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Attraction and Oviposition of *Lucilia eximia* (Diptera: Calliphoridae) to Resources Colonized by the Invasive Competitor *Chrysomya albiceps* (Diptera: Calliphoridae)

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Abstract

The present study aimed to determine if the presence of immatures of the invasive blow fly species *Chrysomya albiceps* (Wiedemann) influences the adult behavior of the native species *Lucilia eximia* (Wiedemann) in Brazil. The level of attraction and oviposition by the native species was assessed in a dual-choice assay. The evaluation was based on sex and stage of ovarian development of *L. eximia* adults to a resource not colonized (NCR) or colonized (RPC) with eggs, different instars, or densities of *C. albiceps*. A significant difference in attraction was observed based on sex and stages of ovarian development. Males and nongravid females were more attracted to RPC, whereas gravid females preferred NCR. Moreover, males exhibited the lowest response in all assays among the three sex categories examined. In general, adults preferably oviposited on NCR rather than RPC. Also, between the eggs and second instar treatments, *L. eximia* laid more eggs on RPC with eggs than second instars (predatory stage). *Lucilia eximia* attraction to second-instar *C. albiceps* at different densities was marginally significant. Overall, results indicate the invasive species, *C. albiceps*, is impacting the behavior of the native blow fly, *L. eximia*, with regards to its attraction and colonization of vertebrate carrion, which could explain why native blow fly populations have significantly decreased since the introduction of *C. albiceps*.

Key words: blow fly, forensic entomology, insect behavior, interspecific interaction, oviposition

Blow flies (Diptera: Calliphoridae) are typically the first arthropods to find and colonize vertebrate carrion. Their oviposition can occur minutes after death of these individuals, and they can be present in all stages of decomposition (Carvalho et al. 2000). Pechal et al. (2014) highlighted the importance of the insect community in the decomposition process and determined that when primary insect colonizers were excluded, carcasses remained in the bloat stage approximately two to three times longer than carcasses available for colonization.

The community structure can vary widely as the resource is decomposed. For instance, Brazilian native species such as *Cochliomyia macellaria* (Fabricius) (Biavati et al. 2010) and *Lucilia eximia* (Wiedemann) are usually found on carrion during the fresh and bloated stages of decomposition rather than resources in advanced decay stage (Grisales et al. 2010). The invasive species, *Chrysomya albiceps* (Wiedemann), generally has been found on carcasses in active and advanced decay stages (Grisales et al. 2010), but also can be present during the fresh and bloated decay stages (Carvalho and Linhares 2001, Biavati et al. 2010, Kosmann et al. 2011). These data indicate interspecific competition between the native and invasive species on carrion due to their overlap in temporal occurrence (see further text).

Lucilia eximia, commonly known as a green bottle fly, is distributed in the Neotropical and Nearctic regions. This species is found in the southern United States and throughout Central and South America, where it is possibly the most common species of Calliphoridae (Prado and Guimarães 1982). This species has a large distribution in Brazil, being found in several environments, such as cerrado (savannah-like) (Rosa et al. 2009), caatinga (Vasconcelos and Salgado 2014), and rainforest biomes, and different climate conditions (e.g., rainy, dry, cold) as well (Oliveira-Costa et al. 2001, Rosa et al. 2009). In addition, this species inhabits both urban (Carvalho et al. 2004) and rural (Oliveira-Costa et al. 2001) areas, exhibiting a high synanthropic index according to previous studies (Vianna et al. 1998, Souza and Von Zuben 2012). Moreover, it has been associated with a variety of vertebrate carrion types, such as pigs (Carvalho and Linhares 2001, Carvalho et al. 2004), dogs (Martins et al. 2013), and even humans (Andrade et al. 2005, Oliveira and Vasconcelos 2010). Needless to say, *L. eximia* is a critical component of the arthropod community that recycles vertebrate remains in various environments in Brazil.

