RENATA RAMOS PEREIRA

CONTROLE BIOLÓGICO NATURAL DE *Chrysodeixis includens*

Tese apresentada à Universidade Federal de Viçosa, como parte das exigências do Programa de Pós-Graduação em Entomologia, para obtenção do título de *Doctor Scientiae*.

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APROVADA: 24 de fevereiro de 2017.

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À meus pais, Eduardo e Edith, meus exemplos;

Aos meus irmãos Aline e Guilherme, meu apoio;

Aos meus sobrinhos Pedro Henrique e Matheus, minhas alegrias;

Aos meus afilhados Isabella e Pedro, meus presentes;

Ao Luis Márcio, meu grande amor;

Aos meus amigos, primos e tios;

Dedico.

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BIOGRAFIA

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RESUMO

PEREIRA, Renata Ramos, D.Sc., Universidade Federal de Viçosa, fevereiro de 2017. **Controle biológico natural de *Chrysodeixis includens***. Orientador: Marcelo Coutinho Picanço. Coorientador: André Luiz Barreto Crespo.

Nos últimos anos têm ocorrido surtos populacionais de *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae) em diversos cultivos agrícolas. Porém as causas desses surtos são desconhecidas. Entre os principais fatores reguladores da intensidade de ataque de pragas nos agroecossistemas estão naturais de mortalidade. As tabelas de vida ecológicas constituem ferramentas ideais de determinação dos fatores que regulam as populações da praga (fatores chave) e em que fase do ciclo de vida da praga eles atuam (estágio crítico). Nestes estudos, também é importante a determinação dos fatores que afetam a atuação desses agentes de controle biológico natural. Assim, com o intuito de preencher parte desta lacuna científica e de gerar conhecimentos a serem incorporados a programas de manejo integrado de *C. includens* nós determinamos: (*i*) os estágios críticos e os fatores chave de mortalidade de *C. includens*, e (*ii*) a variação sazonal dos fatores de mortalidade dessa praga. Para tanto, ao longo de dois anos foram monitorados os fatores de mortalidade natural de *C. includens* em cultivos de feijão usando tabelas de vida ecológicas. A mortalidade total de *C. includens* foi superior a 99%. A mortalidade de ovos foi causada por chuvas, inviabilidade, o parasitóide *Trichogramma* sp., herbívoros desfolhadores e os predadores *Orius* sp e *Franklinothrips* sp. A mortalidade de larvas foi causada por chuvas e os predadores *Orius* sp, *Geocoris* sp., *Franklinothrips* sp., formigas, aranhas e Vespidae. A mortalidade de pupas foi causada pelo parasitóide *Copidosoma* sp. e os predadores Vespidae, formigas e pássaros. Os estágios críticos de mortalidade foram o 2º e 4º ínstar larval. Os fatores chave de mortalidade foram Formicidae (2º ínstar) e Vespidae (4º ínstar). Os Vespidae causaram maiores mortalidades à *C. includens* em épocas quentes, chuvosas e de dias mais longos. Já as maiores mortalidades da praga por Formicidae ocorreram em períodos de maiores umidades relativas do ar. Portanto, este estudo elucida os mecanismos de regulação populacional de *C. includens* e demonstra a importância da preservação das populações de inimigos naturais, sobretudo de Formicidae e

Vespidae. Além disto, possibilita a previsão de ocorrência de maiores ou menores mortalidades da praga em função dos elementos climáticos.

ABSTRACT

PEREIRA, Renata Ramos, D.Sc., Universidade Federal de Viçosa, February, 2017. ***Chrysodeixis includens* natural biological control**. Adviser: Marcelo Coutinho Picanço. Co-adviser: André Luiz Barreto Crespo.

In recent years, there have been *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae) population outbreaks in various agricultural crops. But the causes of these outbreaks are unknown. Among the main regulating factors of pest attack intensity in agroecosystems are natural mortality factors. Ecological life tables are ideal tools of determining the factors that regulate the pest populations (key factors) and at which stage of the pest life cycle they act (critical stage). In these studies, it is also important to determine the factors that affect these natural biological control agents' performances. Thus, in order to fulfill part of this scientific gap and generate knowledge to be incorporated into *C. includens* integrated management programs we determine: (*i*) *C. includens* critical stages and key mortality factors, and (*ii*) seasonal variation of this pest mortality factors. Over two years, *C. includens* natural mortality factors on dry bean crops were monitored using ecological life tables. *C. includens* total mortality was greater than 99%. Egg mortality was caused by rainfall, inviability, the parasitoid *Trichogramma* sp., herbivores and the predators *Orius* sp and *Franklinothrips* sp. Larvae mortality was caused by rainfall and the predators *Orius* sp, *Geocoris* sp., *Franklinothrips* sp., ants, spiders and Vespidae. Pupae mortality was caused by the parasitoid *Copidosoma* sp. and the predators Vespidae, ants and birds. The critical mortality stages were the 2nd and 4th instars. The key mortality factors were ants (2nd instar) and Vespidae (4th instar). Vespidae caused higher *C. includens* mortalities to in hotter, rainy and longer days. On the other hand, the highest mortalities of the pest by ants occurred during periods of higher relative humidity. Therefore, this study elucidates *C. includens* mechanisms of population regulation and demonstrates the importance of the preservation of natural enemies populations, especially of ants and Vespidae. In addition, it allows the prediction of occurrence of higher or lower pest mortalities due to climatic elements.

INTRODUÇÃO GERAL

A lagarta falsa medideira, *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae), é uma importante praga agrícola. Sua importância se dá pelo seu hábito alimentar polífago com capacidade de se desenvolver em 73 espécies de plantas hospedeiras, pertencentes a 29 famílias (CAB, 2016). Dentre os hospedeiros desta praga estão culturas de grande importância econômica como soja, feijão, algodão, fumo, girassol, tomate e batata (Eichlin & Cunningham, 1978; Herzog, 1980).

A polifagia é uma característica extremamente importante para esta espécie, já que, pode colaborar com a dinâmica populacional desta praga e dificultar o seu controle. Insetos polífagos podem se desenvolver simultaneamente em diferentes espécies hospedeiras em uma mesma região e, deste modo permanecer no ambiente em baixas densidades até que a fêmea localize o hospedeiro mais adequado para o desenvolvimento da prole (Fitt, 1989).

Essa espécie está distribuída no hemisfério ocidental, ocorrendo desde o norte dos EUA até o sul da América do Sul (Eichlin & Cunningham, 1978; Alford et. al. 1982; Barrionuevo et al., 2012). Nos Estados Unidos estão presentes na maioria dos estados entre Nova York e a Califórnia (Herzog, 1980). Infestações desta praga causam sérios danos econômicos no norte do Texas, Arkansas, Mississipi, Alabama, Geórgia e Carolina do Sul (Herzog, 1980).

No Brasil, a *C. includens* era considerada praga secundária até a safra agrícola de 2003/2004, porém a partir de então ocorreram surtos populacionais desta praga em várias regiões (Bortolotto et al., 2015). Muitos autores especulam os motivos que levaram à mudança de *status* desta praga (Bueno et al., 2011; Baldin et al., 2014; Bortolotto et al., 2015) porém, ainda não são conhecidas as causas destes surtos.

Nos primeiros ínstares, as lagartas selecionam as folhas mais tenras e se alimentam daquelas com pequena quantidade de fibra (Kogan & Cope, 1974), tornando-se menos exigentes à medida que se desenvolvem. Até o terceiro ínstar, as lagartas deixam intactas algumas regiões da epiderme. A partir do quarto ínstar consomem áreas grandes mantendo íntegras somente as nervuras principais, o que confere um aspecto rendilhado característico às folhas atacadas (Herzog, 1980). Os danos causados por esta praga reduzem a área fotossintética das plantas comprometendo a produção, principalmente se a desfolha ocorrer nos estágios reprodutivos das plantas.

Múltiplos fatores naturais de mortalidade regulam a dinâmica populacional de insetos nos agroecossistemas. Entre estes fatores os mais importantes são os inimigos naturais, os elementos climáticos e a planta hospedeira. O entendimento da magnitude desses fatores é essencial para o estudo da dinâmica populacional de uma espécie. É essencial na previsão de ocorrência de surtos e no desenvolvimento de melhores sistemas de manejo de pragas (Naranjo & Ellsworth, 2005).

Uma importante ferramenta de pesquisa utilizada em estudos de controle natural de insetos é a construção de tabelas de vida ecológicas (Harcourt, 1969). Um método robusto que descreve e quantifica os fatores de mortalidade natural através de medidas que evidenciam as mudanças na população pelo seu ciclo de vida e pelas variações do seu ambiente natural (Harcourt, 1969, Naranjo & Ellsworth, 2005). A identificação e a quantificação das causas de mortalidade nos permite determinar os estágios críticos e os fatores chave de mortalidade de insetos, parâmetros essenciais no entendimento de sua dinâmica populacional. O estágio crítico é aquele que determina o tamanho da população e o fator chave de mortalidade é aquele de maior importância relativa no estágio crítico (Harcourt, 1969; Morris, 1963; Varley et al., 1973; Podoler & Rogers, 1975).

Tabelas de vida ecológicas tem sido desenvolvidas para insetos e suas análises têm provido importantes descobertas sobre a dinâmica e regulação populacional de insetos em diferentes agroecossistemas (Morris, 1959; Varley et al., 1973; Podoler & Rogers, 1975, Royama, 1981; Carey, 1989; Pereira et al., 2007; Semeão et al., 2012). Assim, os objetivos deste trabalho foram determinar: (i) os estágios críticos e os fatores chave de mortalidade de *C. includens*, e (ii) a variação sazonal dos fatores de mortalidade dessa praga.

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CAPÍTULO I: NATURAL BIOLOGICAL CONTROL OF *Chrysodeixis includens*

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 2nd and 4th instars. The key mortality factors were predation by Formicidae in the 2nd instar and predation by Vespidae in the 4th 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.

1. 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).

Climatic elements, in turn, can affect the pest population dynamics directly or indirectly mainly through air temperature, rainfall and photoperiod. Directly, temperature and photoperiod affect the development and reproduction of insects (Mellanby, 1939; Régnière et al., 2012) and rainfall causes mortality by mechanical impact of raindrops. 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).

