

Fumigation with acetic acid and antimicrobials to reduce mortality of *Chrysoperla carnea* due to undetermined infection

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Abstract

Chrysoperla carnea reproduced commercially by a laboratory (CREROB) acquired an infectious disease that kills larvae, pupae and adults. To counteract the infection, fumigation with acetic acid (AA) was evaluated in three doses and four times to eggs and four doses and six times to the pupae; the adult was given six different antimicrobial substances. Of the fumigated eggs of the laceworm, 50% of adults were obtained with respect to the non-fumigated eggs ($p < 0.001$). In contrast, adults from fumigated eggs survived twice as many as those not fumigated ($p < 0.01$). Some adults from fumigated eggs survived, but the fumigation did not prevent them from becoming infected and later the adult lacewings died. After 51 days after keeping the adults of lacewings with antimicrobial substances (in the water they drank), only cefotaxime at a dose of 1 200 $\mu\text{g ml}^{-1}$ of active ingredient allowed to maintain no symptoms of infection and 100% survival. Chrysopters treated with antimicrobial substances survived between 50 and 81% while adults from fumigated eggs had a lower survival between 31 and 34%, possibly due to a greater asepsis of the former. Of the infected adult insects, two strains of *Bacillus thuringiensis* were isolated and their Cry toxins were characterized; although the lacewing infection is not attributed to *B. thuringiensis*, its presence is discussed.

Keywords: *Chrysoperla*, antimicrobial, diseases, fumigation.

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Introduction

Chrysoperla species are widely used as insect predators of insect pests in biological control programs, they are reproduced in multiple commercial laboratories in several countries and are part of the integrated pest management (McEwen *et al.*, 2007). During the reproduction process, lacewings can acquire diseases that decimate their population, associated with bacteria, fungi, protozoa and viruses, although sometimes it has not always been possible to determine the etiological agent (Kleespies *et al.*, 2008; Taber, 2011). Although, for *Chrysoperla* spp., pathogenic bacteria such as *Enterobacter* spp. and *Bacillus* spp., endosymbiotic microorganisms such as yeasts, bacteria and filamentous fungi have also been isolated (Meca *et al.*, 2009; Hemalatha, 2015; Fletcher, 2016).

Likewise, larvae of *Chrysoperla* spp., can be negatively affected by *B. thuringiensis* or its toxins (Hilbeck *et al.*, 1998; Dutton *et al.*, 2003). In the beneficial insect production laboratory (CREROB) in Torreon, Coahuila, Mexico, *Chrysoperla carnea* Stephens reproduces, in the years 2012-2013 there was a deadly infection in all insect states, which decreased by more than 90% commercial production. When dissecting adults of *C. carnea* from the CREROB, within the digestive tract, yeasts, bacteria and some bacteria with crystals were observed, the latter, as described (Carreras-Solis, 2009).

For the above, we ask ourselves; i) if the bacteria with crystals inside the adults of *C. carnea* could belong to *Bacillus thuringiensis* Berliner; and ii) if an alternative to contain *C. carnea* infection could be to fumigate their eggs, pupae or provide antimicrobials to adults. Bacteria, nematodes and insects are susceptible to acetic acid, which has been used as a fumigant to prevent the deterioration or damage of food and agricultural crops by harmful organisms (Momma *et al.*, 2006; Sholberg, 2009; Katase *et al.*, 2009).

Before the contingency presented in the CREROB, it was proposed to evaluate fumigation treatments with acetic acid, eggs and pupae of *C. carnea* and antimicrobials in adults, to contain and potentially prevent infection. Also, look for *B. thuringiensis* within infected *C. carnea* adults.

Materials and methods

Management of insects

During 2013, eggs, pupae and adults of *C. carnea* were provided by CREROB. After receiving them, eggs and pupae were fumigated as indicated, while adults were given antimicrobials in the water they drank. After fumigation, the eggs were incubated at 28 °C and when hatched the larvae were fed with eggs of *Sitotroga cerealella* Olivier, provided by the CREROB. The pupae were incubated at 28 °C until obtaining the adult at laboratory temperature (20-25 °C).

