

# Natural biological control of *Chrysodeixis includens*

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## Abstract

A wide variety of abiotic and biotic factors act on insect pests to regulate their populations. Knowledge of the time and magnitude of these factors is fundamental to understanding population dynamics and developing efficient pest management systems. We investigate the natural mortality factors, critical pest stages, and key mortality factors that regulate *Chrysodeixis includens* populations via ecological life tables. The total mortality caused by natural factors was 99.99%. Natural enemies were the most important mortality factors in all pest stages. The critical stages of *C. includens* mortality were second and fourth instars. The key mortality factors were predation by ants in the second instar and predation by Vespidae in the fourth instar. The elimination of these factors can cause an increase of 77.52 and 85.17% of *C. includens* population, respectively. This study elucidates the importance of natural enemies and other natural mortality factors in *C. includens* population regulation. These factors should be considered in developing and implementing *C. includens* management strategies and tactics in order to achieve effective and sustainable pest control.

**Keywords:** ecological life table, natural enemies, population dynamics, soybean looper, integrated pest management

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## Introduction

Multiple natural mortality factors regulate insect population dynamics in agroecosystems. Among these factors, the most important are natural enemies, climatic elements, and host plants. Studying the spatial distribution, timing, and the magnitude of these mortality factors is essential to understand the population dynamics of a species. It is also important to predict pest outbreaks and to develop better pest management strategies in order to use and improve the natural mortality factors (Naranjo & Ellsworth, 2005).

Each one of these factors acts differently on the insect. Some act directly and others indirectly by interfering with the insect and their relationship with host plants and natural enemies. Natural enemies such as predators, parasitoids, and

entomopathogens are very important in pest population regulation in agroecosystems, and they can cause mortality in different ways. Predators kill their prey immediately on contact, and for this reason, their action is not overlapped by other natural enemies. On the other hand, the action of parasitoids and entomopathogens is overlapped by the action of predators because they cause pest mortality sometime after contact (Hajek, 2004; Semeão *et al.*, 2012).

Among predators, predatory bugs, thrips, and Vespidae are the most common natural enemies of the pest. Parasitoids include egg parasitoids (Hymenoptera: Trichogrammatidae), larval parasitoids (families Hymenoptera: Braconidae and Diptera: Tachinidae), and a pupal parasitoid (*Copidosoma* sp. Hymenoptera: Encyrtidae) (Burleigh, 1972; Daigle *et al.*, 1990). A complex of fungi are reported as entomopathogens of *Chrysodeixis includens*. An example is the fungus *Nomuraea rileyi*, which is highly affected by chemical control (Sosa-Gómez *et al.*, 2003; Spechta *et al.*, 2015).

Climatic elements, in turn, can affect the pest population dynamics directly or indirectly mainly through air temperature, rainfall, and photoperiod. Directly, temperature and

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photoperiod affect the development and reproduction of insects (Mellanby, 1939; Régnière *et al.*, 2012), and rainfall causes mortality by mechanical impact of rain drops. The indirect action of climate occurs because of its influence on natural enemy survivorship and establishment (Norris *et al.*, 2002), and because of its effect on host plant quality.

Host plant characteristics such as morphology, nutritional status, and allelochemicals can cause pest mortality as well. These factors may cause egg viability reduction and affect physiological processes during molting, which can lead to insect mortality (Awmack & Leather, 2002).

Ecological life tables are an important research tool for studying population regulation and have provided important insights into the dynamics and regulation of insect populations in agroecosystems (Carey, 1989). This is a robust method of description and quantification of natural mortality factors throughout the insect life cycle due to variations in its natural environment (Harcourt, 1969; Naranjo & Ellsworth, 2005). Ecological life tables also describe the critical stage and key mortality factors of the insect. The critical mortality stage determines the size of the pest population and the key mortality factor has greater relative importance during the critical stage, that is, the factor that is the most responsible for the changes in a population density in successive generations (Varley & Gradwell, 1960; Morris, 1963; Harcourt, 1969; Podoler & Rogers, 1975).

Soybean looper, *C. includens* (Walker) (Lepidoptera: Noctuidae), is an important agricultural pest that occurs in the Western Hemisphere from the northern United States (US) to southern South America (Eichlin & Cunningham, 1978; Alford & Hammond Junior, 1982). This species is polyphagous and feeds on 73 plant species belonging to 29 different families (CAB International, 2016). Its losses are caused through the larvae feeding on the leaves, especially between the veins, displaying a lacy appearance, which is the pest's fingerprint (Bueno *et al.*, 2011). *Chrysodeixis includens* is an important pest of crops such as soybeans, beans, cotton, sunflower, tomato, and potato (Eichlin & Cunningham, 1978).

In the US, *C. includens* is reported as a key soybean pest in several regions (Kogan & Turnpseed, 1987). In Brazil, only in recent years has this pest become a serious problem when population outbreaks were recorded in different crops (Baldin *et al.*, 2014; Bortolotto *et al.*, 2015). Despite the importance of *C. includens* as a pest of different crops, the stages and factors that regulate its infestation intensity are unknown. This information is very important for the establishment of integrated pest management programs on a sustainable basis. Therefore, our objectives were to determine the natural mortality factors, stages, and key factors that regulate *C. includens* populations.

## Materials and methods

### Experimental conditions

This study was conducted on dry bean (*Phaseolus vulgaris* L.) cultivar Madrepérola in the experimental area of the Universidade Federal de Viçosa, Viçosa, Minas Gerais State, Brazil (20°48'45"S, 42°56'15"W, 600 m a.s.l., tropical climate) in 2014 and 2015. During those 2 years, seven experiments were conducted in the same experimental field. The experiments were conducted in different periods and the crops were planted on 1-14-2014, 4-14-2014, 9-8-2014, 12-3-2014, 2-23-2015, 6-2-2015, and 9-22-2015. These dates were selected

in order to enable the study of *C. includens* mortality factors throughout the year. The experiments were conducted in an area of 3250 m<sup>2</sup> (65 × 50 m), with plants 10 cm apart in rows 50 cm apart. The fields were grown as recommended by EMBRAPA (2005) and pesticides were not used.

### *Chrysodeixis includens* colony

Individuals of *C. includens* used in the experiments were obtained from a laboratory colony which was reared at 25 ± 0.5°C, 70 ± 5% relative humidity, and photoperiod 12:12 (light:dark). For colony establishment, we collected *C. includens* larvae in commercial dry bean fields in Viçosa. The collected larvae were taken to the laboratory where they were reared until pupation in dry bean leaves. These pupae were used to start the colony. In the colony, we used three cages (45 × 45 × 45 cm). The first cage was used for oviposition, the second for egg incubation, and the third for larvae feeding.

