns 2 and 3:

$$\text{Increased}\, R_0 = \left[100 - \left(MT - MI_i\right)\right] \times rs \times f \div R_0. \quad (2)$$

$$R_0 = \left[\left(nsa \times rs \times f\right) / nie\right], \quad (3)$$

where MT = total mortality across the generation, MIi = irreplaceable or indispensable mortality, rs = sex ratio of 0.81 (30), f = fecundity of 118 eggs per female (personal observation), nsa = number of surviving adults and nie = initial number of eggs. The MI is the proportion of the total mortality of the generation that could not occur if a given mortality factor was eliminated (Southwood and Henderson, 2000). Indispensable mortality (%) was estimated for each mortality factor and for each developmental stage according to Naranjo and Ellsworth (2005) using Eqn 4:

$$MI_i = \left\{ \left[1 - \prod_i^j (1 - MM/100) \right] - \left[1 - \prod_i^{j-1} (1 - MM/100) \right] \right\} \times 100. \quad (4)$$

MM is the marginal mortality or the percentage of mortality caused by a factor as if it was the only one. However, MM equals apparent mortality when the factor causes immediate death and does not have its action obscured by another factor, such as predators. For factors that cause delayed mortality of the insect or have their action obscured by other factors, such as parasitoids, $MM = 100q_x/(1 - \sum 100q_x/100)$, where $100q_x$ is the apparent mortality of the factor, and $\sum 100q_x$ is the sum of the apparent mortalities of other factors.

Results

Natural mortality factors

Based on the 64 life tables produced over the eight generations of *P. citri* in this study, we observed that of the 300 individuals starting from the egg stage, only ≈4 reach the adult stage (reproductive female). This value represents 98.79% of the total mortality. The apparent mortality rates ($100q_x$) in the egg, first, second and third instar nymph and adult stages were 67.08, 52.61, 53.06, 71.64 and 41.55%, respectively. The rate of population growth of *P. citri* was close to 1 ($\chi^2 = 8.37$, d.f. = 63, $P < 0.999$) (Table 2).

The deaths of insects were caused by predators, parasitoids, entomopathogenic fungi (*Lecanicillium lecanii* and *Cladosporium* sp.), rain, solar radiation and physiological disorders. The factor that caused the highest *P. citri* mortality at all stages was predation. Predators observed preying on eggs and larvae were the larval stages of Neuroptera: Chrysopidae, Diptera: Syrphidae and Diptera: Dolichopodidae and adults and larvae of Coleoptera: Coccinellidae. The Coleoptera species observed were *Harmonia axyridis* (Pallas, 1773), *Cycloneda sanguinea* (Linnaeus, 1763), and *Azya luteipes* (Mulsant, 1850) (Coccinellidae) and *Diomus seminulus* (Mulsant, 1850), *Diomus sennen* (Gordon), *Cryptolaemus montrouzieri* (Mulsant, 1853), *Cyra loricata* (Mulsant, 1850) and *Hyperaspis festiva* (Mulsant, 1850) (Scymninidae). The Chrysopidae species observed were *Chrysoperla genanigra* (Steinmann, 1964) and *Chrysoperla externa* (Hagen, 1861). Among the observed Diptera were *Allograpta* sp., *Ocyptamus* sp. (Syrphidae) and *Condylostylus* sp. (Dolichopodidae) (Table 1).

Table 2 Ecological life table of *Planococcus citri* in *Coffea arabica*[a]