The adults were fed a mixture of unrefined sugar, milk powder (Nido®) and dry beer yeast (Industrial Rosanco®) in a ratio of 2:1:1 w/w/w, prepared with distilled water and heating until the consistency of a jelly. The jelly was stored for 15 days at 0 °C. The adults were fed with jelly, on

abate-sterilized tongues (renewed every third day) or on the walls of the confinement cells. To the adults, they were provided in the sterile distilled water with the antimicrobials in the head of sterile swabs, replacing them every third day, the control only the water.

Isolation and identification of *Bacillus thuringiensis*

The adults of *C. carnea* were superficially sterilized with 1% sodium hypochlorite and macerated in sterile conditions to subsequently recover the macerate in sterile water. To observe the formation of the parasporal bodies under an optical microscope, bacteria were grown in a nutrient medium until sporulation was induced. After three days of growth, the spores and crystals were recovered and analyzed on an SDS-PAGE gel. This macerate was seeded in Petri dishes containing solid Luria-Bertani medium.

The plates were incubated at 30 °C for 24 h and in these different bacteria were identified, among these two isolates named Cc1 and Cc2 were selected and purified, which presented the typical morphology of bacteria belonging to *B. thuringiensis*. To verify that these bacteria correspond to this species, PCR amplification of the 16S ribosomal gene was analyzed using specific oligonucleotides designed by (Aguino de Muro and Priest, 1993).

To identify both isolates, they were grown for 12 hours in a plate of nutrient medium (Difco). A small volume of cells was transferred to 0.1 ml of H₂O and frozen at -70 °C for 20 min to be then incubated in boiling water for 10 min to lyse the cells. The resulting cell lysate was centrifuged briefly (10 sec at 10 000 rpm in an Eppendorf centrifuge model 5415C) and 15 µl of supernatant was used as a DNA sample in the PCR.

The amplification was carried out in a Perkin-Elmer model 480 thermocycler. The conditions of the PCRs made with oligonucleotides cry1 (gral-cry1) were the following: a single denaturation step of 2 min at 95 °C, followed by a program of 30 cycles in which each cycle consists of denaturation at 95 °C for one min, hybridization at 52 °C for 1 min and extension at 72 °C for one min. At the end of the 30 cycles an additional extension step was made at 72 °C for five min.

The conditions for the PCRs performed with the other oligonucleotides were similar, except that the hybridization temperatures were modified at 49 °C for oligonucleotides of cry8, at 51 °C for oligonucleotides of cry9, cry11 and cyt1 and at 50 °C for the rest of the oligonucleotides. Once the amplification reaction was performed, it was electrophoresed on a 2% agarose gel in Tris-borate buffer (45 mM Tris-borate, 1 mM EDTA (pH 8) at 250 V for 35 min and stained with ethidium bromide. Finally, with the intention of identifying these proteins, an analysis of possible cry genes present in these strains was carried out using a battery of oligonucleotides (Ceron *et al.*, 1995, Bravo *et al.*, 1998). These oligonucleotides allow to identify genes *cry1*, *cry3*, *cry5*, *cry7*, *cry8*, *cry9*, *cry11*, *cry13*, *cry14* and *cyt1A*.

Pathogenicity bioassays in other species

The activities of the different strains of *B. thuringiensis* were analyzed in neonatal larvae of *Spodoptera frugiperda* and *Diatraea magnifactella* (Insecta: Lepidoptera). Dilutions of spore-crystal suspensions were applied on the surface of the diet to a final concentration of 100 and 1 000 ng cm⁻² (Bravo *et al.*, 1998). Mortality was recorded after seven days.