Thus, an important research tool that can be used in studies of pest population regulation by these factors is the ecological life table (Harcourt, 1969). Analysis from the ecological life tables has provided important insights into the dynamics and regulation of insect populations in agroecosystems (Morris, 1959; Varley et al., 1973; Podoler & Rogers, 1975, Royama, 1981; Carey, 1989; Pereira et al., 2007; Semeão et al., 2012). This is because ecological life tables are 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 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 (Varley & Gradwell, 1960; Morris, 1963; Harcourt, 1969; Podoler & Rogers, 1975).

Soybean looper, *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae), is an important agricultural pest that occurs in the Western Hemisphere from the northern United States (U.S.) to southern South America (Eichlin & Cunningham, 1978, Alford & Hammond Junior, 1982, Barrionuevo et al., 2012). This species is polyphagous and feeds on 73 plant species belonging to 29 different families (CAB International, 2016). It is an important pest of crops such as soybeans, beans, cotton, sunflower, tomato and potato (Eichlin & Cunningham, 1978).

In the U.S., *C. includens* is reported as a key soybean pest in several regions (Kogan & Turnipseed, 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 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.

2. MATERIALS AND METHODS

2.1. 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, 600m a.s.l., tropical climate) in 2014 and 2015. During those two years seven experiments were conducted in fields 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^2 (65 x 50 m) and the plants were placed at 50 × 10 cm spacing. The fields were grown as recommended by EMBRAPA (2005) and pesticides were not used.

2.2. *C. includens* colony

Individuals of *C. includens* used in the bioassays 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 the field were transferred to the oviposition cage. When the adults emerged, a 30 days 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 two 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 three 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.

2.3. Determination of mortality factors

The experimental design was completely randomized with 10 experimental plots (replication). Each plot consisted of 10 bean plants 30 days after planting. 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 containing eggs, larvae and pupae.

2.3.1. Evaluation of egg mortality factors

Around each set of 10 plants (plot), we built a wood and organza fabric cage (100 × 50 × 50 cm). Within this structure we release 20 three day old unsexed adults for oviposition. After 24 hours, the adults and the cage around the plants were removed. *C. includens* eggs were recorded (about 50 eggs per plot). 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. 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., 2007).

Each egg that had not hatched after the field evaluation period was placed in a glass tube (10 cm long x 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. 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.

2.3.2. Evaluation of larval mortality factors

For *C. includens* larval cohort establishment we transferred 200 neonates for each field plot. We evaluated the instar and the mortality numbers and causes.

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 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, the number of normal pupae and the parasitoids that emerged from larvae.

2.3.3. Evaluation of pupae mortality factors

For *C. includens* pupae cohort establishment we transferred 15 larvae at the end of the 5th instar for each plot to allow pupation on the plants. 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 who died by physiological disturbance during molting, number of adults and the parasitoids that emerged from the pupae.

2.4. 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.

2.5. 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, 1st, 2nd, 3rd, 4th, 5th instars and pupa.

$$100rx = 100.(dx/L_0) \quad (2)$$

$$100qx = 100.(dx/L_x) \quad (3)$$

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 occurred 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 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 marginal mortality (Elkinton et al., 1992). The marginal mortality 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).

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

$$To_f = \sum 100qxo/100 \quad (5)$$

where MM_f is the marginal mortality 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).

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

$$K = \sum(k) \tag{7}$$

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; 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)

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

3. RESULTS

3.1. 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 egg, 1st, 2nd, 3rd, 4th, 5th 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 *C. includens* eggs. The parasitism of *C. includens* eggs was made by *Trichogramma* sp. (Hymenoptera: Trichogrammatidae) (Table 1).

Larvae 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 wasps (Hymenoptera: Vespidae). Spiders and Formicidae prey on larvae in all instars. *Geocoris* sp. and *Franklinothrips* sp. were found preying on larvae of 1st, 2nd and 3rd instars and Vespidae on 3rd, 4th and 5th instars (Table 1).

Pupal mortality was caused by predation by Vespidae, Formicidae and birds and parasitism by *Copidosoma* sp. (Hymenoptera: Encyrtidae) (Table 1).

The main species of Formicidae 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).

The major causes of egg mortality egg were: inviability (marginal mortality (MM) = 37.4%), parasitism by *Trichogramma* sp. (MM = 23.4%) and predation by *Orius* sp. (MM = 23.1%). The major causes of larvae mortality were predation by Formicidae in 1st and 2nd instars (MM = 64.7 and 54.6%, respectively) and Vespidae in 3rd, 4th and 5th instars (MM = 40.5, 46.2 and 55.3%, respectively). The major cause of pupae mortality was Vespidae (MM = 62.6%) (Table 1).

3.2. 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* (Figure 1). Between these two stages the larvae mortality curve showed higher inclination than the pupae (Figure 2). Mortalities in the 1st, 2nd, 3rd, 4th and 5th instars showed positive and significant correlations ($P < 0.05$) with the larval total mortality (Figure 3). Among the instars, mortality curves of the 2nd and 4th instars showed the highest inclinations (Figure 4). Therefore, the critical stages of *C. includens* mortality were 2nd and 4th instars.

3.3. Key mortality factor of *C. includens*

In the 2nd instar, mortalities caused by Formicidae and rainfall had positive and significant correlations ($P < 0.05$) with total mortality in this instar (Figure 5A). Between these two factors, the mortality curve caused by

Formicidae showed higher inclination than the mortality curve caused by the rainfall (Figure 5B).

In the 4th instar, mortalities caused by Vespidae and rainfall had positive and significant correlations ($P < 0.05$) with total mortality in this instar (Figure 6A). Between these two factors, the mortality curve caused by Vespidae showed higher inclination than the mortality curve caused by the rains (Figure 6B).

Therefore, the key mortality factors of *C. includens* were predation by Formicidae in 2nd instar and predation by Vespidae in 4th instar. Based on the indispensable mortalities caused by predation by Formicidae and by Vespidae, the suppression of these predators action would increase *C. includens* populations at 77.52 and 85.16%, respectively.

4. 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 magnitude over its life cycle.

C. 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 analysis of critical stages and key factors of mortality in ecological life tables are widely used in insect management studies (Morris, 1963; Varley et al., 1973; Podoler & Rogers, 1975; Miranda et al., 1998). The critical stage of *C. includens* mortality was the larval stage (2nd and 4th instars), possibly because of the longer period of insect exposure to natural mortality factors during this stage (Gonring et al., 2002; 2003). This was because larval period was longer (14-20 days) than the egg stage (3 days) and pupal (7 days) (Mitchell, 1967; Reid & Greene, 1973).

Predation by Formicidae was the *C. includens* mortality key factor in 2nd 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 & Carroll, 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 (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 prey, the foraging workers sends information to their partners about their location, and thereby recruits 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 Formicidae 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 periods of morning, afternoon and evening. Foraging characteristics, the large number of species and individuals in the field and its high predation capacity make the 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%.

C. includens mortality key factor in the 4th 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 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 wasps' 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 wasps are opportunistic and generalists. However, in some situations these predators behave individually as facultative specialists (Richter, 2000). This behavior occurs when wasps return to forage in locations where they succeeded to find 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 wasp's 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 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, 2nd and 4th instars are most vulnerable to mortality factors. Mortality key factors are Formicidae in the 2nd and Vespidae in the 4th instar. The action of these two predators 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.

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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	10000	7129±312	71.29	71.29		0.521
Orius sp.		2184±217	21.84	21.84	23.1	0.114
Franklinothrips sp.		1256±199	12.56	12.56	13.3	0.062
Diabrotica speciosa		11±9	0.11	0.11	0.12	0.001
Trichogramma 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
1st Instar	2871±311	2487±268	86.62	24.87		0.578
Orius sp.		86±27	2.99	0.86	3.21	0.014
Geocoris sp.		9±7	0.32	0.09	0.35	0.002
Franklinothrips 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
2nd Instar	384±62	278±46	72.41	2.78		0.459
Geocoris sp.		2±2	0.46	0.02	0.51	0.002
Franklinothrips 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
3rd Instar	106±20	79±16	74.82	0.79		0.405
Geocoris 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
4th 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
5th 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
Copidosoma 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 (±SE) at the beginning of each stage, d_x is the number of insects killed (±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.

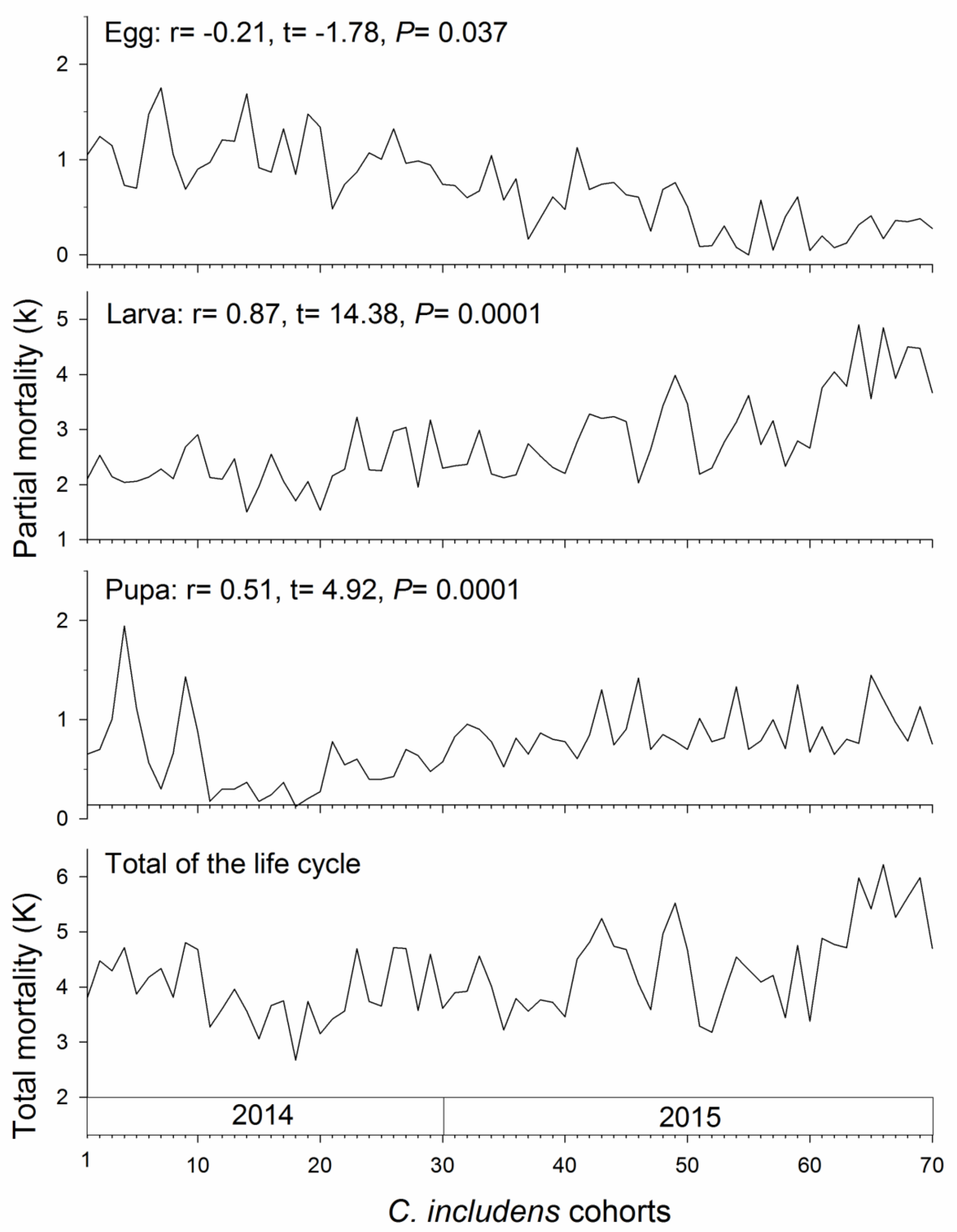

Figure 1. Determination of *Chrysodeixis includens* critical mortality stage using the mortality of egg, larva and pupa correlations with the total mortality of all immature stages combined.