X	L_x	Factors mortality	d_x	$100q_x$	*MM* (%)
Eggs	300	Predators	149.16 ± 7.98	49.72	58.08
		Rainfall	28.38 ± 7.41	9.46	9.46
		Sun	16.65 ± 3.21	5.55	5.55
		Physiological disturbance	1.96 ± 4.90	0.65	1.24
		Fungal diseases	4.70 ± 1.82	1.57	1.66
		Other factors	0.39 ± 0.23	0.13	0.13
			201.24 ± 6.27	67.08	78.12
Nymphs					
First instar	98.76 ± 6.27	Predators	44.77 ± 3.51	45.33	51.07
		Parasitoids	0.16 ± 0.09	0.16	1.42
		Rainfall	3.60 ± 0.94	3.65	3.36
		Sun	0.14 ± 0.09	0.14	0.14
		Physiological disturbance	0.68 ± 0.19	0.69	2.11
		Fungal diseases	2.14 ± 0.51	2.17	1.91
		Other factors	0.49 ± 0.12	0.50	1.21
			51.99 ± 3.75	52.61	61.22
Second instar	46.77 ± 3.90	Predators	21.36 ± 2.09	45.66	48.95
		Parasitoids	0.68 ± 0.17	1.45	6.08
		Rainfall	1.10 ± 0.30	2.34	2.34
		Sun	0.04 ± 0.02	0.09	0.09
		Physiological disturbance	0.44 ± 0.09	0.93	2.29
		Fungal diseases	1.12 ± 0.31	2.39	1.82
		Other factors	0.21 ± 0.05	0.44	1.54
			24.93 ± 2.22	53.06	63.11
Third instar	21.84 ± 2.27	Predators	13.11 ± 1.47	60.04	65.47
		Parasitoids	0.73 ± 0.10	3.32	19.32
		Rainfall	0.78 ± 0.26	3.59	3.59
		Sun	0.04 ± 0.02	0.18	0.18
		Physiological disturbance	0.24 ± 0.05	1.10	5.56
		Fungal diseases	0.67 ± 0.16	3.05	2.06
		Other factors	0.07 ± 0.02	0.33	0.84
			21.84 ± 2.33	71.64	97.02
Adult females	6.20 ± 1.59	Predators	2.13 ± 0.33	34.30	48.11
		Parasitoids	0.22 ± 0.04	3.53	17.25
		Rainfall	0.18 ± 0.07	2.89	2.82
		Sun	0.02 ± 0.00	0.06	0.09
		Physiological disturbance	0.02 ± 0.00	0.35	0.77
		Fungal diseases	0.02 ± 0.00	0.35	0.35
			2.59 ± 0.42	41.55	69.39
Reproductive females	3.60 ± 0.45	Total mortality = 98.79% and $R_0 = 1.16$			

[a]In the column headers, x = life cycle stage; L_x = number of insects alive at the beginning of each x; d_x = number of insects killed by each factor in each x; $100q_x$ = apparent mortality (%). Total mortality = $100*d_x/L_{eggs}$; and R_0 = population growth rate. Values (mean ± standard error) in this table represent the average cause of mortality over 64 insect life tables.

Critical mortality stage

Egg ($r = 0.36$, $P < 0.0027$), nymph ($r = 0.56$, $P < 0.0001$) and adult ($r = 0.85$, $P < 0.0001$) mortality were significantly correlated with the total mortality over the life cycle (Fig. 1A). However, nymph mortality showed the highest angular coefficient and higher slope coefficients than the mortality of either eggs or adults (Fig. 1B). The partial mortality at all nymph stages exhibited a correlation with the total nymph mortality (Fig. 2A); however, the regression analysis mortality curve for the third instar nymphs had a higher angular coefficient (Fig. 2B). Therefore, the critical stage of mortality in *P. citri* was the third nymphal instar.

Key factor of mortality

Mortality caused by predators, parasitoids, entomopathogenic fungi and physiological disturbances in the critical stage (3rd instar) all correlated positively ($r = 0.71$; $P < 0.0001$) with the total mortality at this stage (Fig. 3A). The mortality curve for *P. citri* caused by predation presented the greatest angular coefficient compared to the curves of the other mortality factors of third