The pupae from individuals collected in the field were transferred to the oviposition cage. When the adults emerged, a 30-day-old potted dry bean plant (3 l) was placed inside the cages for oviposition. Honey was provided on a piece of white cloth (9 × 3 cm) for adult feeding. The moths were allowed to oviposit for 2 days, and after this period, the plant was transferred to the egg incubation cage. A new plant was placed inside the adult cage until the death of all adults.

The bean plant with eggs remained in the egg incubation cage until larvae hatching (about 3 days). After this period, this plant was transferred to the larvae-feeding cage. The larvae-feeding cage was monitored daily. When the larvae defoliated the entire plant, a new dry bean plant was placed into the cage. The larvae remained in the feeding cage until pupation. The pupae were then transferred to the oviposition cage.

### Determination of mortality factors

The experimental design was completely randomized with ten experimental plots (replication). Each plot consisted of ten bean plants 30 days after planting in the field. The plots were isolated from each other by 1 m in all directions. The isolation area had no plants, and it was used to prevent larval migration between plots. For the assessment of *C. includens* mortality factors, we have established cohorts in the field containing eggs, larvae, and pupae.

### Evaluation of egg mortality factors

Around each set of ten plants (plot) in the field, we built a wood and organza fabric cage (100 × 50 × 50 cm). Within this structure, we released 20 3-day-old unsexed adults for oviposition. After 24 h, the adults and the cage around the plants were removed. *Chrysodeixis includens* lays its eggs singly and mostly on the bottom side of the leaves. Thus, for each plot, the eggs were recorded, and about 50 eggs were preserved per plot. In this way, we started the egg cohort in the field. The end of the incubation period of eggs was determined when the first larvae hatched.

During the incubation period, we evaluated the number of eggs and their mortality causes in the field (e.g. predator, parasitoids, and abiotic factors such as rainfall). The evaluations were performed three times a day, (i) in the beginning of the morning, (ii) after noon, and (iii) at night. To identify mortality caused by rainfall – removing eggs from the leaves due to the mechanical impact of the raindrops – additional evaluations

were performed in rainy days. These evaluations were performed right after the rainfall ceased. Therefore, eggs that disappeared between two subsequent evaluations in the absence of rain, or only had the corium present were considered dead by predation (Jervis, 2007; Pereira *et al.*, 2007a, b).

Each egg that had not hatched after the field evaluation period was placed in a glass tube (10 cm long  $\times$  2 cm diameter). The tubes were sealed with cotton, transported to the laboratory, and monitored daily. In the tubes that egg parasitoids emerged, we recorded the parasitism and identified the parasitoid to family level. The eggs that were not parasitized and did not hatch were considered dead by inviability.

The hatched larvae were transferred to plastic containers (120 ml) with dry bean leaves. These insects were reared until the emergence of adults for evaluation of *Copidosoma* sp., an egg-pupae parasitoid. We evaluated the numbers of parasitized pupae and *C. includens* emerged adults.

#### Evaluation of larval mortality factors

For *C. includens* larval cohort establishment, a group of individuals of the same species and age, we transferred 200 larvae of newly hatched first instar for each field plot. We evaluated the instar and the mortality numbers and causes twice a day, early in the morning and in the end of the afternoon.

Evaluations of larval mortality by predation and rainfall were performed similarly to evaluations of these factors at the egg stage. Larvae that died attached to their exuviae were assigned as 'physiological disturbance during molting mortality' (Pereira *et al.*, 2007a, b).

To evaluate parasitism, at the end of the larval stage, the remaining larvae were removed from the plants and transported to the laboratory. In the laboratory, the larvae were placed in plastic containers (120 ml) with dry bean leaves for feeding, and they were reared until the pupae stage. We evaluated the number of larvae that died by physiological disturbance during molting, and the number of normal pupae and the parasitoids that emerged from the larvae.

#### Evaluation of pupal mortality factors

For *C. includens* pupae cohort establishment, we transferred 15 larvae at the end of the fifth instar for each plot to allow pupation on the plants in the field. At this stage, we evaluated the mortality numbers and causes. Pupal mortality by predation and rainfall were performed similarly to evaluations of these factors in the egg stage.

Near to the end of the pupal stage, the leaves of plants containing the remaining pupae were removed and transported to the laboratory. In the laboratory, they were placed in cages and monitored until adult emergence. We evaluated the number of pupae that died by physiological disturbance during molting, and the number of adults and the parasitoids that emerged from the pupae.

#### Natural enemies identification

Natural enemy evaluations were recorded during all stages. We collected natural enemies from other plants of the crop and took them to the laboratory. In the laboratory, they were killed, assembled, and separated into morphospecies for further specialists identification.

#### Life table construction and analysis

The life table was constructed using the classical models presented by Varley *et al.* (1973) and Southwood & Henderson (2000). They were composed of the following variables: life cycle stage ( $x$ ), mortality factor,  $L_x$  (number of living insects at the beginning of each stage),  $d_x$  (number of dead insects in each stage),  $100qx$  (apparent mortality rate),  $100rx$  (real mortality rate),  $MM$  (marginal mortality rate), and  $k$  (partial mortality). These variables were calculated using the following equations:

$$L_{x+1} = L_x - d_x \quad (1)$$

where  $L_x$  and  $L_{x+1}$  are the numbers of living insects at the beginning of each stage ( $x$ ) and in the beginning of the next stage ( $x + 1$ );  $x = 0, 1, 2, 3, 4, 5$ , and 6 correspond to the egg, first, second, third, fourth, fifth instars, and pupa.

$$100rx = 100 \cdot \left( \frac{dx}{L_0} \right) \quad (2)$$

$$100qx = 100 \cdot \left( \frac{dx}{L_x} \right) \quad (3)$$

Real mortality rate is the percentage of mortality with respect to the number of insects that started their life cycle (i.e. the initial number of eggs). This mortality parameter determines the factors that caused more or less absolute mortality to the insect. However, the magnitude of the real mortality occurring in the last stages of the insect's life cycle is highly influenced by mortality occurred in the early stages. To remove this influence in later stages, we calculated apparent mortality rate (Southwood, 1978).