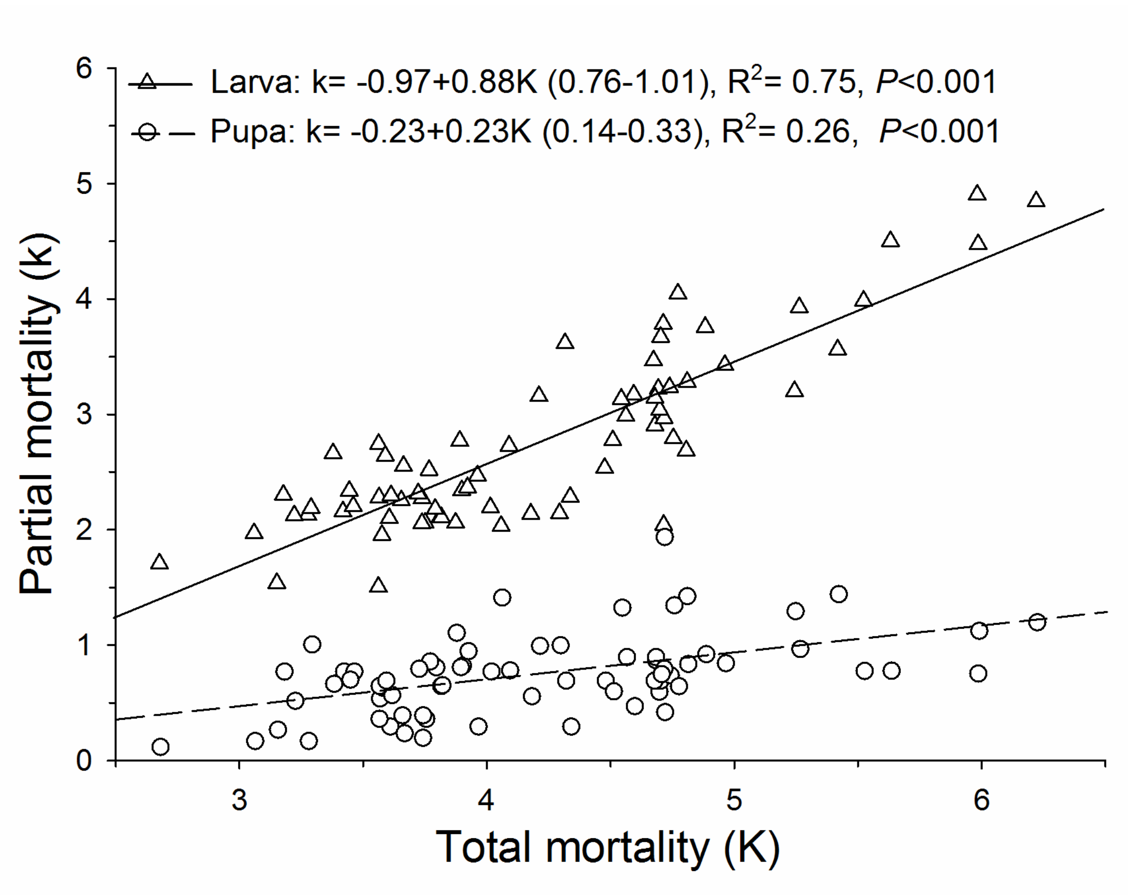

Figure 2. Determination of *Chrysodeixis includens* critical mortality stage using simple linear regression analysis between larva and pupa mortalities with the total mortality of all immature stages combined. Numbers in parentheses represent 95% confidence interval for the slope of the curves.

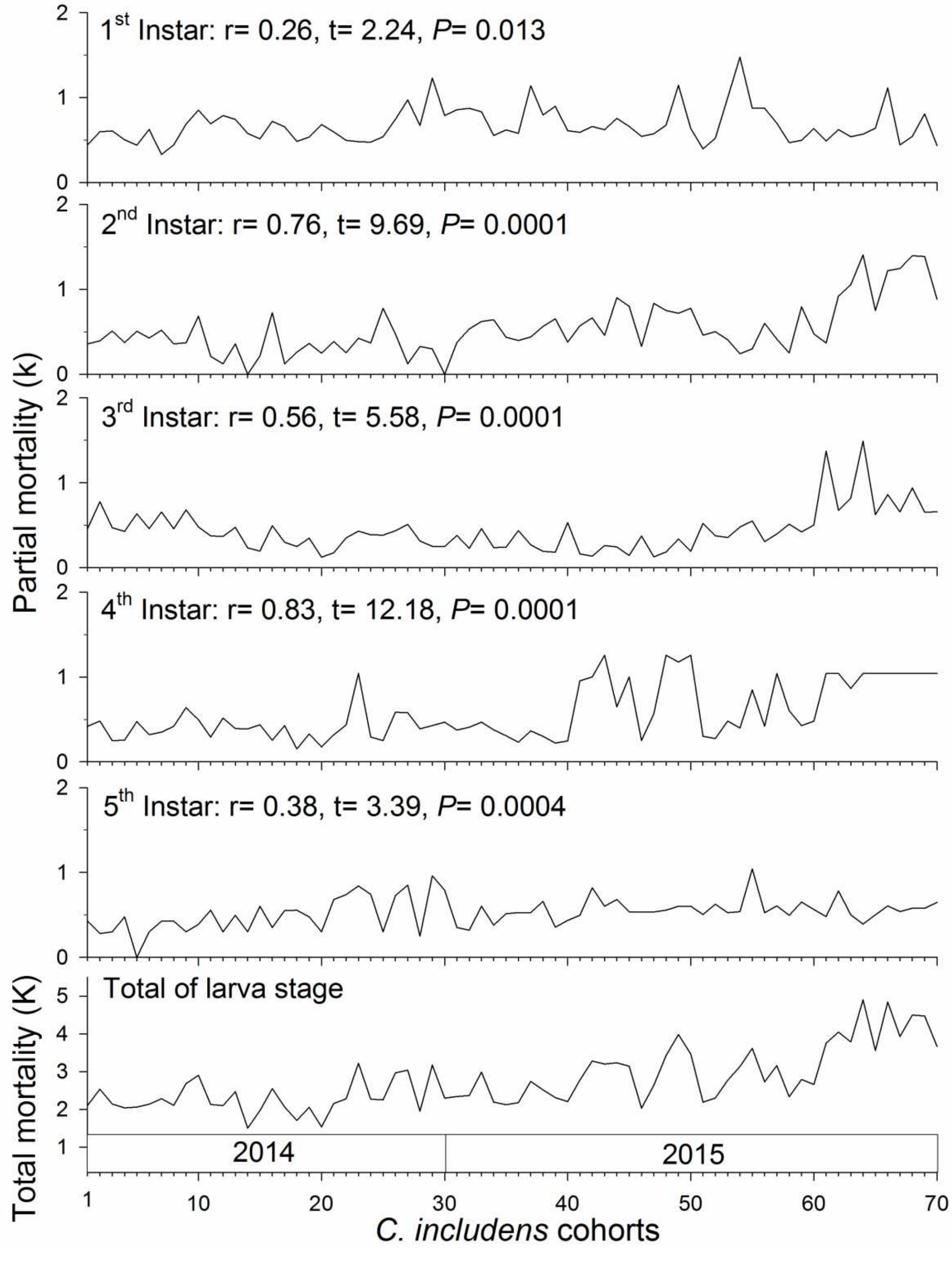

Figure 3. Determination of *Chrysodeixis includens* critical mortality stage using correlations between partial mortality of each instar with the total mortality of larvae.