Approximately 40 years ago, three blow fly species were introduced into the Americas: *Chrysomya megacephala* (Fabricius),

Chrysomya putoria (Wiedemann), and *C. albiceps* (Guimarães et al. 1978). These species now are known to occur in several countries in South America, including Brazil (Grella et al. 2015). According to Guimarães et al. (1978), these species were probably introduced in the southern region of Brazil by ships coming from Angola, Africa. As previously discussed, these exotic blow fly species arrive at decaying resources at similar times as the native species, resulting in competition. Furthermore, *C. albiceps* larvae are predators and greatly impact the survival rate of other blow fly species (e.g., 74% reduction in larval *L. eximia* present on the resource; Andrade et al. 2002, Faria et al. 1999). Such competition and predation success by the *Chrysomya* species most likely explain why *L. eximia* and *Co. macellaria* populations have been displaced in Brazil.

Interspecific interactions, such as predation and resource competition, are being systematically investigated in studies examining immature and adult relationships of *Chrysomya* species with native species, such as *Co. macellaria* and *L. eximia* (Faria et al. 1999, Reis et al. 1999, Faria and Godoy 2001, Andrade et al. 2002, Gião and Godoy 2006). However, the mechanism utilized by *L. eximia* to locate and colonize potential food resources is largely unstudied and seems crucial to enlighten the outcome of this interspecific interaction. Thus, this study aimed to determine if: 1) attraction and oviposition of *L. eximia* adults on a food resource colonized with eggs, second instars, and third instars (i.e., predaceous stages) of *C. albiceps* are based on their sex or stage of ovarian development; and 2) if their responses are partially governed by the larval density of *C. albiceps*.

Materials and Methods

The study was carried at the Laboratory of Entomology of the Federal University of Pelotas (UFPel), State of Rio Grande do Sul, Brazil. The methods applied in this study for evaluation of attraction and oviposition behavior of *L. eximia* and *C. albiceps* were based on those described in the works by Ma et al. (2012) and Tomberlin et al. (2012).

Insect-Rearing Method

Blow flies used in the bioassays resulted from a colony established with specimens captured in the field using a trap described by Ferreira (1978) and modified by Moretti et al. (2009). Adults of *L. eximia* and *C. albiceps* were collected in a rural area of the UFPel campus (31° 52′00″ S, 52° 21′24″ W), three times per week for a 1-mo period (May 2015). Adults collected in traps were separated by species (Grella and Thyssen 2011). Adults of each species were placed in transparent plastic cages (30 [length] by 30 [width] by 50 [height] cm) and maintained in a room at 26.0 ± 2.0 °C, 70.0 ± 10.0% RH, and a photoperiod of 12:12 (L:D) h. Flies were fed a blend of sugar, powdered milk, and yeast mixed in equal proportions (1:1:1) offered in a petri dish (90 by 15 mm, diameter and height, respectively). Water was provided *ad libitum* in a glass container (50 [D] by 100 [H] mm) with a moistened piece of cotton inserted through the top. Stimulation of ovarian development was necessary for oviposition. Therefore, 50 g fresh bovine liver was offered for 2 h during three consecutive days prior to egg collection with a similar amount of tissue.

After collection, eggs were placed in a plastic container (500 ml), with dimensions of 120 (D) by 78 (H) cm, until hatch. Larvae were transferred to another 500-ml plastic container, covered with white synthetic fabric and placed inside a larger cylindrical container (1,000 ml), with dimensions of 150 (D) by 94 (H) cm, with the top covered with white synthetic fabric and sawdust on the bottom to provide a dry place for pupation. Larvae were fed minced fresh beef *ad libitum* until pupation. Pupae were removed daily and transferred into a clean 500-ml container, as described previously. Emerged adults (F_1 generation) were released into transparent plastic cages (30 [L] by 30 [W] by 50 [H] cm), and both larvae and adults of the F_1 generation were kept under the same controlled conditions previously described. For both species, the trials were conducted with the F_1 and F_2 generation.