Another difficulty in the life table construction is that, in the field, the mortality factors act simultaneously. So, one mortality factor can obscure the performance of another. To prevent the action of a factor to obscure another action, we use  $MM$  (Elkinton *et al.*, 1992). The  $MM$  estimates the mortality of a factor as if it were the only one. These estimates are based on the concepts proposed by Royama (1981) and adapted by Elkinton *et al.* (1992).

$$MM_f = 100qx_f / (1 - To_f) \quad (4)$$

$$To_f = \sum^1 100qx_o / 100 \quad (5)$$

where  $MM_f$  is the  $MM$  caused by factor  $f$ ;  $f$  is a factor of mortality (predation, parasitism, rainfall, or egg inviability);  $To_f$  is the factor obscuration rate. For rainfall,  $To = 0$  since its action is not obscured by any other factor. Predation is obscured by rainfall. Parasitism is obscured by the action of rainfall and predation. Eggs inviability is obscured by the action of rainfall, predation, and parasitism (Semeão *et al.*, 2012).

For critical stages and key factors of mortality determination, partial ( $k$ ) and total mortalities ( $K$ ) were calculated using equations (6) and (7) (Southwood & Henderson, 2000).

$$k = -\log_{10} \left( 1 - \left( \frac{MM_f}{100} \right) \right) \quad (6)$$

$$K = \sum (k) \quad (7)$$

Correlation and linear regression analysis were conducted between partial and total mortality. Critical stages or mortality key factors were those in which the partial mortalities showed significant correlations with total mortality by  $t$  test at  $P < 0.05$ . When more than one mortality stage or factor correlated significantly, we performed linear regression analysis between

partial and total mortalities at  $P < 0.05$ . The critical stage or key factor of mortality was the one whose regression line showed the greatest slope to  $P < 0.05$ . The difference between the slopes was verified by comparing their confidence intervals to 95% probability (Podoler & Rogers, 1975; Royama, 1996; Pereira et al., 2007a, b).

Indispensable mortality is the mortality that would not occur if a mortality factor was eliminated (Southwood & Henderson, 2000) and was calculated according to Naranjo & Ellsworth (2005):

$$MI_i = \left\{ \left[ 1 - \prod_i^j (1 - MMf/100) \right] - \left[ 1 - \prod_i^{j-1} (1 - MMf/100) \right] \right\} \cdot 100 \quad (8)$$

## Results

### Natural mortality factors of *C. includens*

The average total mortality of immature stages of *C. includens* was 99.99%. The mortalities for each stage were: 71.29, 24.87, 2.78, 0.79, 0.19, 0.06, and 0.0097% for the egg, first, second, third, fourth, fifth instars, and pupa, respectively. Thus, for every one million eggs of *C. includens* that initiated its life cycle, approximately 31 reached its adulthood (table 1).

Mortality of *C. includens* was caused by multiple biotic and abiotic factors. Egg mortality was caused by predation, parasitism, rainfall, and inviability. The predators were *Orius* sp. (Hemiptera: Anthocoridae), *Franklinothrips* sp. (Thysanoptera: Aeolothripidae), and *Diabrotica speciosa* (Germar) (Coleoptera: Chrysomelidae). Predation by *D. speciosa* occurred when the adults of this beetle ate leaves containing

Table 1. Ecological life table of *Chrysodeixis includens* on dry beans.

Stage/mortality factor	$L_x$	$d_x$	$100q_x$	$100r_x$	MM	$k$
Egg	10,000	7129 ± 312	71.29	71.29		0.521
<i>Orius</i> sp.		2184 ± 217	21.84	21.84	23.1	0.114
<i>Franklinothrips</i> sp.		1256 ± 199	12.56	12.56	13.3	0.062
<i>Diabrotica speciosa</i>		11 ± 9	0.11	0.11	0.12	0.001
<i>Trichogramma</i> sp.		1400 ± 210	14.00	14.00	23.4	0.116
Rainfall		562 ± 129	5.62	5.62	5.62	0.025
Inviability		1715 ± 183	17.15	17.15	37.4	0.203
First instar	2871 ± 311	2487 ± 268	86.62	24.87		0.578
<i>Orius</i> sp.		86 ± 27	2.99	0.86	3.21	0.014
<i>Geocoris</i> sp.		9 ± 7	0.32	0.09	0.35	0.002
<i>Franklinothrips</i> sp.		232 ± 53	8.08	2.32	8.70	0.040
Ants		1725 ± 258	60.07	17.25	64.7	0.452
Spiders		232 ± 51	8.08	2.32	8.70	0.040
Rainfall		203 ± 40	7.08	2.03	7.08	0.032
Second instar	384 ± 62	278 ± 46	72.41	2.78		0.459
<i>Geocoris</i> sp.		2 ± 2	0.46	0.02	0.51	0.002
<i>Franklinothrips</i> sp.		20 ± 5	5.24	0.20	5.91	0.026
Ants		186 ± 37	48.40	1.86	54.6	0.343
Spiders		27 ± 13	7.02	0.27	7.92	0.036
Rainfall		43 ± 16	11.29	0.43	11.3	0.052
Third instar	106 ± 20	79 ± 16	74.82	0.79		0.405
<i>Geocoris</i> sp.		0.28 ± 0.28	0.27	0.003	0.28	0.001
Vespidae		41 ± 10	39.12	0.41	40.5	0.225
Ants		27 ± 7	25.44	0.27	26.3	0.133
Spiders		7.1 ± 3	6.67	0.07	6.90	0.031
Rainfall		3.5 ± 1	3.32	0.04	3.32	0.015
Fourth instar	27 ± 5	18 ± 4	69.68	0.19		0.385
Vespidae		12 ± 4	45.89	0.12	46.2	0.269
Ants		5.6 ± 1	21.22	0.06	21.4	0.104
Spiders		0.52 ± 0.17	1.95	0.01	1.97	0.009
Rainfall		0.16 ± 0.08	0.61	0.002	0.61	0.003
Fifth instar	8 ± 1.6	6.8 ± 1.46	84.21	0.07		0.497
Vespidae		4.4 ± 1.04	54.27	0.04	55.3	0.350
Ants		1.9 ± 0.37	22.94	0.02	23.4	0.116
Spiders		0.41 ± 0.32	5.09	0.004	5.19	0.023
Rainfall		0.15 ± 0.08	1.91	0.001	1.91	0.008
Pupa	1.28 ± 0.2	0.97 ± 0.17	75.97	0.01		0.517
Vespidae		0.80 ± 0.16	62.60	0.01	62.6	0.427
Ants		0.02 ± 0.01	1.48	0.0002	1.48	0.006
Birds		0.12 ± 0.06	9.23	0.001	9.23	0.042
<i>Copidosoma</i> sp.		0.03 ± 0.01	2.40	0.0003	8.98	0.041
Adult	0.31 ± 0.08					
Total mortality = 99.9969%						

$L_x$  is the number of insects alive ( $\pm$ SE) at the beginning of each stage,  $d_x$  is the number of insects killed ( $\pm$ SE) in a stage or killed by a factor in a stage,  $100q_x$  is the apparent mortality (%),  $100r_x$  is the real mortality (%), MM is the marginal mortality (%) and  $k$  is the partial mortality = log (MM). This life table represents the mean of 70 cohorts.