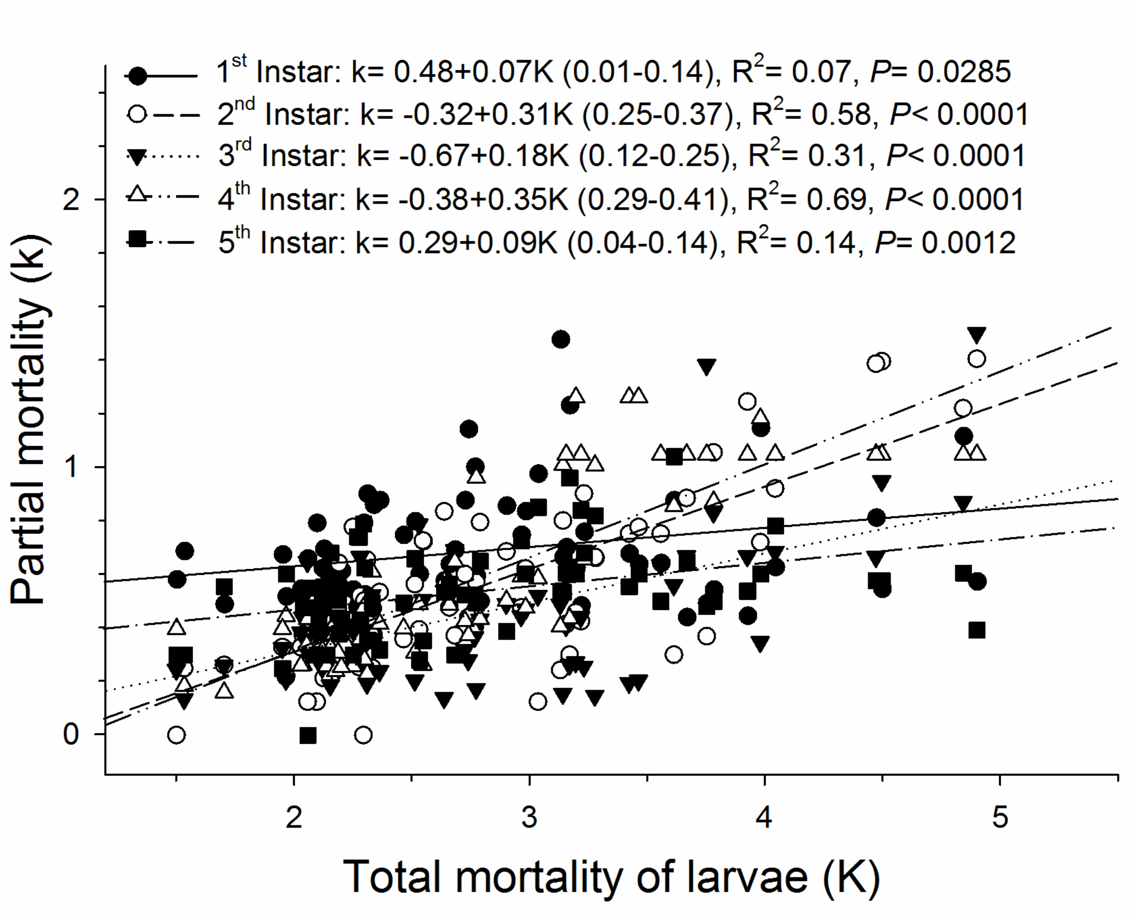

Figure 4. Determination of *Chrysodeixis includens* critical mortality stage using simple linear regression analysis between the mortality of each instar with the total mortality of larvae. Numbers in parentheses represent 95% confidence interval for the slope of the curves

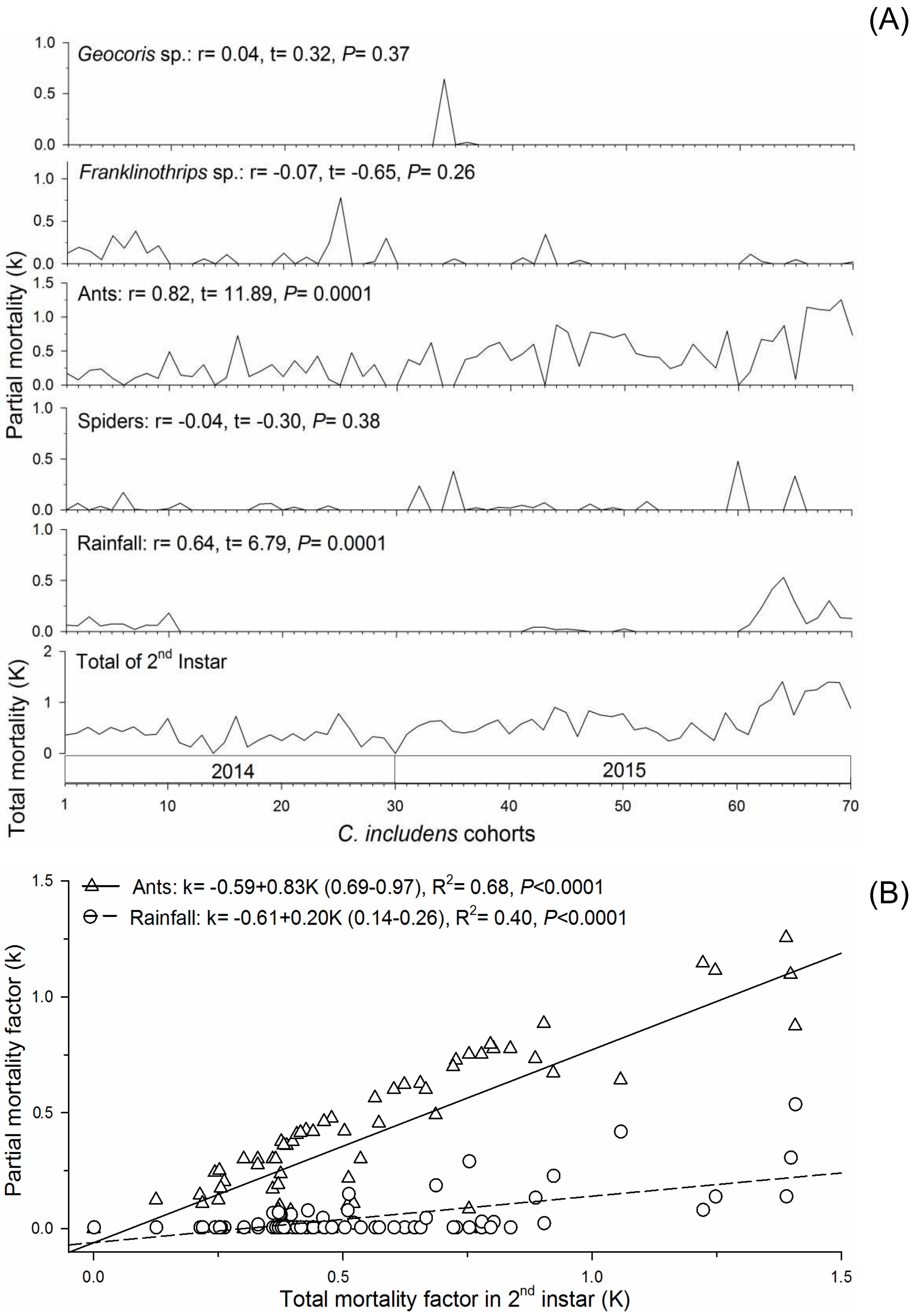

Figure 5. Determination of *Chrysodeixis includens* key mortality factors of 2nd instar using correlations between partial mortality of each factor with the total mortality (A). Simple linear regression analysis between the mortality of each factor with the total mortality (B). Numbers in parentheses represent 95% confidence interval for the slope of the curves.

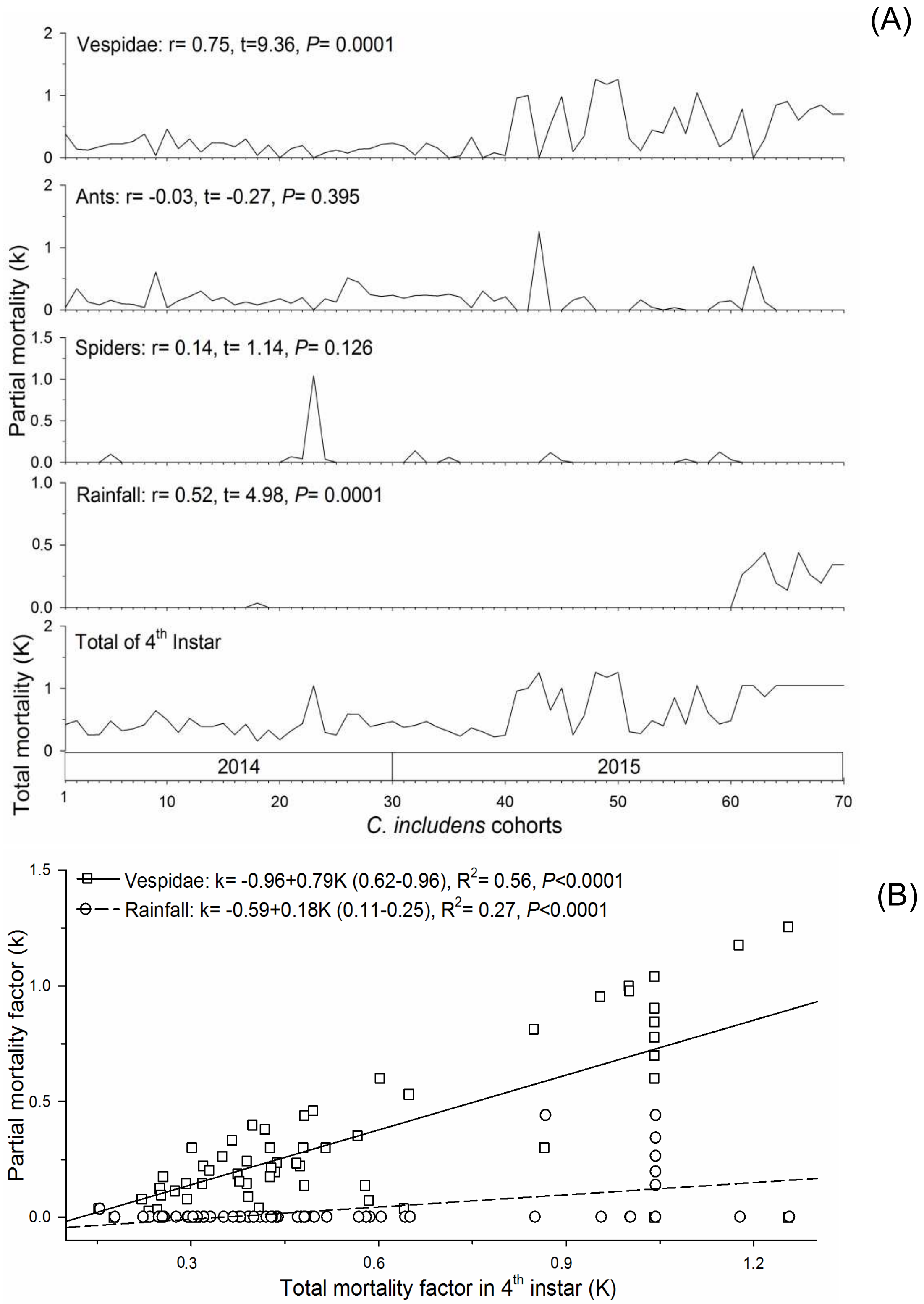

Figure 6. Determination of *Chrysodeixis includens* key mortality factors of 4nd instar using correlations between partial mortality of each factor with the total mortality (A). Simple linear regression analysis between the mortality of each factor with the total mortality (B). Numbers in parentheses represent 95% confidence interval for the slope of the curves.