Olfactometer Design

Methods were adapted from Ma et al. (2012) and Tomberlin et al. (2012). The olfactometer (Fig. 1-a) used consisted of a Plexiglas cube (90 [L] by 40 [W] by 60 [H] cm) with 10-cm openings on opposite sides of each other. Polyvinyl chloride (PVC) pipes (both white, with 10 cm [D] and 15 cm [L]) were connected to each opening. The distal ends of the pipes were covered with nylon fabric to prevent flies from contacting assigned treatments. A funnel was placed in the proximate end of the pipe, with the larger end facing the interior of the cube and narrowing into the interior of the pipe, thus minimizing the chance of flies that entered the pipe from returning to the cube. The inside of the pipes was lined with two odor-free sticky traps, which captured flies entering the pipes. The distal end of each pipe was also connected to a 90° PVC pipe, with its distal end capped with a 9-cm-diameter petri dish on which the assigned treatment was placed (Fig. 1a). The Plexiglas cage and all components were washed with odorless soap and dried prior to use for each replicate.

Attraction Bioassays

For this assay, 7–10-d-old *L.eximia* adults were used, per Tomberlin et al. (2012) and Ma et al. (2012), as they determined that this age-group is most concise in response to such stimuli. For each replicate of the experiment, 60 flies, including 20 males, 20 nongravid females, and 20 gravid females, were introduced into the cube (Fig. 1b). Gravid and nongravid females were dissected and classified based on follicle development (Linhares 1988). Flies were allowed to acclimate for 1 h before initiating the experiment. Four replicates for each experimental group were organized as follows: 50 g of minced meat inoculated in a sterile petri dish (90 [W] by 15 [H] mm) with either 1 g of eggs or 100 larvae of second- and third-instar *C. albiceps* (RPC = resource colonized). A control group consisted only of the substrate (NCR = resource not colonized). Larvae were allowed to acclimate for 2 h on the tissue in the petri dish, which was placed in the rearing room prior to implementation in the experiment. Each replicate of the experiment was conducted for 24 h. Each petri dish with larvae with tissue, or tissue alone, was used only once.

To avoid side biases, treatment and control were alternated across the olfactometer arms between replicates. Experiments were conducted under the controlled conditions previously described. No other flies were present in the room during the experiments. Both RPC and NCR treatments were examined together in a given replicate. At the conclusion of each replicate, all flies on the sticky traps were counted and categorized (i.e., male, gravid females, or nongravid females). Flies remaining in the cube (no-choice group) at the conclusion of the experiment were also catalogued.

The effect of larval density of *C. albiceps* on adult *L. eximia* attraction was examined. The methodology for these experiments followed the same design previously described for the attraction assay. Four replicates of each density paired with a control were