*C. includens* eggs. Parasitism of *C. includens* eggs was made by *Trichogramma* sp. (Hymenoptera: Trichogrammatidae) (table 1).

Larval mortality factors were rainfall and predation. These two factors caused mortality in all *C. includens* instars. The predators observed were spiders, ants (Hymenoptera: Formicidae), *Orius* sp., *Geocoris* sp. (Hemiptera: Lygaeidae), *Franklinothrips* sp., and Vespidae (Hymenoptera). Spiders and ants preyed on larvae in all instars. *Geocoris* sp. and *Franklinothrips* sp. were found preying on larvae of first, second, and third instars and Vespidae on third, fourth, and fifth instars (table 1). We did not observe *C. includens* mortality caused by physiological disturbance. This also occurred in

previous experiments evaluating host plant effects on larvae (personal observation).

Pupal mortality was caused by predation by Vespidae, ants, and birds and parasitism by *Copidosoma* sp. (table 1).

The main species of ants observed preying on *C. includens* larvae were: *Camponotus crassus* (Mayr), *Crematogaster evallans* (Forel), *Pseudomyrmex gracilis* (Fabr.), *Pseudomyrmex termitarius* (Smith), *Solenopsis geminata* (Fabr.), and *Solenopsis saevissima* (Smith). The main species of Vespidae predators of *C. includens* were: *Polistes simillimus* (Zikan), *Polistes versicolor* (Olivier), *Polybia ignobilis* (Haliday), and *Polybia occidentalis* (Olivier).

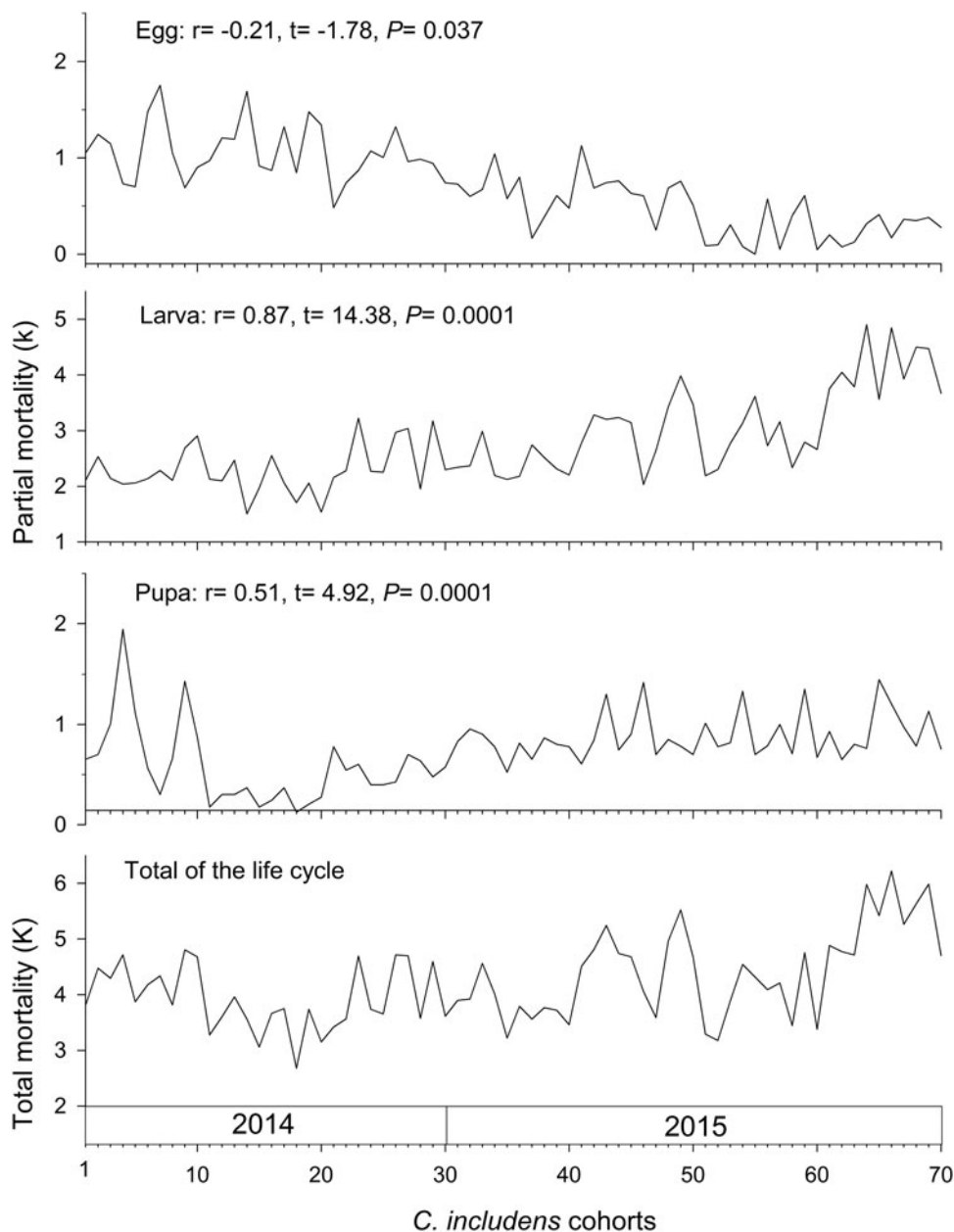


Fig. 1. Determination of *Chrysodeixis includens* critical mortality stage using the mortality of egg, larva and pupa correlations with the total mortality of all immature stages combined.

The major causes of egg mortality were: inviability (MM = 37.4%), parasitism by *Trichogramma* sp. (MM = 23.4%), and predation by *Orius* sp. (MM = 23.1%). The major causes of larval mortality were predation by ants in first and second instars (MM = 64.7 and 54.6%, respectively) and Vespidae in third, fourth, and fifth instars (MM = 40.5, 46.2, and 55.3%, respectively). The major cause of pupal mortality was Vespidae (MM = 62.6%) (table 1).