CAPÍTULO II: FACTORS AFFECTING *Chrysodeixis includens* NATURAL CONTROL AGENTS

ABSTRACT - In recent years, there have been frequent reports of *Chrysodeixis includens* outbreaks, but their causes are unknown. Thus, in order to fill part of this scientific gap and to generate knowledge to be incorporated into integrated pest management programs, we investigate the natural mortality agents of *C. includens* eggs, larvae and pupae and the influence of climatic elements on these agents. The natural mortality factors of eggs were predation by *Orius* sp., *Franklinothrips* sp. and herbivores, rainfall, the parasitoid *Trichogramma* sp. and inviability. The causes of larval mortality were predation by *Orius* sp., *Geocoris* sp., *Franklinothrips* sp., ants, spiders and Vespidae and rainfall. Pupal mortality was caused by the predators Vespidae, ants and birds and the parasitoid *Copidosoma* sp. Overall, these factors were variable during the different seasons of the years. In addition, environmental factors varied over time and years and were correlated with the natural mortality agents of *C. includens*. This research promotes a better understanding of the *C. includens* population dynamics, a fundamental step for the management of this pest.

Keywords: outbreak; soybean looper; population dynamics; integrated pest management; biological control; population regulation.

1. INTRODUCTION

The soybean looper, *Chrysodeixis includens* (Walker) (Lepidoptera: Noctuidae), is an important agricultural pest. Its importance is highlighted by the fact that this insect is polyphagous, with a capacity to develop on 73 host plants belonging to 29 families (CAB 2016). This feature may contribute to its pest condition, since its populations may develop at the same time on different host plants in the same environment or persist in a region at low densities until the female finds a suitable host for its offspring development. Among the hosts of this pest are crops of great economic importance, such as soybeans, beans, cotton, tobacco, sunflowers, tomatoes and potatoes (Eichlin & Cunningham 1978, Herzog 1980).

C. includens occurs in the Western Hemisphere from the northern United States (U.S.) to southern South America (Eichlin & Cunningham 1978, Herzog 1980, Alford & Hammond Junior 1982). In the United States, it is present in most states between New York and California (Herzog 1980). Infestations of this pest cause serious economic damage in northern Texas, Arkansas, Mississippi, Alabama, Georgia, and South Carolina (Herzog 1980).

In the early instars, larvae select leaves with more water content, feeding on those with small amounts of fiber (Kogan & Cope 1974), and become less demanding as they develop. Up to the third instar, the larvae consume a small amount of the leaf. However, from the fourth instar, they consume large areas of the leaf, keeping the main veins intact, which gives a lacy appearance to the attacked leaves (Herzog 1980). The damage caused by this species reduces the photosynthetic area of the plants and compromises their production, especially if defoliation occurs in the plant’s reproductive stages.

This species was considered a secondary pest until the 2003/2004 growing season, but since then, there have been population outbreaks of this pest in several regions of Brazil (Bortolotto *et al.* 2015). Many authors have speculated on the reasons that led the status change of this pest (Bueno *et al.* 2011, Baldin *et al.* 2014, Bortolotto *et al.* 2015); however, the causes of these outbreaks are unknown.

The occurrence of pest outbreaks is related to the factors regulating the intensity of their attacks on crops (Hunter 2002). These factors include a reduction in natural mortality factors, cultivation in seasons and regions favorable to pest development, and control failure (Luck *et al.* 1977, Berryman 1982).

Overall, the natural control agents that cause mortality in pests are their natural enemies (Rosenheim 1998). Among them, the most important are predators, parasitoids and entomopathogens (Hajek 2004). Natural enemy populations are regulated by their prey abundance, the existence of alternative food (non-pest herbivores, pollen and nectar) (Landis *et al.* 2000), climatic elements (Hance *et al.* 2007) and the impact of the methods used in pest control (Croft & Brown 1975).

To determine the causes of *C. includens* outbreaks, it is important to understand the population dynamics of this pest by studying the factors that affect its natural control agents. Thus, the objectives of this study were to quantify and identify the natural mortality agents of *C. includens* eggs, larvae and pupae and to relate these mortalities to the climatic elements in different seasons and years of cultivation.

2. MATERIAL AND METHODS

Experiments were conducted 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), during 2014 and 2015. The factors considered in the study were the rainy and dry seasons of these two years. In tropical climate regions, these are the two characteristic seasons of climatic element variation (Alvares *et al.* 2014). Each of these seasons lasts six months. In the region where the experiments were conducted, the rainy season extends from October to March and the dry season from April to September (Alvares *et al.* 2014). We conducted two experiments to evaluate the factors that affect the natural control agents of *C. includens* in each season. In the rainy season, the experiments were conducted in November and January, and in the dry season, they were conducted in May and August.

This study was conducted on dry beans (*Phaseolus vulgaris* L.), cultivar Madrepérola, in an area of 3250 m^2 (65 × 50 m). Crops were planted with 50 × 10 cm spacing. The fields were grown as recommended by EMBRAPA (2005), and pesticides were not used.

2.1 Experimental conditions

The experimental design was completely randomized with 10 replicates. Each replicate consisted of 30 bean plants 30 days after planting. The replicates were isolated from one another by an area of 1 meter in width with no plants. This was done to prevent larval migration from the plants of one replicate to the others. Individuals of *C. includens* used in the experiments were obtained from a laboratory colony.

For the infestation of plants with *C. includens* eggs, in each replicate, we built a wood and organza fabric cage (100 × 50 × 50 cm) around a set of 10 plants (two rows with five plants each). Within this cage, we released 20 *C. includens* adults for oviposition on the plants. After 24 hours, the adults and the wooden structure were removed, and the number of eggs was recorded (approximately 50 eggs per replicate). When the first larva hatched, this was considered the end of the egg stage.

For the evaluation of larval natural control agents, 200 neonates were transferred into each replicate. When the first larva became a pupa, this was considered the end of this phase.

For the evaluation of pupae, we added 15 larvae at the end of the 5th instar to pupate on the plants in each replicate. When the first pupa became an adult, this was considered the end of the pupal stage.

2.2 Quantification of the action of natural control agents on *C. includens*

The causes of mortality and the numbers of individuals in each stage of the *C. includens* life cycle were monitored daily in the field. Eggs, larvae and pupae that disappeared during rainfall were considered to have died as a result of this factor (Harcourt 1969). On the other hand, mortality by predators was observed by the direct observation of these natural enemies killing *C. includens* eggs, larvae and pupae (Hawkins *et al.* 1997). Individuals of each observed predator morphospecies were stored in glass vials (10 mL) with a 70% ethanolic solution.

At the end of each stage of the *C. includens* life cycle (eggs, larvae and pupae), the remaining insects were taken to the laboratory to evaluate their mortality by parasitoids. Eggs that did not hatch during the field evaluation period were placed into glass tubes (10 cm long x 2 cm in diameter) sealed with cotton. Eggs that did not hatch and were not parasitized were considered dead by inviability.

The larvae that hatched from the eggs were reared in plastic containers (120 mL) with dry bean leaves until the pupal stage to evaluate the mortality caused by egg-larval parasitoids. Parasitoids that emerged from the eggs and at the end of the larval stage were evaluated, separated into morphospecies and stored in Eppendorf tubes (2 mL) with a 70% ethanolic solution. Larvae and pupae from the field were reared in the laboratory to verify the occurrence of mortality in these stages by parasitoids. *C. includens* predators and parasitoids were forwarded to taxonomists for further identification.

2.3 Statistical analysis

We calculated the mortality percentages caused by the natural control agents at each stage of the *C. includens* life cycle using the following equation:

$$M_{xi} = (100 \times d_{xi}) \div L_x$$

where M_{xi} = mortality (%) caused by control agent i (predator, parasitoid or rainfall) in *C. includens* stage x (egg, larva or pupa), d_{xi} = number of *C. includens* individuals dead by agent i in stage x, and L_x = number of living *C. includens* individuals at the beginning of stage x.

Mortalities caused by each natural control agent at each *C. includens* stage were subjected to analysis of variance at $P < 0.05$ using the GLM procedure in SAS. Averages of *C. includens* mortality in each season and year of cultivation were compared by Tukey's test at $P < 0.05$ (SAS 2013).

A redundancy analysis (RDA) using the CANOCO 4.0 system was performed to determine the relationship between climatic elements with total mortalities and the mortalities caused by major natural control agents in each *C. includens* stage (Ter Braak 1995). Canodraw 3.0 generated the biplot ordination gradient from this analysis (Ter Braak 1995). The response gradients were represented by vectors originating from the center point of the two axes of the ordination diagram. The vector length is proportional to the importance of the variable. Vectors with the same direction and orientation represent variables with positive correlation, while vectors with the same direction and opposite orientation have negative correlation. Variables are not correlated when the angle between the vectors is 90° (Ter Braak 1995).

3. RESULTS

C. includens egg mortality agents included predators (*Orius* sp., *Franklinothrips* sp. and herbivores), rainfall, the parasitoid *Trichogramma* sp. and inviability (Fig. 1 and Fig. 2A). *C. includens* egg mortality was caused by herbivores when they were ingested together with leaves. The causes of larval mortality were predators (*Orius* sp., *Geocoris* sp., *Franklinothrips* sp., ants, spiders and Vespidae) and rainfall (Fig. 3 and Fig. 2B). The causes of pupal mortality were predators (Vespidae, ants and birds) and the parasitoid *Copidosoma* sp. (Fig. 4 and Fig. 2C).

Egg predation ($F_{3, 66}$ = 2.08, P = 0.1116) and inviability ($F_{3, 66}$ = 0.79, P = 0.5039) were similar in all seasons and years (Fig. 1A, D). Mortality by rainfall was higher in the wet season of year 2 ($F_{3, 66}$ = 10.59, P < 0.0001) (Fig. 1B). Egg parasitism by *Trichogramma* sp. was higher in both seasons of year 1 ($F_{3,}$

$_{66} = 18.66$, $P < 0.0001$) (Fig. 1C). Egg total mortality varied between years, and it was higher in year 1 ($F_{3, 66} = 13.38$, $P < 0.0001$) (Fig. 1E).

Egg predation by *Orius* sp. ($F_{3, 66} = 1.85$, $P = 0.1470$), *Franklinothrips* sp. ($F_{3, 66} = 1.82$, $P = 0.1527$) and herbivores ($F_{3, 66} = 1.08$, $P = 0.3617$) was similar in all seasons. The first two of these were the most important predators of *C. includens* eggs (Fig. 2A).