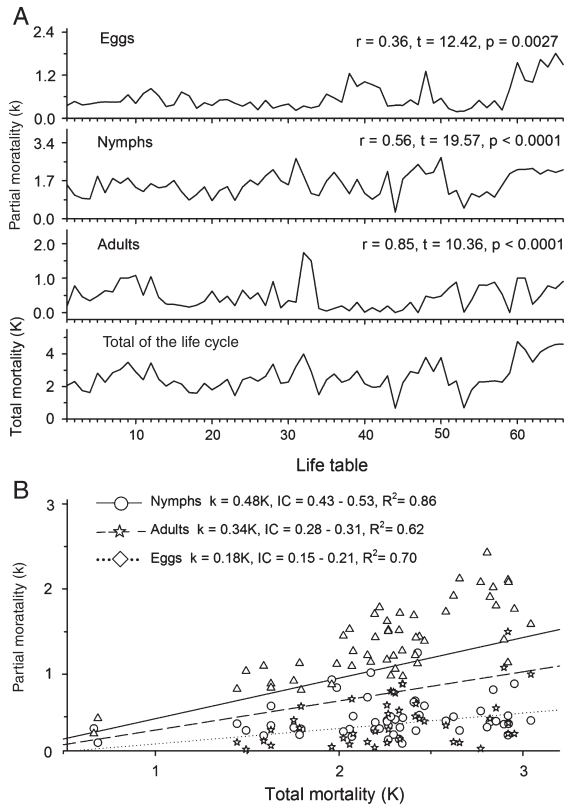

Figure 1 Determination of the critical mortality stage for *P. citri* using the correlation of mortality of eggs, nymphs and adults with the total mortality (A) and the linear regression mortality curves between the mortality of eggs, nymphs and adults depending on total mortality (B). CI 95% = Confidence interval of the slope of the regression curves at the 95% probability level. Total mortality is the mortality for each generation (i.e. sum of the mortalities observed in the different developmental stages (egg, nymph, and adult). Partial mortality is the mortality observed at each stage of development (egg, nymph, or adult).

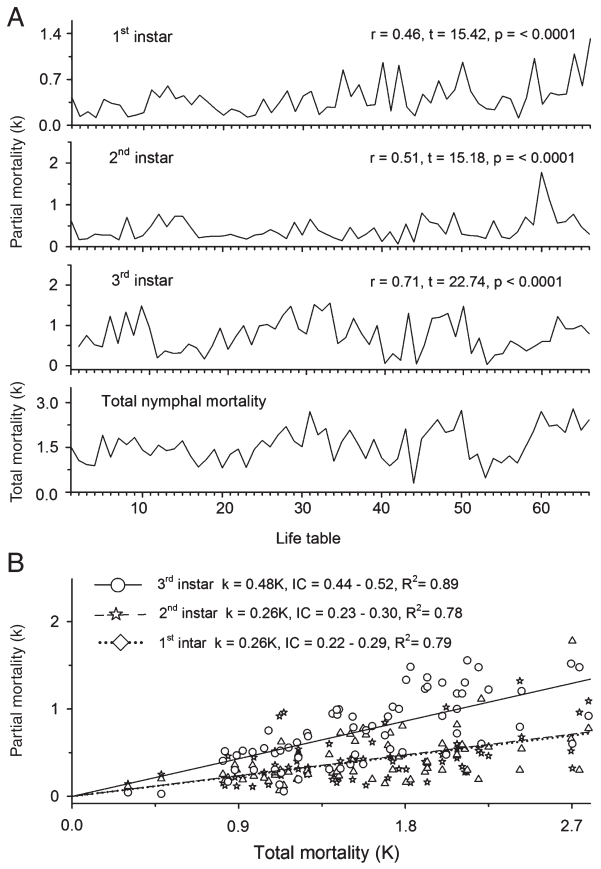

Figure 2 Determination of the critical instar of mortality of *P. citri* using the correlations of mortality in the first, second and third with the total mortality of nymphs (A) and linear regression mortality curves in the first, second and third instars depending on the total mortality of nymphs (B). CI 95% = confidence interval of the slope of the regression curves at the 95% probability level. Total mortality is the sum of the mortalities observed in all nymph stages, and partial mortality is the mortality observed at each nymphal instar (1st, 2nd or 3rd instar).