#### Critical mortality stages of *C. includens*

Mortalities in larval and pupal stages have positive and significant correlations ( $P < 0.05$ ) with the total mortality of *C. includens* (fig. 1). Between these two stages, the larvae mortality curve showed higher inclination than the pupae (fig. 2). Mortalities in the first, second, third, fourth, and fifth instars showed positive and significant correlations ( $P < 0.05$ ) with the larval total mortality (fig. 3). Among the instars, mortality curves of the second and fourth instars showed the highest inclinations (fig. 4). Therefore, the critical stages of *C. includens* mortality were second and fourth instars.

#### Key mortality factor of *C. includens*

In the second instar, mortalities caused by ants and rainfall had positive and significant correlations ( $P < 0.05$ ) with total mortality in this instar (fig. 5a). Between these two factors, the mortality curve caused by ants showed higher inclination than the mortality curve caused by the rainfall (fig. 5b).

In the fourth instar, mortalities caused by Vespidae and rainfall had positive and significant correlations ( $P < 0.05$ ) with total mortality in this instar (fig. 6a). Between these two

factors, the mortality curve caused by Vespidae showed higher inclination than the mortality curve caused by the rains (fig. 6b).

Therefore, the key mortality factors of *C. includens* were predation by ants in the second instar and predation by Vespidae in the fourth instar. Based on the indispensable mortalities caused by predation by ants and by Vespidae, the suppression of these predators action would increase *C. includens* populations at 77.52 and 85.16%, respectively.

## Discussion

For the development of efficient pest management systems for *C. includens*, it is essential to identify and quantify the factors affecting its population dynamics. The results of this study show that *C. includens* populations were regulated by the mortalities caused by biotic and abiotic factors that varied in the magnitude over its life cycle.

*Chrysodeixis includens* total mortality was very high (99.99%), indicating that natural mortality factors are sufficient to regulate its population density. Therefore, while managing the crops, we should adopt practices that allow the preservation of natural mortality agents for this pest.

The critical stage of *C. includens* mortality was the larval stage (second and fourth instars) because in these instars the natural mortality is very high. The high mortality can be due to the longer period of insect exposure to natural mortality factors during the larval stage comparing to the others stages (Gonring et al., 2002, 2003). *Chrysodeixis includens* larval period is longer (14–20 days) than the egg (3 days) and pupal (7 days) stages (Mitchell, 1967; Reid & Greene, 1973).

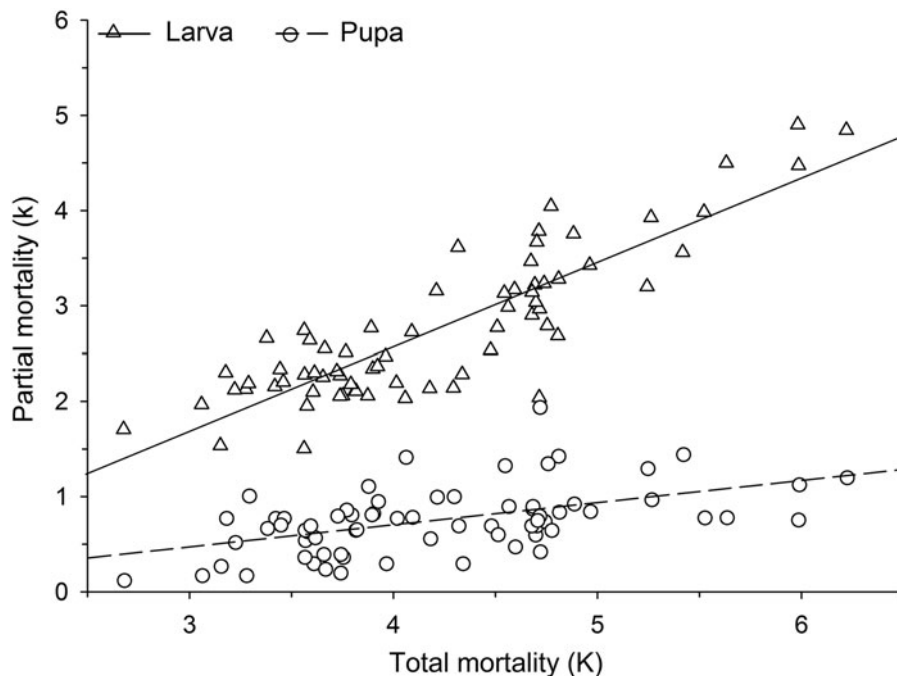


Fig. 2. Determination of *Chrysodeixis includens* critical mortality stage using simple linear regression analysis between larva and pupa mortalities with the total mortality of all immature stages combined. Linear regression curves of Larva:  $k = -0.97 + 0.88K(0.76 - 1.01)$ ,  $R^2 = 0.75$ ,  $P < 0.001$  and pupa:  $k = -0.23 + 0.23K(0.14 - 0.33)$ ,  $R^2 = 0.26$ ,  $P < 0.001$ . Numbers in parentheses represent 95% confidence interval for the slope of the curves.