Larval predation was higher in the dry season of year 2 ($F_{3, 66} = 15.88$, $P < 0.0001$) (Fig. 3A). Rainfall caused significantly higher mortality in the wet season in both years ($F_{3, 66} = 20.23$, $P < 0.0001$) (Fig. 3B). Larval total mortality was lower in the dry season of year 1 ($F_{3, 66} = 11.56$, $P < 0.0001$) (Fig. 3C).

In larvae, predation by *Geocoris* sp. ($F_{3, 66} = 0.91$, $P = 0.4417$) and spiders ($F_{3, 66} = 0.77$, $P = 0.5129$) did not differ between seasons or years. However, *Orius* sp. caused higher larval mortality ($F_{3, 66} = 3.17$, $P = 0.03$) in the dry season of year 1 than in this season the following year. Larval mortality by *Franklinothrips* sp. was higher in the wet season of year 1 ($F_{3, 66} = 9.03$, $P < 0.0001$). Ants caused higher mortality in the dry season of year 2 compared to the wet season of year 1 ($F_{3, 66} = 4.73$, $P = 0.0048$). Larval predation by Vespidae was higher in the dry season of year 2 ($F_{3, 66} = 12.30$, $P < 0.0001$). Ants and Vespidae were the main predators of *C. includens* larvae (Fig. 2B).

Pupal predation was higher in year 2 in both seasons ($F_{3, 66} = 71.83$, $P < 0.0001$) (Fig. 4A). Pupal parasitism by *Copidosoma* sp. was similar in all seasons and years ($F_{3, 66} = 2.57$, $P = 0.0613$) (Fig. 4B). Pupal total mortality was higher in year 2 in both seasons ($F_{3, 66} = 69.22$, $P < 0.0001$) (Fig. 4C).

Pupal predation varied among all predators. Predation by Vespidae did not occur in the dry season of year 1. This predator caused higher mortality in the wet season of year 2 than in this season in year 1 ($F_{3, 66} = 146.74$, $P <$

0.0001). Ants caused higher predation in the dry season of year 2 ($F_{3, 66} = 8.10$, $P = 0.0001$), and birds only occurred in the dry season in year 1 ($F_{3, 66} = 336.11$, $P < 0.0001$). Vespidae was the main predator of *C. includens* pupae (Fig. 2C).

Environmental factors varied across seasons and years. Rainfall was higher in the wet seasons in both years ($F_{3, 296} = 5.18$, $P = 0.0017$). Means of rainfall in the rainy and dry seasons was 5.23 ± 0.93 $mm.day^{-1}$ and 0.75 ± 0.33 $mm.day^{-1}$, respectively. Temperature changes in tropical regions are not very drastic, but there were significantly differences in temperature ($F_{3, 296} = 182.12$, $P < 0.0001$). Temperature was higher in the wet season of year 2 (23.45 ± 0.11°C) compared with the other seasons (19.87 ± 0.74°C). Variation in the relative humidity was observed, and higher percentages occurred in the dry season of both years (79.86 ± 0.47%) ($F_{3, 296} = 20.30$, $P < 0.0001$) than in the rainy season (73.07 ± 0.79%). The highest photoperiod occurred in the wet season of year 2 ($F_{3, 296} = 995.53$, $P < 0.0001$) (12.90 ± 0.21 hours) compared to the other seasons (11.55 ± 0.06 hours).

The redundancy analysis (RDA) of *C. includens* mortality and the environmental factors generated four axes explaining 97.4% of the variance observed. The occurrence of rainfall, the photoperiod and temperature were positively correlated with the mortality in all stages caused by rainfall, the total mortality of larvae and pupae and predation by Vespidae. These environmental factors were negatively correlated with the mortality caused by *Orius* sp. Further, they were relatively independent of the mortality by ants, *Trichogramma* sp. and *Franklinothrips* sp. and also did not affect the egg total mortality (Fig. 5).

Relative humidity was positively correlated with predation by ants. Additionally, this factor was relatively independent of predation by Vespidae and pupal and larval total mortality (Fig. 5).

4. DISCUSSION

Herbivore insect populations are regulated in time and space by multiple natural mortality factors (Miranda *et al.* 1998, Pustejovsky & Smith 2006). In this study, we verified that *C. includens* mortality was caused by biotic and abiotic factors. Biotic factors were natural enemies and egg inviability, and the abiotic factor was rainfall. The determination of the influence of the seasons, years of cultivation and pest life cycle stage on natural mortality agents is important for understanding the population dynamics of these herbivores and in the development of integrated pest management programs (Pereira *et al.* 2007, Semeão *et al.* 2012).

Rainfall caused *C. includens* mortality due to the mechanical impact of its drops, which knocked eggs and larvae onto the soil. Egg mortality caused by rainfall was low (up to 14.8%). This was possibly because the eggs are attached in the leaves. In addition, *C. includens* females oviposit preferentially on the abaxial surface of the leaves (Mascarenhas & Pitre 1997), which protects them from the action of raindrops. This attachment occurs due to the shape of the egg (circular, flattened and striated) (Peterson 1964) and the spumaline (adhesive substance) that fixes eggs onto the leaf surface (Al Bitar *et al.* 2012).

Larval mortality by rainfall was low (0.6 to 13.2%), a fact that is unusual in Lepidoptera in regions with a tropical climate (Caldas 1992, Pereira *et al.* 2007), where rainfall is torrential. This low mortality should be related to a behavior that we observed in *C. includens* larvae. In this behavior, the larvae migrated to the leaves of the canopy interior, mainly those in the basal portion of the plant. In these places, larvae are less exposed to raindrops.

Rainfall did not cause pupal mortality. This was possibly because *C. includens* is protected against raindrops at this stage. They are found inside a silk cocoon on the abaxial surface of the leaves (Reed & Bass 1980).

The predators *Orius* sp. and *Franklinothrips* sp. and the parasitoid *Trichogramma* sp. were the main natural enemies of *C. includens* eggs. Predatory ants and wasps were the main natural enemies of larvae, and wasps were the main natural enemy of pupae. Thus, the mortality factors were different in the different stages of the *C. includens* life cycle. This diversity of factors causing mortality results in a minimization of interspecific competition and the complementary action of these factors (Pereira *et al.* 2007, Semeão *et al*. 2012), which allowed a high rate of natural pest control.

C. includens predation by ants was higher in seasons and periods with higher relative humidity. This fact indicates that the ants avoid drier seasons and periods of the day due to their high susceptibility to desiccation (Carrol & Janzen 1973). In addition, during the experiments, it was observed that *C. includens* predation by ants occurred at the end of the day and during the night, when the relative humidity is higher.

Wasps had greater predation activity during hot, rainy and longer days. In tropical climate regions, the occurrence of these conditions at the same time is common (Alvares *et al.* 2014). Because we did not observe wasp activity during rainfall, we believe that only the air temperature and the day length positively affected these predators. High temperatures can positively affect wasps because they exhibit higher flight activity at warmer times (Picanço *et al.*

2011). On the other hand, on longer days, the wasps remain active for longer periods of time, which contributes to the greater *C. includens* predation.

C. includens predation by *Orius* sp. was higher during drier periods with lower temperatures and shorter days. This was possibly due to the impact caused by rainfall on these predators. The mechanical action of raindrops causes insect mortality, especially in those of small size (Pereira *et al.* 2007), such as the insects in this genus.

Egg predation rates by *Franklinothrips* sp. and parasitism by *Trichogramma* sp. did not vary according to the climatic elements. Thus, the mortality rates of *C. includens* eggs caused by these two natural enemies may have been regulated by other factors. *Trichogramma* sp. adults feed on nectar and pollen (Zhang *et al.* 2004), while *Franklinothrips* sp. nymphs and adults feed on pollen (Hoddle *et al.* 2001). Therefore, the abundance of other environmental resources, such as the existing food sources in flowers in the area surrounding the crop where this study was conducted, may have influenced the population variation in these two natural enemies.

Due to the great importance of natural enemies in *C. includens* population regulation, as verified in this research, the adoption of cultural preservation practices for these biological control agents is necessary. Thus, plant diversity must be preserved in agroecosystems. This can be achieved by using polycultures and preserving native vegetation and weeds in the vicinity (Picanço *et al.* 2011, Ramos *et al.* 2012). These plants positively affect natural enemy populations by providing food (nectar, pollen and non-pest herbivores) and shelter to natural enemies (Landis *et al.* 2000). In addition, the decision to control pests should be made using a sampling plan and by considering the economic injury level. The use of this decision-making system reduces the number of pesticide applications (Kogan 1998), which contributes to the preservation of natural enemy populations.

On the other hand, when insecticide application is carried out in the crops, it must be carried out based on the selectivity principles. This selectivity may be ecological or physiological. In ecological selectivity, insecticides are applied to have greater contact with the pest than with the natural enemy (Ripper *et al.* 1951). To achieve this objective, it is necessary to know the behavior of the pests and their natural enemies. To preserve predatory wasp populations, pesticide applications should be avoided at warmer times, since the activity of these natural enemies is greater in these periods (Picanço *et al.* 2011). For the preservation of predatory ant populations, nocturnal applications should be avoided, since this is the period of their greatest activity. In addition, the application of insecticides to the soil should also be avoided because this is the main nesting site of these insects (Ramos *et al.* 2012).

Physiological selectivity consists of the use of insecticides that are more toxic to the pest (*C. includens*) than to its natural enemies (Riper *et al.* 1951, Ramos *et al.* 2017). Thus, before the application of insecticides, the farmer must verify which natural enemies are the most abundant in the crop and use products that are selective against these organisms.

Our research elucidates the main natural mortality agents of *C. includens* during different season of the year and the influence of climatic elements on these agents. We also highlighted the main natural enemies of the different life cycle stages of this pest. Management strategies may improve these natural enemy populations, and their levels should be considered during the pest control decision-making process. Understanding the importance of the multiple agents of *C. includens* natural mortality represents the first step toward a better understanding of its population dynamics, an essential step for the management of this pest.