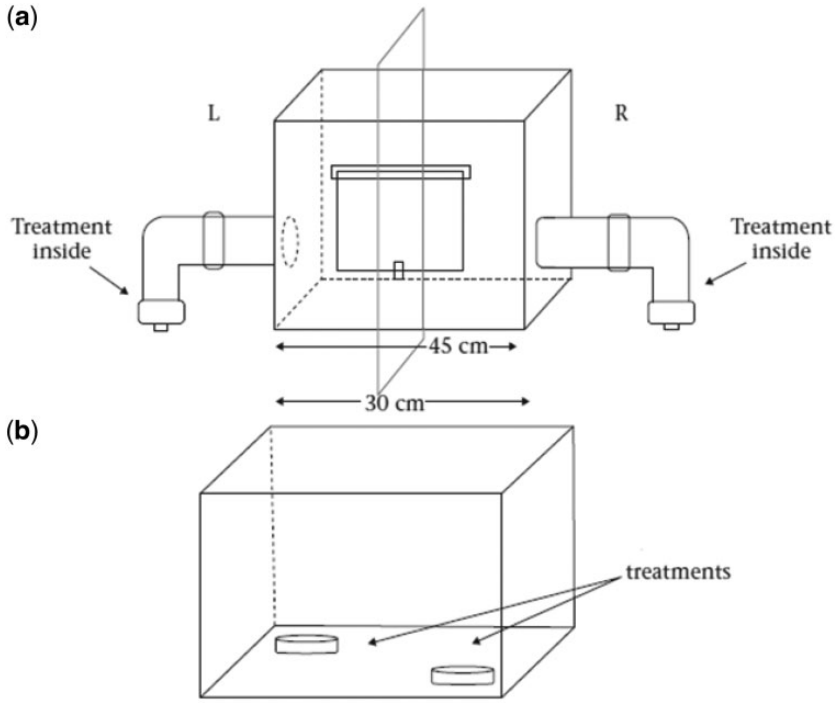

Fig. 1. Olfaction device used (Tomberlin et al. 2012) to measure *L. eximia* (a) attraction to control (resource not colonized treatment) and treatment (resource colonized by heterospecifics) with eggs or second or third instars of *C. albiceps* and (b) oviposition on control (resource not colonized treatment) and treatment (resource colonized by heterospecifics) with eggs or second instars of *C. albiceps* (images not to scale).

conducted. The environmental conditions mentioned previously were applied to the attraction assay. For these experiments, 20 gravid females (13–15-d-old) were placed in the olfactometer. Fly response to five larval densities (0 g, 1 g [$n = 100$], 2 g [$n = 200$], 4 g [$n = 400$], 5 g [$n = 500$]) of second-instar *C. albiceps* was determined (Queiroz et al. 1997, Faria and Godoy 2001).

Oviposition Response of *L. eximia*

For each replicate of this experiment, 40 gravid females of *L. eximia* (13-15-d-old) were used. Flies were placed in a cage (Fig. 1-b) for 1 h before being exposed to the treatment and control. The treatments consisted of two petri dishes (90 by 15 mm), one with minced bovine meat (50 g), as substrate, containing 1 g of eggs or 100 second-instar (approximately 1 g) *C. albiceps* and the other with 50 g minced bovine meat without eggs or larvae. Both substrates were acclimated for 2 h before exposure to *L. eximia* gravid females. In this experiment, only second-instar *C. albiceps* was examined due the results from the attraction experiments indicating greater response to this stage of development. The petri dishes with the resources were placed inside the cage, and flies were allowed 24 h to oviposit. Treatment and controls were rotated across positions across each replicate to minimize a position bias. The experiment was replicated five times and conducted under the same conditions as previously described. The number of *L. eximia* eggs was counted in each treatment after 24 h. Egg viability also was confirmed after each experiment.

Statistical Analysis

Four replicates of the attraction assay, and five replicates of the oviposition assay, examining *L. eximia* attraction to larvae or eggs of *C. albiceps* were conducted. Assessments were based on responses of males, gravid females, nongravid females, and all flies (summary of flies to respond) following the methodology proposed by Tomberlin et al. (2012). Data were analyzed using PROC logistic (SAS Institute Inc, 2002, Cary, NC), and the model output for each analysis was used to construct model statements that are presented in the tables. The probability (P) of attraction responses by *L. eximia* to the control or treatment (eggs, second instars, or third instars of *C. albiceps* and second instars at different densities) was examined with sex and stages of ovarian development. Also, it was considered the probability (P) of oviposition response to the control and treatment (eggs or second instars of *C. albiceps*). Only statistically significant ($P < 0.05$) variables are presented in the tables.

Results

Attraction Bioassays

Lucilia eximia attraction to a resource was influenced by the presence–absence of different life stages of *C. albiceps* (Table 1). *Lucilia eximia* adults, regardless of gender and gravid status, tended to respond more to the treatment, with 62.2% and 53.4% for eggs and third instars, respectively, rather than the control, with the exception of second instars, where they had a slight preference (55.2%) for the control (Table 1).

Estimated probability values, adjusted for replicate, for making a choice or not (staying in the center of the olfactometer) are presented in Table 2. Replicate was significant for adult response to the second instar ($F_{3,12} = 5.093$; $P = 0.0016$) treatment, with Replicates 1 and 4 being significant ($F_{3,12} = 2.3555$; $P = 0.0079$ and $F_{3,12} = 6.3837$; $P < 0.0001$, respectively), with 54.4% and 57.1%, respectively, of flies preferring the control. However, replicate was not significant for eggs ($F_{3,12} = 2{,}1087$; $P = 0.0968$) or third instars ($F_{3,12} = 0.9342$; $P = 0.4231$).