Egg fumigation bioassays

250 ml glass jars were used to put acetic acid on the bottom as a fumigant, inside this bottle was placed a 25 ml bottle where the eggs of *C. carnea* were placed. The doses and times of fumigation of the eggs were 0 (control) 0.04 and 0.4 $\mu\text{l ml}^{-1}$ acid-air during 8, 16, 24 and 48 h, respectively. In total, four repetitions were used for each dose and time, each repetition was made up of 50 eggs. After the fumigation, the eggs were placed individually in cells of about one cm^3 , where when the larvae hatched they were fed with eggs of *S. cerealella*.

The formed pupae were placed in individual confinement cells of three cm^3 , where the jelly was previously impregnated on the walls to feed the adults that emerged, also supplying them with sterile water and incubating at laboratory temperature (20-25 °C) for 35 days. The count of living adults without symptoms and dead with symptoms was carried out weekly and daily dead adults were removed to avoid the spread of the infection as much as possible. This and subsequent experiments were repeated twice. Another experiment was performed identically, except that larvae were provided with heat-sterilized *S. cerealella* eggs.

Fumigation bioassays in pupae

The pupae were fumigated with acetic acid at a dose of 0 (control), 0.04, 0.4 and 4 $\mu\text{l ml}^{-1}$ acid-air at fumigation times of 10, 20, 30, 60 and 120 min. Then, they were confined in one liter polyurethane cups, and the emerged adults were kept for two weeks at laboratory temperature (20-25 °C), at which time dead adults with symptoms of the disease and adults without symptoms were counted. The adults were fed the jelly in the abbot-tongues and the water supply in the swabs. Dead pupae and adults were removed daily. In total, four repetitions for each dose and time of fumigation of the pupae were performed, each repetition with 15 pupae.

Bioassays with antimicrobials in adults

In the CREROB, we were given ten units each with 700-800 cells occupied by pupae, from which it was expected to obtain at least 4 200 adults, to evaluate six antimicrobials for three doses each (one control) with four repetitions. Of the units, only 530 pupae were recovered from where the adults would be born. With the available pupae, 21 treatments were established with (25 pupae) without repetitions, six antimicrobial substances with three doses and the control without antimicrobial. After the dead adults were discarded in the first two days, the treatments had a different number of adults (Table 1).

The antimicrobial products of the commercial type that were used were Amylamcin Amk[®] Ampicillin Binotal[®]; Biosint[®] cefotaxime, Ilosone[®] erythromycin, Flagelase[®] metronidazole, vancomycin Vancocin[®] in addition to the control.

Pupae and emerged adults were confined in 2 L polyurethane cups, dead adults were removed daily when there were. The lacewings were given food in abate-tongues with the jelly, while the antimicrobials in the water retained the swabs. The insects were temporarily transferred to another vessel, while the source vessel was cleaned using cotton impregnated with a 0.3% commercial chlorine solution. The first cleaning was done seven days after the adults emerged, the subsequent ones every five days.

Table 1. Treatments and doses of antimicrobial substances evaluated against adults of *Chrysoperla carnea*.

Antimicrobial substance	Dose ($\mu\text{g ml}^{-1}$)	No. of initial adults
Amikacin	25	15
	50	12
	100	19
Ampicillin	150	15
	300	8
	600	9
Cefotaxime	300	14
	600	5
	1 200	13
Erythromycin	100	15
	200	13
	400	16
Metronidazole	100	20
	200	7
	400	14
Vancomycin	50	16
	100	13
	200	10
Control 1		7
Control 2		10
Control 3		16

Analysis of data

The adult data obtained from the egg spray experiments were analyzed by the chi square test (χ^2). The experiment with pupae was analyzed with a completely random design, a factorial arrangement and with the GLM procedure, where the time of fumigation and the dose were the factors; each analysis was performed separately for the incubation times of 7 and 14 d. The experiment of adults treated with antimicrobials was not analyzed statistically due to the lack of repetitions.