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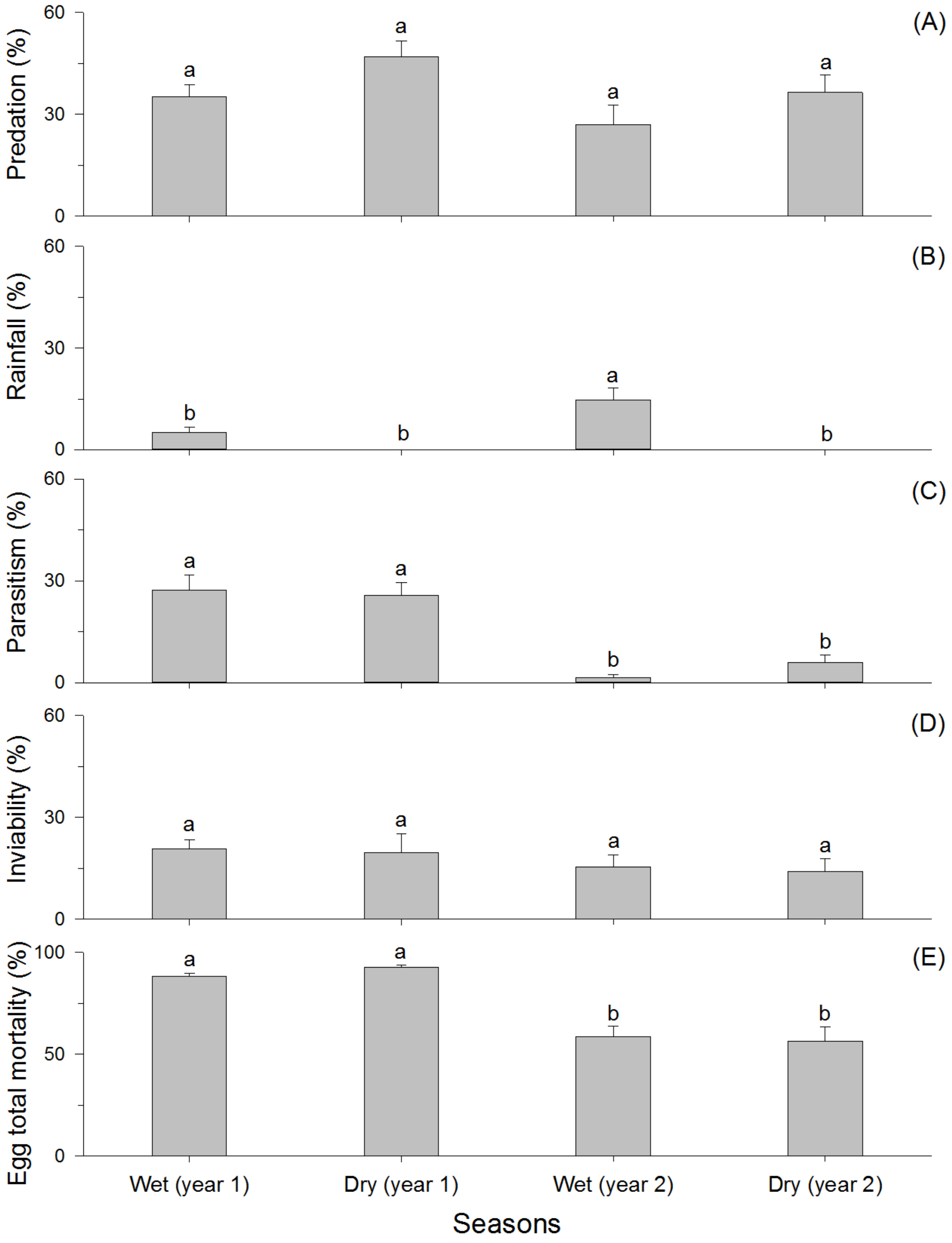

Fig. 1 Percentage of *Chrysodeixis includens* egg mortality caused by (A) predation, (B) rainfall, (C) parasitism by *Trichogramma* sp., and (D) inviability and (E) the total mortality in the wet and dry seasons of two years. Egg mortality in different seasons was compared using ANOVA. Different letters on the tops of bars indicate significant differences at $P < 0.05$ using Tukey's test.

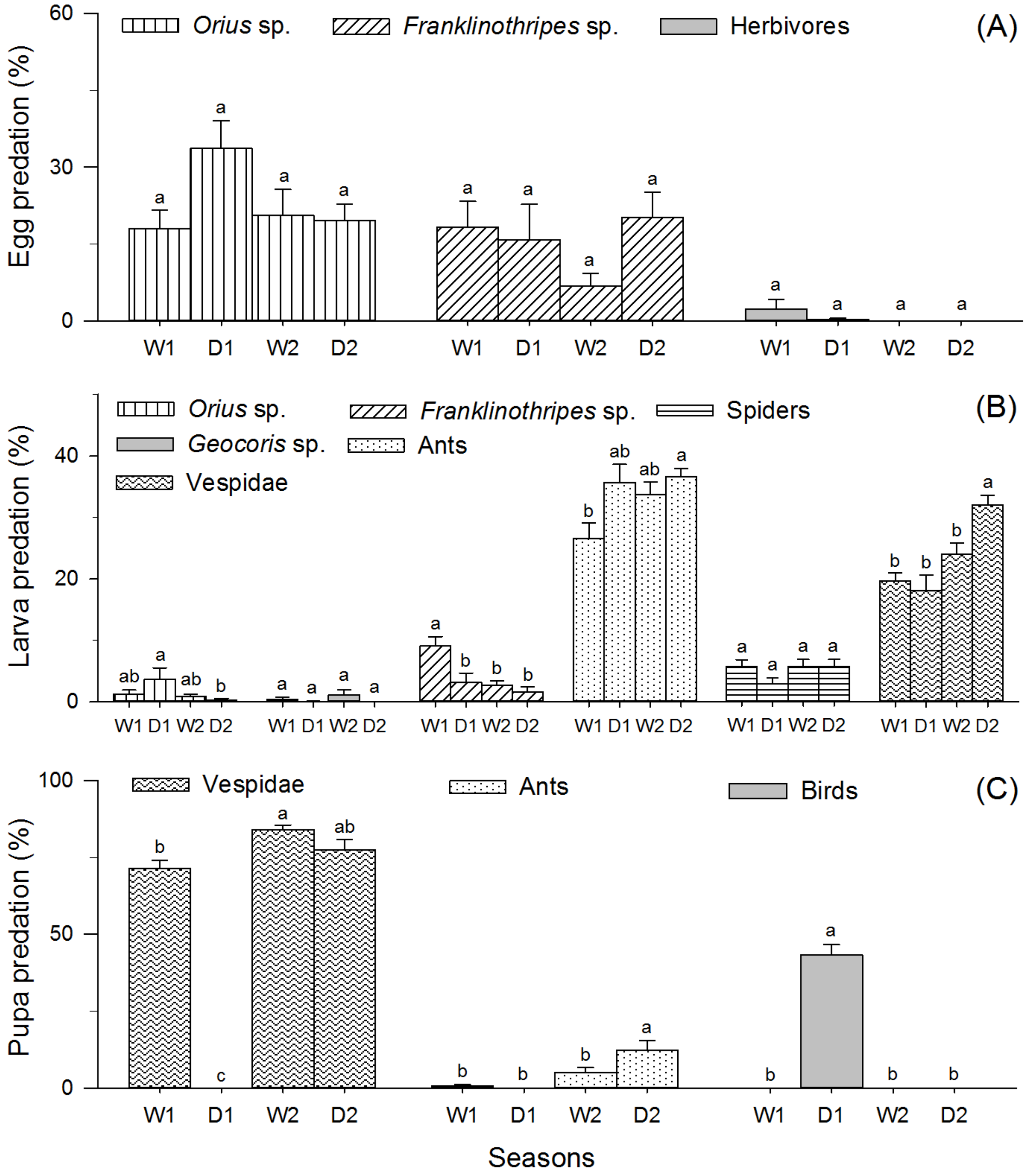

Fig. 2 Egg, larval and pupal mortality caused by different predators in each season. W1 = wet season and D1 = dry season of year 1; W2 = wet season and D2 = dry season of year 2. Predation in different seasons and stages was compared using ANOVA. Different letters on the tops of bars indicate significant differences at *P* < 0.05 using Tukey's test.

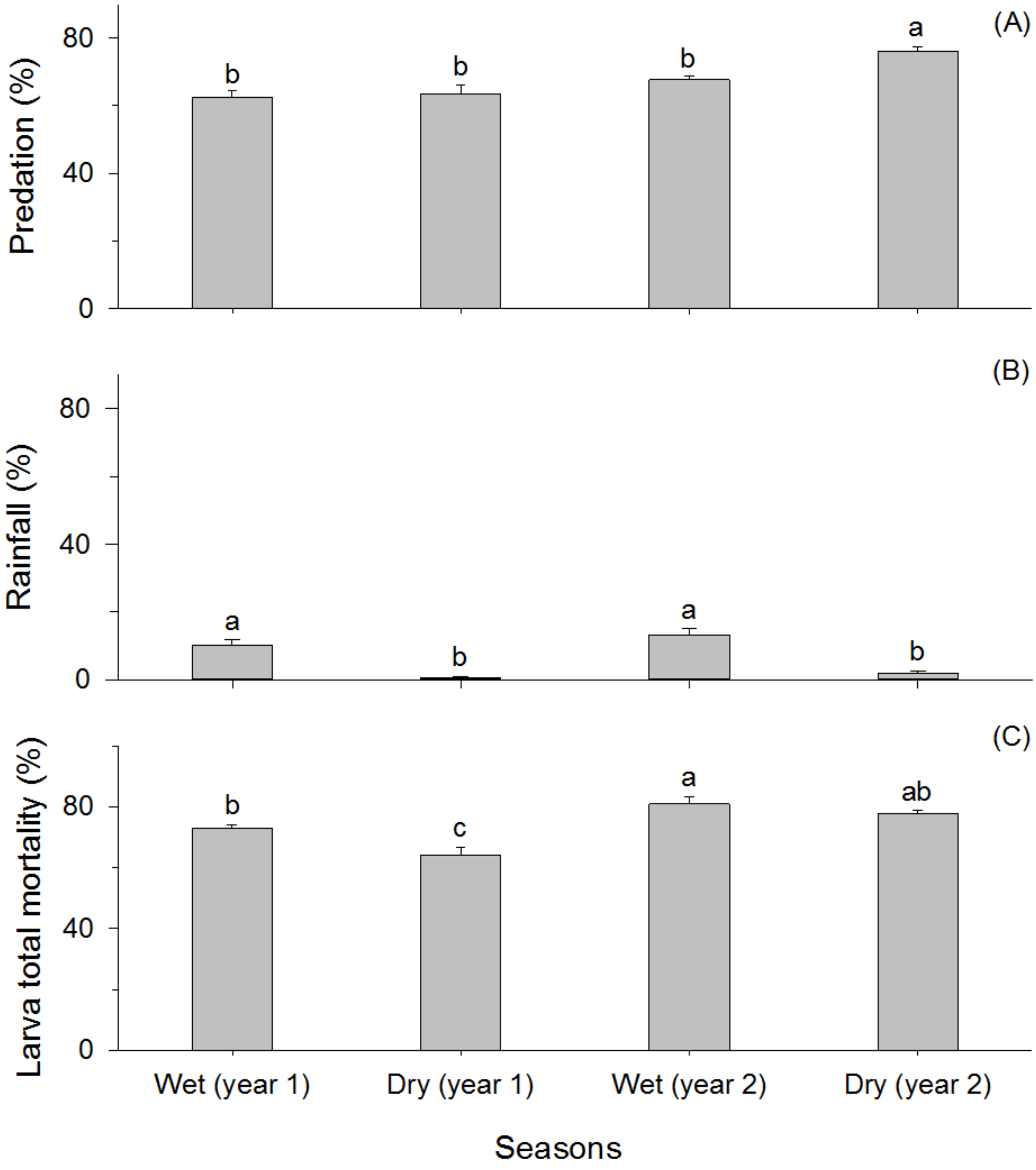

Fig. 3 Percentage of *Chrysodeixis includens* larval mortality caused by (A) predation and (B) rainfall and (C) the total mortality in the wet and dry seasons of two years. Larval mortality in different seasons was compared using ANOVA. Different letters on the tops of bars indicate significant differences at $P < 0.05$ using Tukey's test.