Flies were also examined for the influence of sexual group and stage of ovarian development on the probability of making a choice

Table 1. Mean ± SEM percentage attraction of *L. eximia* adults of different sexual groups and stage of ovarian development to control (resource not colonized treatment) and treatment (resource colonized by heterospecifics)* in attraction assays ($N^1 = 4$)

Treatment	Sexual group	Stage of ovarian development	Mean percentage attraction ± SEM (n^2)	
			Control	Treatment
Eggs	All		37.2 ± 5.4 (33)	62.2 ± 4.5 (57)
	Female	Gravid	85.2 ± 5.4 (23)	14.7 ± 5.4 (4)
		Nongravid	10.0 ± 7.0 (4)	90.0 ± 7.0 (31)
	Male		18.5 ± 7.8 (6)	84.0 ± 6.6 (22)
Second instar	All		55.2 ± 1.3 (52)	44.7 ± 1.4 (41)
	Female	Gravid	95.0 ± 5.0 (24)	5.0 ± 4.3 (1)
		Nongravid	43.5 ± 3.7 (18)	59.0 ± 4.6 (23)
	Male		24.7 ± 9.3 (8)	75.2 ± 9.3 (16)
Third instar	All		43.7 ± 5.1 (42)	53.4 ± 4.5 (49)
	Female	Gravid	80.0 ± 7.7 (27)	19.9 ± 7.7 (7)
		Nongravid	26.5 ± 3.6 (10)	78.5 ± 6.0 (28)
	Male		28.0 ± 7.5 (5)	75.0 ± 7.5 (14)

The treatment consists of eggs or larvae of the predator *C. albiceps*.

*100 eggs or larvae of second and third instars placed on 50 g minced beef; [1]number of flies to respond; [1]trials; [2]number of flies to respond.

Table 2. Estimated probability (SEM) and odds, adjusted for replicate, for choice (attracted to treatment or control) or no-choice (remaining in central cage of olfactometer) of *L. eximia* adults of different sexual groups and stage of ovarian development to control (resource not colonized treatment) or treatment (resource colonized by heterospecifics) in attraction assays ($N^1 = 4$, $n^2 = 20$)

Treatment	Sexual group	Stage of ovarian development	Estimated *P* (SEM)	Estimated odds (*P*/1-*P*)
Eggs	Female	Gravid	0.0538 (0.2364)	0.5094
	Male	Nongravid	0.1581 (0.2242)	0.7609
			0.1636 (0.2321)	0.5091
Second instar	Female	Gravid	0.1759 (0.2411)	0.4546
	Male	Nongravid	0.1654 (0.2182)	0.9535
			0.0589 (0.2427)	0.4138
Third instar	Female	Gravid	0.1876 (0.2261)	0.7392
	Male	Nongravid	0.1850 (0.2202)	0.8445
			0.0682 (0.2612)	0.2969

The treatment consists of eggs or larvae of the predator *C. albiceps*.

Estimated probability values per each treatment (eggs; second instars; third instars) can be obtained from the following model: $\text{Logit}(\pi) = \beta o + \beta_1 \times \text{Sex}_1 + \beta_2 \times \text{Sex}_2$, being $\text{Logit}(\pi) = -0.6751 + 0.4018 \times \text{Sex}_1 + 0.000674 \times \text{Sex}_2$ for eggs; $\text{Logit}(\pi) = -0.8823 + 0.8347 \times \text{Sex}_1 + 0.0939 \times \text{Sex}_2$ for second instars; $\text{Logit}(\pi) = -1.2144 + 1.0454 \times \text{Sex}_1 + 0.9122 \times \text{Sex}_2$ for third instars, where $\text{Sex}_1 = 1$ and $\text{Sex}_2 = 0$ for nongravid females; $\text{Sex}_1 = 0$ and $\text{Sex}_2 = 1$ for gravid females; $\text{Sex}_1 = 0$ and $\text{Sex}_2 = 0$ for males. Values calculated can then be computed with $P = \exp(\text{LO})/(1 + \exp(\text{LO}))$; [1]trials; [2]number of each sex per trial.