instars (Fig. 3B). Among predators, only mortality caused by the Coccinellinae ($r = 0.43$, $P = 0.0003$), Scymninae ($r = 0.61$, $P = 0.0001$), Chrysopinae ($r = 0.51$, $P = 0.0001$), Syrphinae ($r = 0.27$, $P = 0.0274$) and Lebiinae ($r = 0.58$, $P = 0.0001$) subfamilies correlated positively with the total mortality for third instar *P. citri* (Fig. 4A). However, the regression curves for the mortality caused by Coccinellinae, Scymninae and Chrysopinae showed similar slope coefficients (Fig. 4B). Thus, we conclude that the generalist predators belonging to the subfamilies Coccinellinae, Scymninae and Chrysopinae are the key factor of mortality for *P. citri*.

Marginal rates of mortality

Pooled over all developmental stages, the highest marginal mortality rate was observed due to predators and varied from 48% to 65%, and higher predation occurred in the third instar (65%) (Table 2). The second highest marginal mortality occurred due to parasitism in the third instar (19.32%) followed by the adult (17.25%), second instar (6.08%) and first instar (1.42%) stages. On average, the marginal mortality values due to parasitism were 4- to 9-fold higher than the apparent mortality (Table 2). On the other hand, the other mortality factors caused marginal mortality values below 6% (Table 2).

Irreplaceable mortality

Indispensable mortality results in the absence of the action of predators on third-instar nymphs of *P. citri*, resulting in a 42.84% increase in R_0. Moreover, removing the key factors Coccinellinae, Scymninae and Chrysopinae individually would cause population

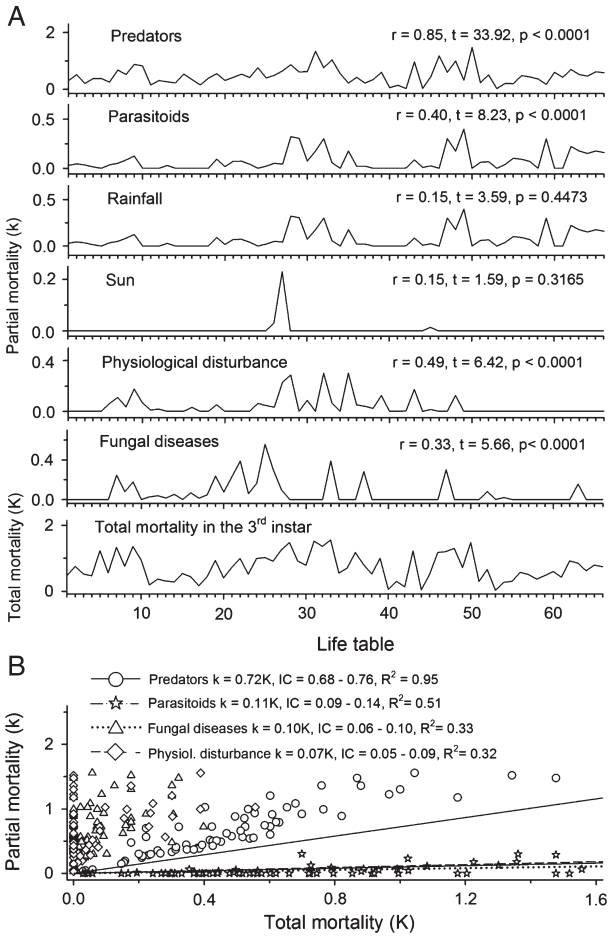

Figure 3 Key factors determining the mortality at the nymph third instar of *P. citri* using the (A) correlations between the mortality factors and total mortality in the third instar and (B) regression mortality curves of the factors depending on total mortality in the third instar. CI 95% = confidence interval of the slope of the regression curves at the 95% probability level. Total mortality is the sum of the mortalities caused by all mortality factors on the third instar, and partial mortality is the mortality caused by each mortality factor on the third instar.