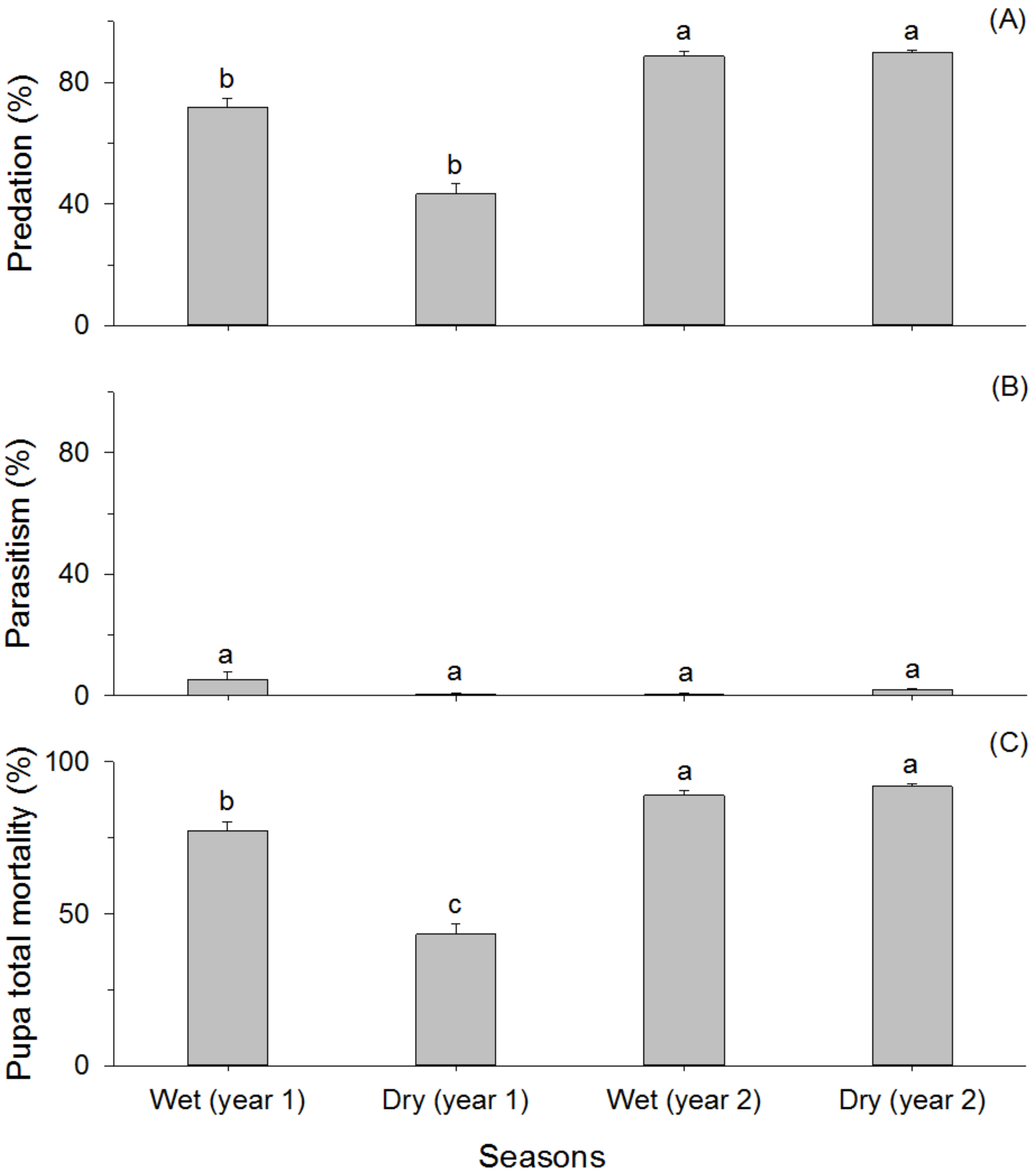

Fig. 4 Percentage of *Chrysodeixis includens* pupal mortality caused by (A) predation and (B) parasitism by *Copidosoma* sp. and (C) the total mortality in the wet and dry seasons of two years. Pupal mortality in different seasons was compared using ANOVA. Different letters on the tops of bars indicate significant differences at $P < 0.05$ using Tukey's test.

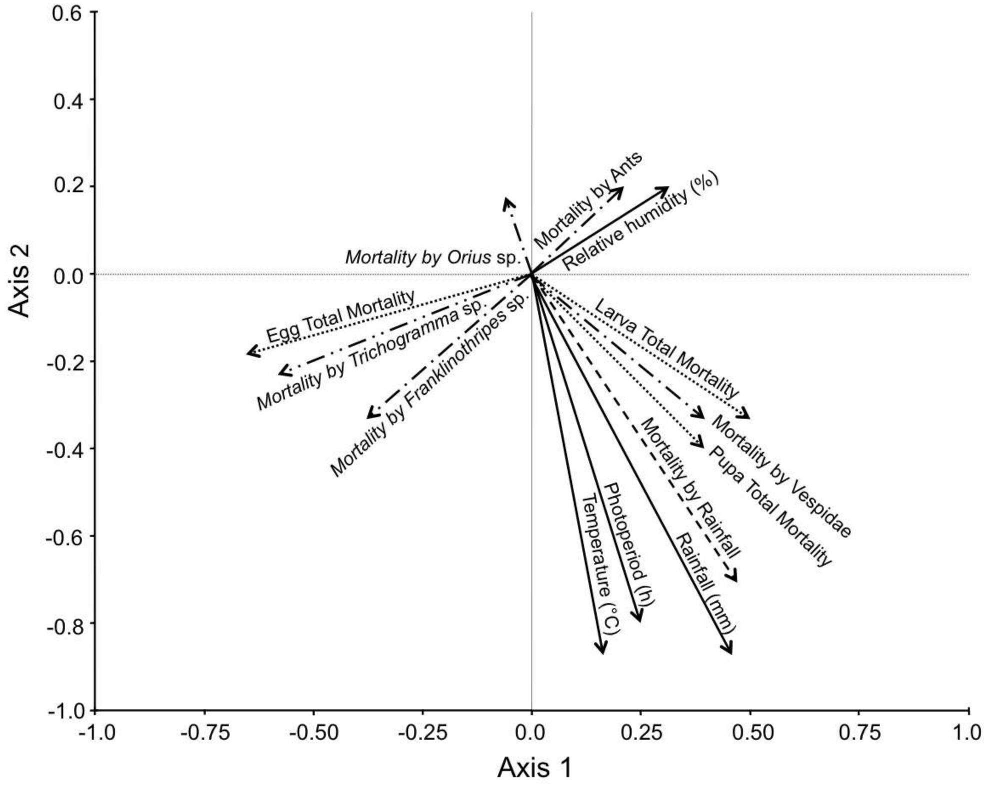

Fig. 5 Diagram of the redundancy analysis (RDA) of the effects of natural mortality factors on *Chrysodeixis includens* egg, larval and pupal stages. The vector length is proportional to the importance of a variable. Variables with positive correlation have vectors with the same direction and orientation. Variables with negative correlation have vectors with the same direction and opposite orientation. Variables are not correlated when the angle between the vectors is 90°.

CONCLUSÕES GERAIS

Este estudo elucida os mecanismos de regulação populacional de *C. includens* e demonstra a importância da preservação das populações de inimigos naturais. Ainda, possibilita a previsão de ocorrência de maiores ou menores mortalidades da praga em função dos elementos climáticos.

A mortalidade natural de *C. includens* é alta e os fatores de mortalidade são suficientes para reduzir sua densidade populacional. Os fatores de mortalidade natural de ovos foram predação por *Orius* sp, *Franklinothrips* sp. e herbívoros desfolhadores, chuvas, o parasitóide *Trichogramma* sp. e inviabilidade. As causas de mortalidade de larvas foram predação por *Orius* sp, *Geocoris* sp., *Franklinothrips* sp., formigas, aranhas e Vespidae e chuvas. Os causadores de mortalidade de pupas foram os predadores Vespidae, formigas e pássaros e o parasitóide *Copidosoma* sp.

O estágio crítico de mortalidade de *C. includens* é o estágio larval e, os 2° e 4° ínstares são os mais vulneráveis aos fatores de mortalidade. Os fatores chave de mortalidade são formigas no 2° ínstar e Vespidae no 4° ínstar. A ação de Vespidae e formigas é fundamental para a redução das populações de *C. includens* já que, a supressão destes fatores causaria um aumento populacional de 77.52 e 85.17%, respectivamente.

Estudos futuros visando a determinação de estratégias e táticas de manejo desta praga devem levar em consideração as descobertas proporcionadas por este estudo. Os inimigos naturais, sobretudo formigas e Vespidae, devem ser favorecidos pelas táticas de manejo de pragas e seus níveis populacionais devem ser considerados durante o processo de tomada de decisão, visto que estes são essenciais para o controle desta praga. A determinação da importância de múltiplos agentes de mortalidade natural de *C.*

includens representa o primeiro passo para o melhor entendimento de sua dinâmica populacional, etapa fundamental para o manejo dessa praga.