Of the adult insects that came from the fumigated eggs, their coefficients of linear determination R^2 and their slopes were established, this for their survival versus incubation time. R^2 was also obtained between the slopes obtained for each treatment (in each experiment) including the controls versus the initial number of adult insects. All analyzes were performed with the Statistical Analysis System (SAS version 9.1, 2003).

Results

Symptomatology of the disease

The adults of infected *C. carnea* slow down their movement, their thorax becomes dark, later also the whole digestive tract; the insect does not eat and lose weight until death (Figure 1 A and B), when this occurs, the decomposition of the insect generates a foul odor. Pupae and larvae also turn dark and die (Figure 1C and 1D).

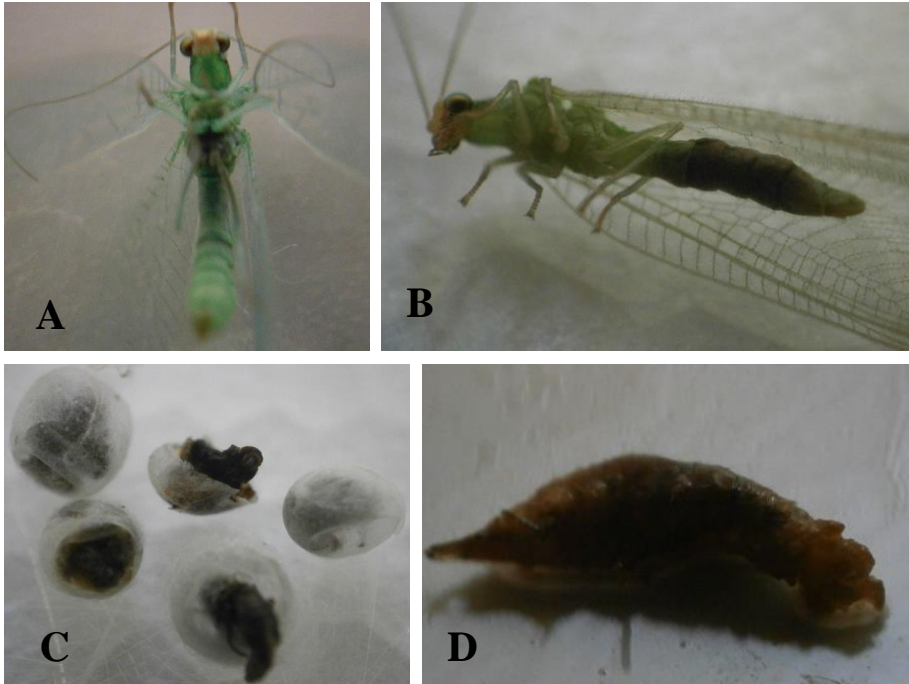


Figure 1. A-D) signs of infection in *Chrysoperla carnea*. A) adult with darkened thorax and tight abdomen; B) adult with very darkened abdomen; C) pupae with dead and darkened product; and D) larva dead and darkened.

Isolation and identification of *Bacillus thuringiensis*

The bacterial colonies isolated were white, opaque, starry and flat. The PCR product obtained showed the size (data not shown) of the *Bacillus* spp. The parasporal bodies, unique characteristic of *B. thuringiensis* (where the Cry or Cyt proteins accumulate) were also observed.

Figure 2 shows that the Cc1 strain produces a 150 kDa protein and the Cc2 strain has a very small crystal, so it is difficult to identify the crystal band on the SDS-PAGE gel, noting only that it has a 65 kDa protein that resembles the weight of other Cry proteins reported in *B. thuringiensis*.

Strain Cc2 presented amplification with the oligonucleotides of the *cry3* gene. However, no protein of similar weight was observed as observed with the control strain expressing *Cry3Aa*. Strain Cc1 did not amplify with any of these oligonucleotides. These data suggest that these strains could contain genes different from those analyzed by means of these PCR assays or could contain a possible new *cry* gene.