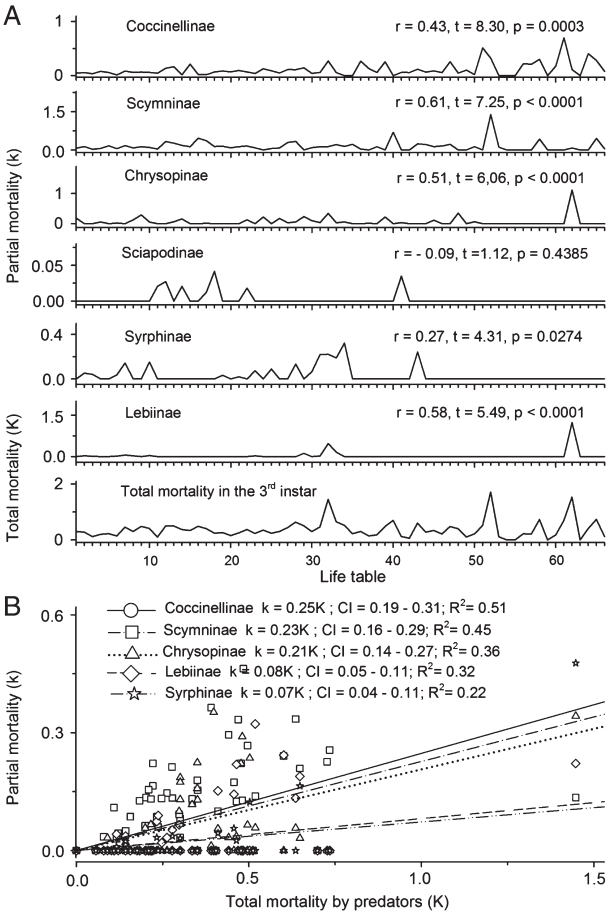

Figure 4 Determination of the key predator mortality at the nymph third instar of *P. citri* using the (A) correlations of mortality caused by predators and total predation in the third instar and (B) regression curves of mortality caused by predators depending on total predation on the third instar. CI = confidence interval of the slope of the regression curves at the 95% probability level. Total mortality is the sum of the mortalities caused by all mortality factors on the third instar, and partial mortality in the mortality caused by each mortality factor on the third instar.

increases of 16.75%, 16.70% and 9.15%, respectively (Fig. 5).

Discussion

Key factor analysis has been widely used in life table studies to identify the primary mortality factors responsible for changes in population density. Despite the problems discussed by Royama (1996), our analysis of the key factors was consistent with the life table data. As reported in this study, predation was the most important factor in the mortality of *P. citri* at every stage of development (Table 2). Various predator species were observed preying on *P. citri* in the field (Table 1), but the magnitude of control provided by each predator species was different. The rate of population growth of *P. citri* remained stable ($R_0 \approx 1$) across the generations evaluated in the field. In addition to predation, other top-down forces also contributed to maintaining this balance. Top-down forces have been reported as the most important factors in regulating phytophagous arthropods in tropical regions (Hairston *et al.*, 1960; Miranda *et al.*, 1998; Gonring *et al.*, 2003*a*,*b*; Pereira *et al.*, 2007*a*,*b*; Semeão *et al.*, 2012*a*,*b*; Rosado *et al.*, 2014).

The number of natural enemy species reported in our study (Table 1) was greater than those previously reported in other life table studies (Pereira *et al.*, 2007*b*; Semeão *et al.*, 2012*b*; Rosado *et al.*, 2014). We did not conduct nightly evaluations; however, the daily evaluations allowed the identification and quantification of the