versus not making a choice (Table 3). The data indicated sexual group and physiological state significantly influence adult responses to second instars ($F_{2,12} = 4.0687$; $P = 0.0178$) and third instars ($F_{2,12} = 5.2488$; $P = 0.0053$); meanwhile, eggs did not significantly ($F_{2,12} = 1.0367$; $P = 0.3546$) explain choice or no-choice in the olfactometer. Nongravid adults tended to respond more to these treatments than males (Table 3), preferring the treatment side (59.0% and 78.5% for second and third instar treatments, respectively) instead of the control. Gravid adults were more attracted to the control than the treatment (95.0% and 80.0% for second and third instar treatments, respectively).

Attraction data for *L. eximia* to the treatment (eggs, second instar, and third instar) and control are presented in Tables 2 and 3. Estimated values for the probability of attraction, adjusted for replicate, are presented in Table 3. Replicate was not significant ($F_{3,12} = 0.7633$, $P = 0.5145$; $F_{3,12} = 0.3288$, $P = 0.9865$; $F_{3,12} = 0.6884$, $P = 0.5590$, for eggs, second instars, and third instars, respectively) for explaining attraction of *L. eximia* adults of different sexual groups and stage of ovarian development to the control and treatment. Adult responses were 62.2% to the egg treatment, 44.7% to the second instar treatment, and 53.4% to the third instar treatment.

There was a significant attraction of adults based on the sexual group to the tissue inoculated with eggs ($F_{2,12} = 14.2599$; $P < 0.0001$), second instars ($F_{2,12} = 6.9909$; $P = 0.0009$), and third instars ($F_{2,12} = 9.7084$; $P < 0.0001$). Regarding the eggs treatment, gravid females were more attracted to the control (85.2%), whereas nongravid ones were more attracted to the treatment (90.0%). Similarly, for the second instar treatment, gravid females were also more attracted to the control (95.0%), and nongravid females were more attracted to the treatment (59.0%). In addition, for the third instar treatment, gravid females also showed a preference for the control (80.0%), whereas nongravid females were more attracted to the treatment (78.5%). Males showed more attraction in all experiments for the treatment side, with 84.0%, 75.2%, and 75.0% for eggs, second instars, and third instars, respectively (Table 1).

The estimated probability values indicated nongravid females tended to respond more to minced beef inoculated with eggs or third instars than males and gravid females (Table 3). Gravid adults had the least response to the treatments. The probability estimates for gravid flies indicate they were significantly repelled (95.0%) from a resource inoculated with second instars. This pattern was repeated for eggs and third instar larval treatment with 85.2 and 80.0%,

Table 3. Estimated probability (SEM) and odds, adjusted for replicate, for attraction of *L. eximia* adults of different sexual groups and stage of ovarian development to control (resource not colonized treatment) and treatment (resource colonized by heterospecifics) in attraction assays ($N^1 = 4$, $n^2 = 20$)

Treatment	Sexual group	Stage of ovarian development	Estimated *P* (SEM)	Estimated odds (*P*/1-*P*)
Eggs	Female	Gravid	0.7177 (0.5417)	0.1739
		Nongravid	0.7065 (0.5312)	7.7500
	Male		0.2121 (0.4605)	3.6667
Second instar	Female	Gravid	0.0869 (0.7371)	0.9184
		Nongravid	0.4740 (0.3146)	1.2777
	Male		0.1875 (0.4330)	1.9999
Third instar	Female	Gravid	0.9021 (0.4241)	0.2592
		Nongravid	0.8579 (0.3683)	2.7999
	Male		0.3611 (0.6009)	2.2499

The treatment consists of eggs or larvae of the predator *C. albiceps*.