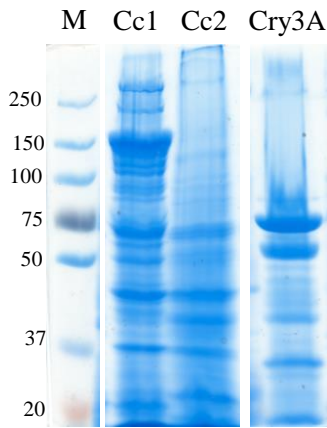


Figure 2. SDS-PAGE gel showing a different molecular weight of the crystal-forming proteins in the Cc1 and Cc2 strains with respect to the Cry3A proteins of *B. thuringiensis*.

Pathogenicity bioassays in other species

The toxicity of strains of *Bacillus* spp. including two strains of *B. thuringiensis* tested against *S. frugiperda* and *D. magnifactella* showed that they are not toxic to these insect species, although the toxicity of *B. thuringiensis* strains was not tested against larvae and adults of *C. carnea*.

Egg fumigation bioassays

In the first and second experiments, the control treatments without fumigation and fumigated were recovered 19, 17 and 8, 8% of adults, respectively (Figure 3 left). The differences between the treatments were significant ($\chi^2 = 74.6$, $gl = 8$, $p < 0.001$) for the first experiment and ($\chi^2 = 109.6$, $gl = 8$, $p < 0.001$) for the second experiment. After five weeks, the survival of the adults that came from fumigated eggs was double that of the control egg not fumigated (Figure 3 right) in the first experiment ($\chi^2 = 36.9$, $gl = 9$, $p \leq 0.001$) and ($\chi^2 = 26.6$, $gl = 9$, $p \leq 0.001$) in the second.

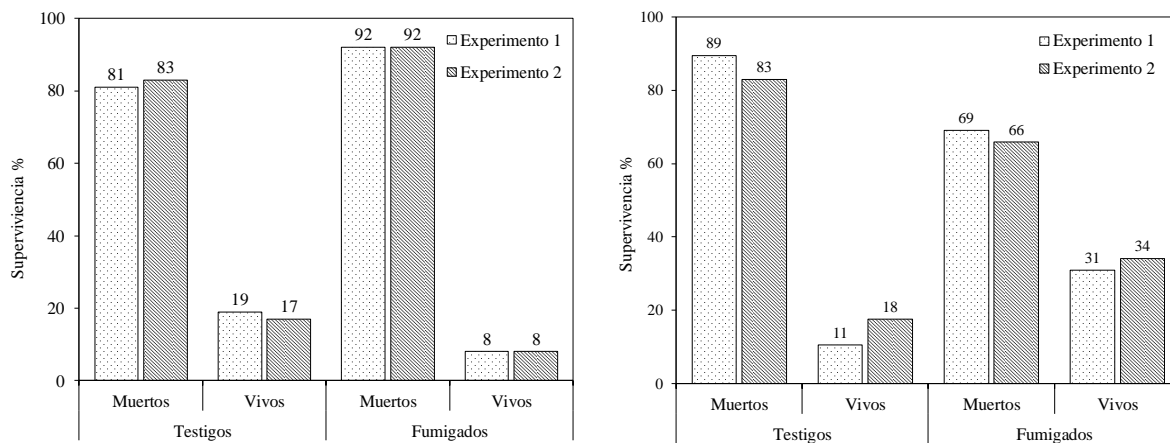


Figure 3. Survival of live and dead adults of *C. carnea*. Left, live or dead adults that came from 1 600 fumigated eggs or 200 undamaged control eggs in two experiments. Right, adults alive or dead after five weeks of incubation.

For each curve (linear) of survival versus initial number of healthy adults, we obtained their slopes, R^2 and their goodness of fit (Table 2), there it is observed that in both experiments the fumigated egg treatment with $0.4 \mu\text{l ml}^{-1}$ acid-air for 16 h was not significant goodness of adjustment of its slopes, the rest of the treatments and control were significant.