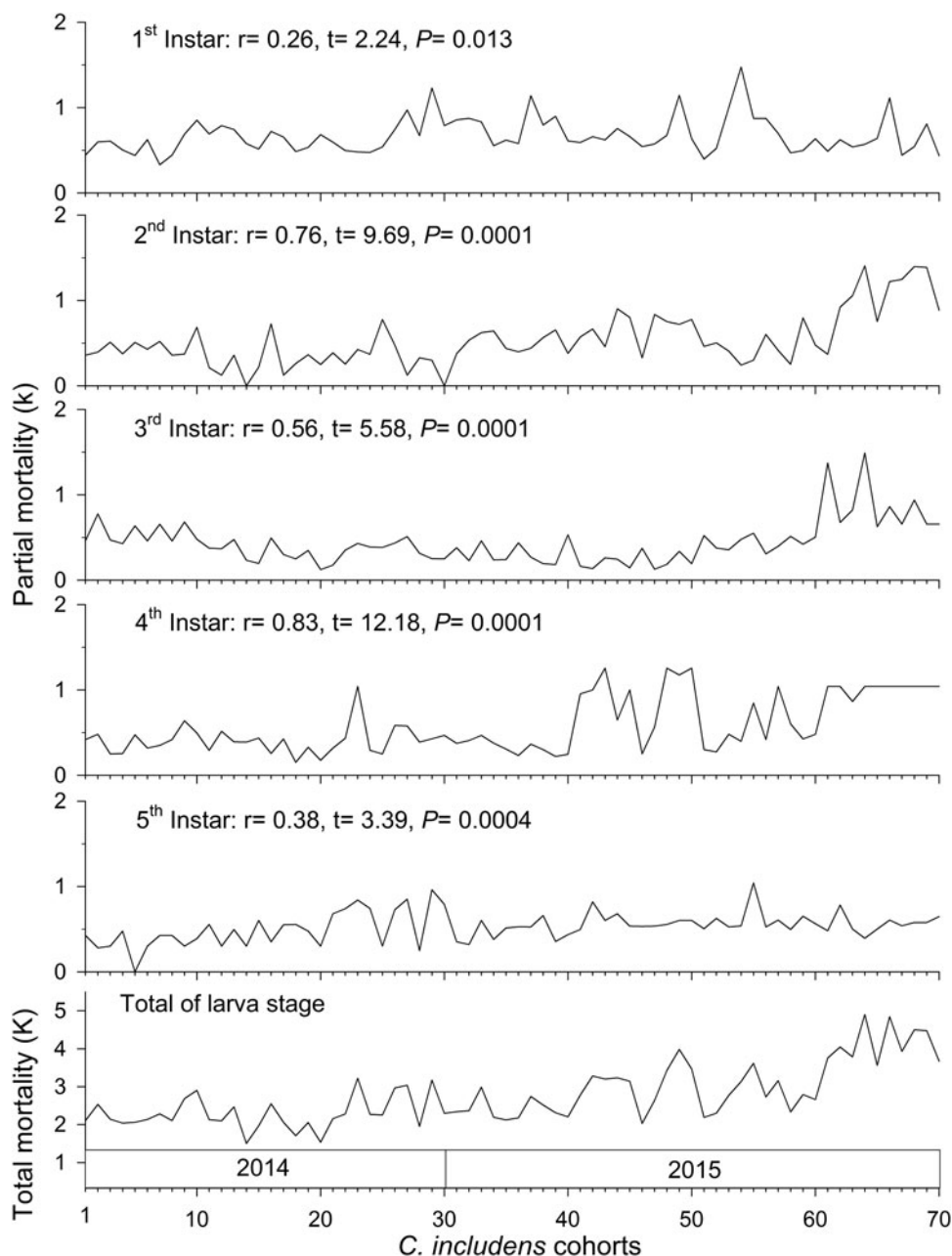


Fig. 3. Determination of *Chrysodeixis includens* critical mortality stage using correlations between partial mortality of each instar with the total mortality of larvae.

Predation by ants was the *C. includens* mortality key factor in the second instar. Ants have important attributes that are responsible for their success as predators. For example, they: (a) respond to the prey density; (b) remain plentiful even when the prey is scarce because they can cannibalize their offspring and feed from alternative sources such as honeydew and flower nectar; (c) store food and continue capturing prey even when they are not immediately consumed; (d) prey on different sizes; and (e) they can be managed to increase their abundance, distribution, and contact with their prey (Risch & Carrol, 1982).

Predatory ants can be generalists or specialists. The generalist species are important biological control agents, but the specialists do not seem to play a significant role in agroecosystems (Way & Khoo, 1992). They are abundant and successful in different environments (Hajek, 2004). As their nests are usually built on the ground, soil preparation and soil application of pesticide can cause significant negative impact on these predators (Ramos *et al.*, 2012).

As foraging strategy, ants can perform individual or in-group search. The first is the most common strategy and includes the species with mass recruitment behavior

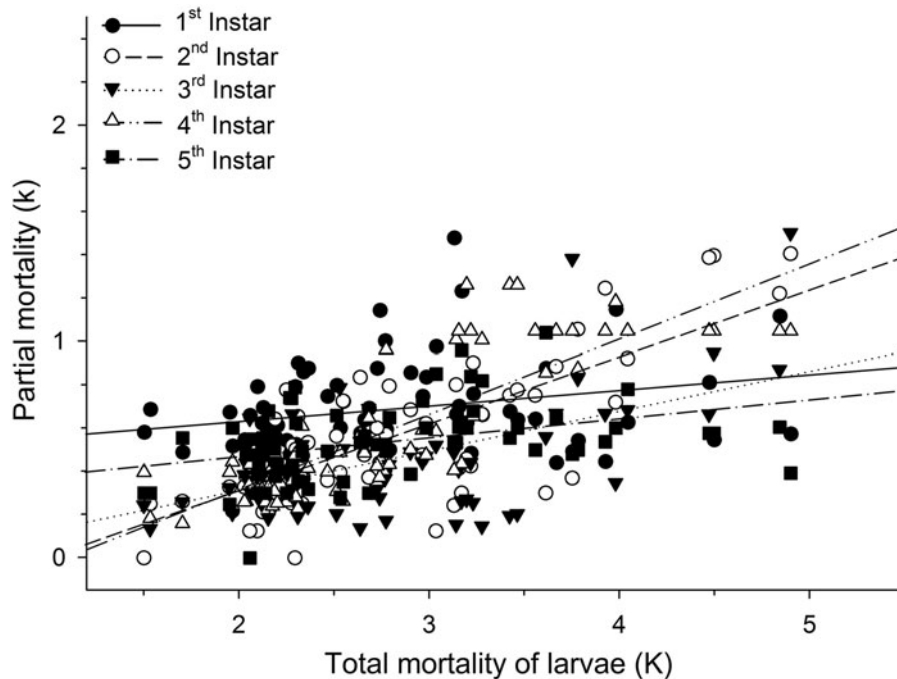


Fig. 4. Determination of *Chrysodeixis includens* critical mortality stage using simple linear regression analysis between the mortality of each instar with the total mortality of larvae. Linear regression curves of first instar:  $k = 0.48 + 0.07K(0.01 - 0.14)$ ,  $R^2 = 0.07$ ,  $P = 0.0285$ ; second instar:  $k = -0.32 + 0.31K(0.25 - 0.37)$ ,  $R^2 = 0.58$ ,  $P < 0.001$ ; third instar:  $k = -0.67 + 0.18K(0.12 - 0.25)$ ,  $R^2 = 0.31$ ,  $P < 0.001$ ; fourth instar:  $k = -0.38 + 0.35K(0.29 - 0.41)$ ,  $R^2 = 0.69$ ,  $P < 0.001$ ; fifth instar:  $k = 0.29 + 0.09K(0.04 - 0.14)$ ,  $R^2 = 0.14$ ,  $P = 0.0012$ . Numbers in parentheses represent 95% confidence interval for the slope of the curves.