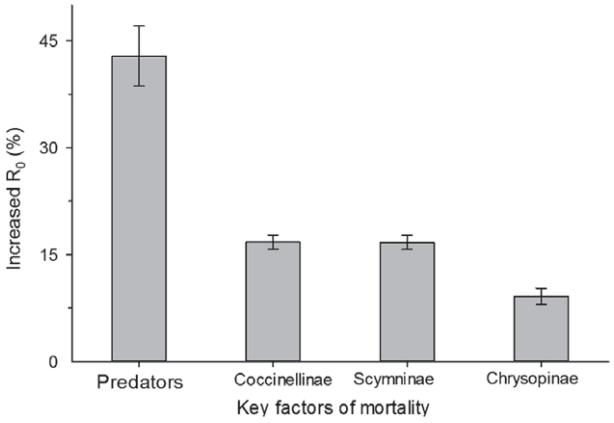

Figure 5 Percentage (mean ± standard error) increase in population growth rate (R_0) of *Planococcus citri* in the absence of the key factors of mortality. Increased of $R_0 = [100 - (MT - MI_i)] \times rs \times f \div R_0$, and $R_0 = [(\text{No. survival adults} \times rs \times f) \div \text{No. initial eggs}]$. MT = total mortality across the generation, MIi = irreplaceable or indispensable mortality, rs = sex ratio of 0.81 (30), f = fecundity of 118 eggs per female (personal observation). Bar in graph represents the mean of increased of R_0 for each mortality factor. The error bars show the standard error of the mean values.

relative abundance of predator taxa in this study. Most predatory taxa exhibit diurnal or nocturnal predatory activity patterns or in some cases both activity patterns (Woltz & Landis, 2014), making their identification difficult in the field. Furthermore, Pfannenstiel (2005) and Novotny *et al.* (1999) showed that the rate of nocturnal predation is similar to that occurring throughout the day, but the abundance of predators is lower at night. In the egg and nymph stages (particularly in 1st and 2nd instar nymphs), mortality was primarily caused by diurnal Coccinellidae and Syrphidae predators and by nocturnal Chrysopidae predators (Woltz & Landis, 2014). *H. axyridis* and *Chrysoperla* spp. were the primary predators of the third instar nymphs and adults. The relative predation importance in *P. citri* control could be explained by the densities of several larval and adult predator taxa identified during this study; this information can be confirmed by the lowest level of marginal mortality estimates (approximately 1.2-fold) compared to the apparent mortality (Table 2). These small marginal mortality estimate values suggest that predation may not subsequently be replaced by rain or other factors.

Parasitism has been reported to be one of the main factors in mealybug mortality (Mgocheki & Addison, 2010; Walton & Pringle, 2004; Whitehead, 1957); however, in this study, mortality caused by parasitism was far inferior to mortality caused by predation (Table 2). The parasitism observed in this study was primarily due to the action of *Coccidaxenoides perminutus*, *Leptomastix dactylopii* and *Anagyrus* spp., which are commonly associated with the control of *P. citri* (Triapitsyn *et al.*, 2007; Fernandes *et al.*, 2016). However, the levels of mortality caused by parasitism of *P. citri* in this study were generally low, and parasitism was therefore not a decisive mortality factor. The estimates of marginal mortality showed that parasitism was responsible for 17.25% and 19.32% of the mortality occurring in adults and third instar nymphs, respectively. On average, the marginal mortality values for parasitism were 4- to 9-fold higher than for the apparent mortality (Table 2). Despite the great values found in the marginal rate estimates, much of the mortality caused by parasitism may subsequently be replaced by predation or other factors. Several studies have highlighted the role of *P. citri* parasitoids as biological control agents in other crops (Triapitsyn *et al.*, 2007; Mgocheki & Addison, 2010; Fernandes *et al.*, 2016), but these studies, unlike life table studies, are generally poor predictors of the overall impact of parasitoids on insect pest population dynamics (Naranjo, 2001).

Abiotic factors such as rain and insolation caused mortality throughout the life cycle of *P. citri*. Observations on several occasions clearly showed that *P. citri* disappearance was caused by the impacts of raindrops near egg masses and nymphs and sometimes by the action of wind.

The absence of the waxy layer surrounding both the egg masses and nymphs caused by rainfall increased the dehydration mortality by solar radiation, especially during the spring and summer seasons. Several studies have reported that insolation affects insect developmental rates, insect size, egg production rates, adult maturation rates, and insect orientation to the sun (Begon, 1983; Lactin & Johnson, 1996). There is little information about the impact of rain on the population dynamics of phytophagous insects, especially in tropical regions (Pereira *et al.*, 2007*a*,*b*). Factors such as rainfall, winds and solar radiation were shown to be important mortality factors for *P. citri*; however, future studies should be conducted to increase the knowledge about these mortality factors.