Table 2. Slopes, coefficients of determination R^2 and adjustment χ^2 of survival relationships versus incubation time of adults of *C. carnea*. Adults are from eggs that were applied fumigation treatments for different times.

Treatments €	Experiment 1			Experiment 2		
	Slopes	R^2	£ χ^2	Slopes	R^2	χ^2
0.04 T8	-0.759	0.991	***	-1.033	0.977	***
0.4 T8	-0.416	0.957	***	-0.208	0.957	***
0.04 T16	-0.314	0.953	***	-0.188	0.856	***
0.4 T16	0	0	ns	-0.037	0.771	ns
0.04 T24	-0.294	0.912	***	-0.131	0.757	***
0.4 T24	-0.033	0.686	***	-0.053	0.852	***
0.04 T48	-0.310	0.945	***	-0.457	0.971	***
0.4 T48	-0.682	0.966	***	-0.269	0.982	***
Control	-0.988	0.966	***	-0.833	0.982	***

€= the treatments were eggs that were fumigated with acetic acid at a dose of 0.0 or control, 0.04 and 0.4 of $\mu\text{l ml}^{-1}$ acid-air, in fumigation times of 8 T8, 16 T16, 24 T24 and 48 T48 hours; £= the χ^2 test was applied to determine its goodness of fit. ***= significant with $p \leq 0.001$; ns= not significant.

In relation to the R^2 obtained between the initial number of adults of *C. carnea* versus its survival slope for both experiments, the treatment $0.04 \mu\text{l ml}^{-1}$ acid-air for 8 hours (of the second experiment) had the steepest slope (-1.033) followed by the control treatments -0.833 and -0.988 of the first and second experiments, respectively (Table 2 and Figure 4). In the two experiments, in which the larvae were fed as heat-sterilized eggs of *S. cerealella*, they did not form any pupae.

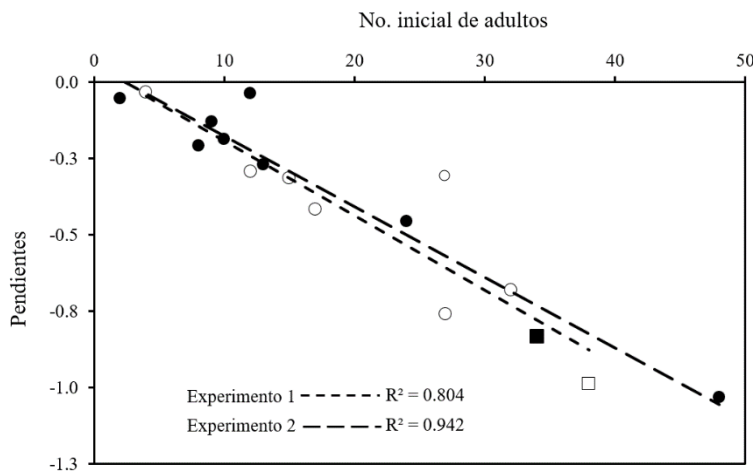


Figure 4. Coefficients of determination between the initial number of *C. carnea* adults and their survival slope. The open and closed squares indicate the control or non-fumigated treatments of the first and second experiments, respectively; the open and closed circles represent the fumigation treatments of the first and second experiments, respectively.

Fumigation bioassays in pupae

The time and dose of fumigation were significant $p \leq 0.001$, this occurred for the pupae that remained 7 or 14 days, as they increased the time and dose of fumigation of the pupae their formation of adults decreased.

The adults formed after fumigating the pupae were not in agreement with the doses and times of fumigation, for example, in the incubation times 7 or 14 days, 30 min of fumigation and doses of $4 \mu\text{l ml}^{-1}$ acid-air were not generated adults, but in times of fumigation less than or greater than 30 min (Table 3). The survival of fumigated pupae that was reflected in the emergence of adults, suggests that acetic acid does not cross the outer layer of the pupa, because if it did kill or disinfect it.