(Carroll & Janzen, 1973). During foraging, the workers seek prey, so each worker is responsible for a small search area. When prey is located, the worker returns to the nest by visual guidance and establishes an odor trail wherever it goes (Carroll & Janzen, 1973). Depending on the species, the size of the ant, the density, and quality of the prey, the foraging workers send information to their partners about their location, and thereby recruit additional foragers (Traniello, 1989). The other forager follows the trail to the prey and locates the food. Predation occurs until the food source is exhausted or until the food is sufficient to feed the colony (Carroll & Janzen, 1973).

During this research, we observed ants preying on larvae during the day and at night. Ramos *et al.* (2012) had a similar result and found predation of *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) by ants during the periods of morning, afternoon, and evening. Foraging characteristics, the large number of species and individuals in the field, and its high predation capacity make predatory ants important natural enemies for *C. includens* control. Further evidence of the importance of ants as *C. includens* predators is that the suppression of these predators' action would increase *C. includens* populations by 77.52%.

*Chrysodeixis includens* mortality key factor in the fourth instar was predation by Vespidae. This was because this predator feeds preferably on Lepidoptera larvae (Rabb, 1960) in advanced instars (Picanço *et al.*, 2011; Santana Junior *et al.*, 2012). The reason for this is the larger body size of the late instar larvae. The larger body size makes these larvae a good source of protein and facilitates its location by predators (Picanço *et al.*, 2010).

Acquired experience, distribution, and abundance of prey are important factors in Vespidae predator foraging (Richter, 2000; Picanço *et al.*, 2010). Some Vespidae species choose foraging locations influenced by the prey density (Richter, 2000) and previous experience of other foragers (Picanço *et al.*, 2010). Social Vespidae are opportunistic and generalists. However, in some situations, these predators behave individually as facultative specialists (Richter, 2000). This behavior occurs when Vespidae return to forage in locations where they succeeded to find the prey (Suzuki, 1978; Richter & Jeanne, 1985; Picanço *et al.*, 2010), and so they can feed repeatedly on the same prey species (Picanço *et al.*, 2011). During this research, we observed the constant return of Vespidae to plants with *C. includens* larvae. We also observed the behavior of recruitment of new individuals to foraging on these plants. According to O'Donnell & Jeanne (1992), foraging success increases with Vespidae age and experience. In this research, Vespidae foraging was intense and predation was high even in times where the population density of *C. includens* was low. This demonstrates the high efficiency of prey location by these predators.

The habitat of Vespidae must provide suitable nesting sites and food resources within reach of foraging to ensure their reproductive success (Sutherland, 1996). For this reason, nests are built in trees or shrubs in forests located near agricultural crops, usually where prey availability is high. Lawton (1983) demonstrated that an environment with more complex structure enables the establishment and survival of most species of social Vespidae. Our experiments were conducted in areas near the native vegetation. This