Other mortality factors such as fungal diseases and physiological disorders also caused mortality in *P. citri*. Mortality caused by the fungal species *Lecanicillium lecanii* and *Cladosporium* sp. increased during the fall and winter seasons, likely because the temperature and humidity favour the action of fungal spores during this period. Solar radiation, particularly ultraviolet radiation, is the main factor responsible for the mortality of fungal conidia in the field (Smits *et al.*, 1996; Maniania, 1998). Physiological disorder mortality occurs due to the toxic secondary compounds of coffee plants, such as caffeine alkaloids, flavonoids, chlorogenic and neochlorogenic acid, pyrazinic acid and coumarins (Maxemiuc Naccache & Dietrich, 1985; Zuluaga *et al.*, 1971), and the biological characteristics of the insects (Pereira *et al.*, 2007*a*,*b*). The

compositions and proportions of defence compounds in plants are variable, but additional studies should be conducted to determine the effects of these on *P. citri*.

The critical phase of mortality was the third nymphal instar of *P. citri*. Therefore, changes to this parameter can have the greatest direct influence on the rate of population growth of *P. citri*. Thus, efforts to develop biological control programmes or integrated pest management (IPM) strategies should be directed toward this phase to maximise the chances of success.

Identifying the role of natural enemies in an agroecosystem is a complex task; however, the calculation of indispensable mortality allows the estimation of the value of a factor or its contribution in a biological control program (Huffaker & Kennett, 1966; Van Driesche *et al.*, 1991). The estimated values of indispensable mortality indicate that in the absence of predators, population growth would increase by approximately 44%, and in the absence of the subfamilies Coccinellinae, Scymninae, and Chrysopinae, the R_0 would increase by 17%, 17% and 10%, respectively (Fig. 5). These results suggest that the preservation of this mortality component may be the key to managing the *P. citri* population growth rates in the field. In addition, we showed results of key factor and marginal mortality analyses that support the important contribution of predation to the regulation of this insect population. Furthermore, Morris (1957) showed that adding a mortality factor that already occurs at high levels in agroecosystems will have a greater effect in reducing the survival of insect pests than adding it to a low-level factor. Thus, the adoption of practices aimed at the preservation and population growth of predators will allow the continued suppression of *P. citri* populations in fields.

Our results showed that the major predators of *P. citri* include beetles (Coccinellidae) and neuropterans (Chrysopidae). These natural enemies should be favoured by pest management tactics, and their population levels should be considered when making control decisions. Possible tactics include cultural control, which can be used to make the environment less favourable for pest development and reproduction. Including the provision of insectary plants that provide natural enemies with an alternative food source, such as pollen and nectar, is known to increase predator and parasitoid density and thus improve biological control (Davidson & Evans, 2010; Landis *et al.*, 2000; Morandin *et al.*, 2011; Rosado *et al.*, 2014). IPM practitioners can use selective insecticides with short residual times or compatible insecticides (such as horticultural oil and insect growth regulators), and insecticide spraying should be done in periods with low temperatures (when the foraging of predators is reduced) to minimise impacts on pollinators, natural enemies, and other nontarget organisms (Bacci *et al.*, 2009; Camacho & Chong, 2015; Kabashima & Dreistadt, 2014; Kosztarab & Kozár, 1988; Picanço *et al.*, 2010).

In this study, we mainly reported on factors responsible for the natural mortality of *P. citri* in the field as well as the identification of key factors that should be considered when developing management strategies and control tactics for this pest. Moreover, predators belonging to the subfamilies Coccinellinae, Scymninae and Chrysopinae are the key mortality factors for *P. citri* in coffee plantations. Our results also suggest that the presence of different kinds of predators in the field can favour the natural biological control of *P. citri* in the field; however, more refined studies are needed about their biology, viability of mass rearing and release in field, as well as, evaluate their compatibility with other control methods.

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