Table 3. Survival (%)[‡] of *C. carnea* adults at 7 and 14 d. The origin of adults was pupae sprayed with three doses of acetic acid.

Minutes of fumigation	Evaluation days	Fumigation dose in $\mu\text{l ml}^{-1}$ acid-air		
		0.04	0.4	4
10	7	€100 a	56 e	33 g
20	7	89 b	50 f	39 g
30	7	92 b	54 e	0 i
60	7	63 d	44 f	56 e
120	7	75 c	25 h	58 d
Control	7	36 g		
10	14	22 d	28 d	0 f
20	14	56 b	44 c	28 d
30	14	77 a	54 b	0 f
60	14	13 e	19 e	56 b
120	14	58 b	0 f	17 e
Control	14	23 d		

[‡]= each value represents the average of four repetitions, each repetition was made up of 15 pupae; €= number with a different letter indicates statistical difference according to the test Duncan mean separation with $p=0.05$.

Bioassays with antimicrobials in adults

Only the treatment of cefotaxime at a dose of $1\ 200 \mu\text{g ml}^{-1}$ allowed all adults to survive until day 51. The 267 adults without symptoms at the beginning of the application of antimicrobial substances, represents less than 5% of pupae that formed adults. In contrast, of the initial number (267) of adults treated with antimicrobial substances including the control survived 183, after 51 days, that is, around 69%, although 80% of the treatments with erythromycin and metronidazole survived.

Discussion

Fumigation of the egg

Fumigation with acetic acid has been found to kill pathogenic microorganisms of bees and insect pests (Van Engelsdorp *et al.*, 2008; Randall *et al.*, 2011). Although fumigation of lacewings improved survival, it was not enough to completely eliminate the infection, since 100% of adults did not survive.

The infectious agent of *C. carnea* was manifested in larvae, pupae and adults. Commonly, the toxins of *B. thuringiensis* or the bacteria itself infect only larval stages of the insects causing their death from 2 to 5 and from 7 to 10 days, respectively (Broderick *et al.*, 2006).

Of the non-fumigated eggs, twice as many adults were born as fumigated eggs (Figure 3. left), which suggests that the fumigation favors the mortality of the eggs and at the same time a selection of adults that will survive longer and in proportion (Figure 3, right). However, the greater the initial number of adults who were confined in individual cells, the death rates were more accentuated (Table 2, Figure 4).

A possible cause of the infection that explains the death of lacewings that remained in individual cells after two weeks, could be the food contained in the cells, where the possible infectious agent could reproduce and reinfect the adults. Consider that this food was not replaced during the five weeks in which the adult insects remained under observation.

The food could favor the development of microorganisms directly or indirectly related to the infection and death of the lacewings. This is suggested by the survival of almost 100% of adults at 16 days in 18 of 21 treatments where antimicrobials were evaluated (data not shown) and where there was food replacement and asepsis of the vessels where the lacewings remained. Although in this work, the presence of *B. thuringiensis* cannot be attributed as a cause of infection of adults or another stage of *C. carnea*, it is known that this bacterium alone or in the presence of enteric bacteria can kill lacewing larvae (Broderick *et al.*, 2009; Johnston and Crickmore, 2009).

Within eggs, larvae and adults of insects including *Chrysoperla* spp., they inhabit yeasts, bacteria that help them digest their food (Chen *et al.*, 2006), supplement their diet or nutritional deficiencies (Gibson and Hunter, 2005; Ben-Yosef *et al.*, 2010). In sum, there are at least ten extrinsic factors that affect the selectivity and dose-response relationship of *B. thuringiensis* toxins to insects, such as their microbiota, nutritional factors, immune response, among others (Lawo *et al.*, 2010; Then, 2010; Grenier, 2012). The eggs of *S. cerealella* used to feed the larvae that came from the fumigated eggs could also harbor infectious inoculum or microorganisms that favored the infection.