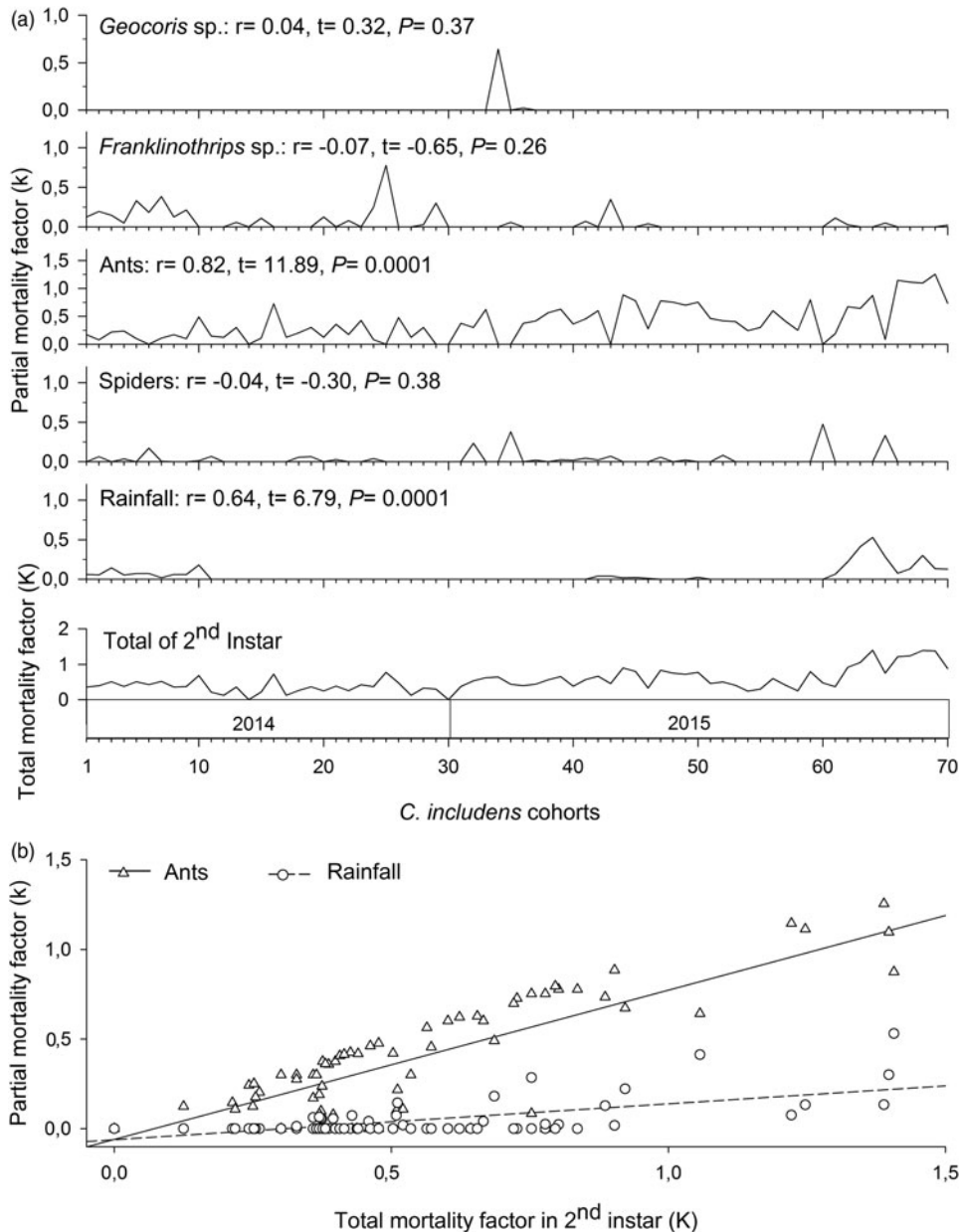


Fig. 5. Determination of *Chrysodeixis includens* key mortality factors of second instar using correlations between partial mortality of each factor with the total mortality (A). Simple linear regression analysis between the mortality of each factor with the total mortality (B). Linear regression curves of ants:  $k = -0.59 + 0.83K(0.69 - 0.97)$ ,  $R^2 = 0.68$ ,  $P < 0.001$  and rainfall:  $k = -0.61 + 0.20K(0.14 - 0.26)$ ,  $R^2 = 0.40$ ,  $P < 0.001$ . Numbers in parentheses represent 95% confidence interval for the slope of the curves.

possibly contributed to the high number of Vespidae foraging during the evaluations and, therefore, to the high *C. includens* mortality.

This study elucidates the factors and mechanisms involved in *C. includens* population regulation, so these factors should be considered in planning strategies and management tactics for this pest. The natural mortality of *C. includens* is high and the mortality factors are sufficient to reduce its population density. The mortality critical stage of *C. includens* is the larval stage, and second and fourth instars are most vulnerable to mortality factors. Mortality key factors are ants in the second

instar and Vespidae in the fourth instar. The action of Vespidae and ants is crucial to the reduction of *C. includens* populations. The elimination of these factors can cause an increase of 77.52 and 85.17% of its population, respectively. Favoring these natural enemies through pest management tactics and the maintenance of their population levels should be considered during the decision-making process. Thus, efficient pest control becomes possible. Therefore, determining the importance of multiple mortality factors in the management of *C. includens* is the first step to a better understanding of their population dynamics.

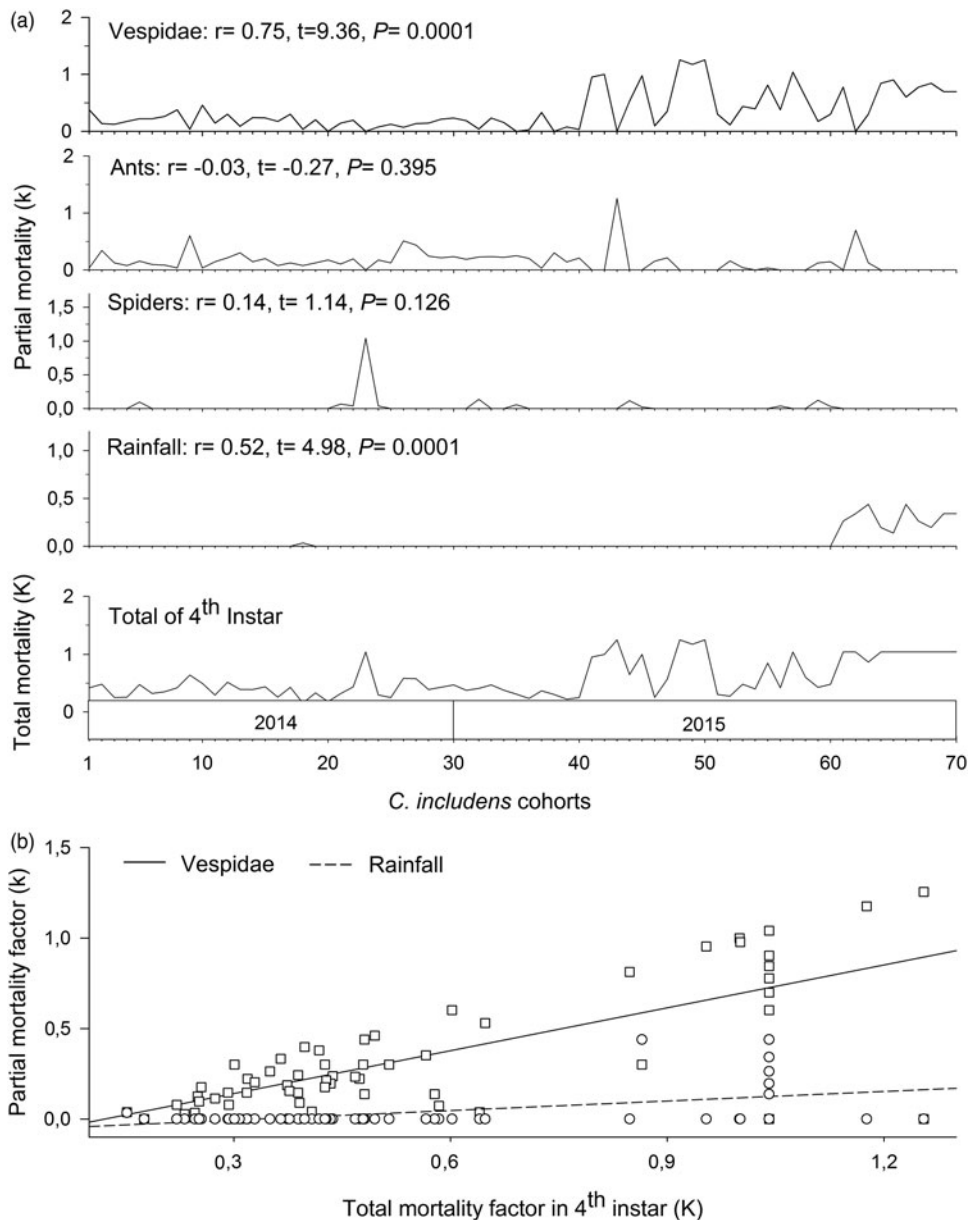


Fig. 6. Determination of *Chrysodeixis includens* key mortality factors of fourth instar using correlations between partial mortality of each factor with the total mortality (A). Simple linear regression analysis between the mortality of each factor with the total mortality (B). Linear regression curves of Vespidae:  $k = -0.96 + 0.79K(0.62 - 0.96)$ ,  $R^2 = 0.56$ ,  $P < 0.001$  and rainfall:  $k = -0.59 + 0.18K(0.11 - 0.25)$ ,  $R^2 = 0.27$ ,  $P < 0.001$ . Numbers in parentheses represent 95% confidence interval for the slope of the curves.

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