In this work, an attempt was made to eliminate the potential infection by sterilizing the eggs of *S. cerealella* to feed the larvae of *C. carnea*, but these did not survive with this food. Results obtained by Fletcher (2016) show that *C. carnea* larvae were infected and died by feeding on eggs of the insect *Adalia bipunctata* L. infected with the pathogen *Nosema adaliae* Steele & Bjørnson. The

lacewings can also become infected and die when in contact with bacteria, for example, *Chrysoperla externa* Hagen died from bacteria isolated from *Phyllocnistis citrella* Stainton (prey of lacewings) (Meca *et al.*, 2009), while *C. carnea* died by pathogenic bacteria of humans *Pseudomonas aureoginosa* and *P. putida* (Fernández *et al.*, 2015).

Fumigation of pupae

Some pupae that were fumigated up to two hours, which was the maximum time and at the dose of 4 $\mu\text{l ml}^{-1}$ acid-air survived, suggesting that some tolerate fumigation. In contrast, the marked emergence and survival variability of the lacewings from the pupae exposed to different doses-time of fumigant suggests that they had a marked deterioration and/or death before applying the treatments (Table 3).

The vertical transmission (adult→egg→larva→pupa→adult) of beneficial or harmful microorganisms of the *Chrysoperla* species is known (Sung-Oui *et al.*, 2004; Hemalatha, 2015), this could explain the transmission of the infectious agent with the subsequent mortality of eggs, pupae, larvae and adults.

Application of antimicrobial substances in adults

The asepsis and the elimination of lacewings that died during the first two days during the treatments of antimicrobial substances, possibly allowed keeping *C. carnea* with the higher survival rates than in the previous experiments. Even in the treatment with 1 200 $\mu\text{g ml}^{-1}$ of cefotaxime the death of adults or symptoms of infection did not manifest themselves. Antibiotics have been used to eliminate bacteria and at the same time, determine their role including endosymbiotic bacteria in insects; the doses used that affected the insects were from 10 $\mu\text{g ml}^{-1}$ to 5 $\mu\text{g ml}^{-1}$ (Broderick *et al.*, 2006; Ben-Yosef *et al.*, 2010; Lin *et al.*, 2015).

Entomophagous insects such as *Chrysoperla* spp., have negative effects on their life cycle when they feed on their prey if the latter have fed on transgenic crops that express toxins of *B. thuringiensis* (Hilbeck *et al.*, 1998; Dutton *et al.*, 2003). In contrast, other studies indicate that *Chrysoperla* is not affected by preying on prey that have consumed transgenic plants (Carvalho *et al.*, 2012; Tian *et al.*, 2013; Meissle *et al.*, 2014), therefore, it is not entirely clear *B. thuringiensis* paper on the lacewings.

It is also do not know the role of *B. thuringiensis* found in dead adults of *C. carnea*, but knowing this could help clarify whether the bacteria could be pathogenic or not on these insects from egg to adult. The tolerance of eggs and pupae to fumigation with AA and that of adults to antimicrobials could be evaluated in the future to eliminate undesirable organisms in the mass production of beneficial insects other than lacewings.

Our quantitative ignorance of the infection and its lack of control in egg, pupa and adult generated an inconsistency dose-times of fumigation and dose of antimicrobials versus survival of adults.

Conclusions

In this work, *B. thuringiensis* isolated from carcasses of *C. carnea* is not sufficient to associate it as the cause of the infection and a more detailed study would be required to determine its role in the death of these insects. The effect of acetic acid in the fumigated eggs resulted in fewer adults obtained compared to non-fumigated eggs, but the adults of fumigated eggs survived twice as many adults of non-fumigated eggs, after five weeks showing therefore a certain beneficial effect on the survival of *C. carnea*.

Some pupae managed to survive, generate adults and survive these up to 14 days, after fumigating the pupae up to 120 min with acetic acid dose of 4 $\mu\text{l ml}^{-1}$ acid-air. The treatments with antimicrobial substances together with the asepsis allowed the greater survival of lacewings compared to pupae and fumigated eggs.

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