Estimated probability values per each treatment (eggs; second instar; third instar) can be obtained from the following model: $\text{Logit}(\pi) = \beta o + \beta_1 \times \text{Sex}_1 + \beta_2 \times \text{Sex}_2$, being $\text{Logit}(\pi) = 1.2993 + 0.7484 \times \text{Sex}_1 + (-3.0485) \times \text{Sex}_2$ for eggs; $\text{Logit}(\pi) = 0.6931 + (-0.4480) \times \text{Sex}_1 + (-3.1354) \times \text{Sex}_2$ for second instars; $\text{Logit}(\pi) = 0.8109 + 0.2187 \times \text{Sex}_1 + (-2.1608) \times \text{Sex}_2$ for third instars, where $\text{Sex}_1 = 1$ and $\text{Sex}_2 = 0$ for nongravid females; $\text{Sex}_1 = 0$ and $\text{Sex}_2 = 1$ for gravid females; $\text{Sex}_1 = 0$ and $\text{Sex}_2 = 0$ for males. Values calculated can then be computed with $P = \exp(\text{LO})/(1 + \exp(\text{LO}))$; *100 eggs or larvae of second and third instars placed on 50 g minced beef;[1]trials; [2]number of each sex per trial.

Table 4. Odds ratio estimates, adjusted for replicate, for choice or no-choice of *L. eximia* adults of different sexual groups and stage of ovarian development to control (resource not colonized treatment) or treatment (resource colonized by heterospecifics) in attraction assays ($N^1 = 4$, $n^2 = 20$)

Treatment	Sexual group effect	Estimated *P*	Estimated odds (*P*/1-*P*)
Eggs	Nongravid vs male	0.8168	1.495
	Gravid vs male	0.7312	1.001
Second instar	Nongravid vs male	0.9092	2.304
	Gravid vs male	0.7498	1.098
Third instar	Nongravid vs male	0.9450	2.844
	Gravid vs male	0.9234	2.490

The treatment consists of eggs or larvae of the predator *C. albiceps*.

Estimated probability values per each treatment (eggs; second instar; third instar) can be obtained from the following model: exp (−0.6751 + 0.4018)/exp (−0.6751) for males vs nongravid and exp (-0.6751 + 0.000674)/exp (−0.6751) for males vs gravid in eggs treatment; exp (−0.8823 + 0.8347)/exp (−0.8823) for males vs nongravid and exp (−0.8823 + 0.0939)/exp (−0.8823) for males vs gravid in second instar treatment; exp (−1.2144 + 1.0454)/exp (-1.2144) for males vs nongravid and exp (−1.2144 + 0.9122)/exp (−1.2144) for males vs gravid in third instar treatment; [1]trials; [2]number of each sex per trial.

Table 5. Odds ratio estimates, adjusted for replicate, for response of *L. eximia* adults of different sexual groups and stage of ovarian development to control (resource not colonized treatment) and treatment (resource colonized by heterospecifics) in attraction assays ($N^1 = 4$, $n^2 = 20$)

Treatment	Effect	Estimated *P*	Estimated odds (*P*/1-*P*)
Eggs	Nongravid vs male	0.8922	2.114
	Gravid vs male	0.5117	0.047
Second instar	Nongravid vs male	0.6545	0.639
	Gravid vs male	0.5107	0.043
Third instar	Nongravid vs male	0.7762	1.244
	Gravid vs male	0.5287	0.115

The treatment consists of eggs or larvae of the predator *C. albiceps*.

Estimated probability values per each treatment (eggs; second instar; third instar) can be obtained from the following model: exp (1.2993 + 0.7484)/exp (1.2993) for males vs nongravid and exp (1.2993 + −3.0485)/exp 1.2993) for males vs gravid in eggs treatment; exp (0.6931 + −0.4480)/exp (0.6931) for males vs nongravid and exp (0.6931 + −3.1354)/exp (0.6931) for males vs gravid in second instar treatment; exp (0.8109 + 0.2187)/exp (0.8109) for males vs nongravid and exp (0.8109 + −2.1608)/exp (0.8109) for males vs gravid in third instar treatment; [1]trials; [2]number of each sex per trial.

respectively. Nongravid females, on the other hand, were more attracted to the treatment side in all cases (90.0%, 59.0%, and 78.5% for eggs, second instars, and third instars, respectively). Males preferred the treatment in each experiment (84.0%, 75.2%, and 75.0% for eggs, second instars, and third instars, respectively).

The probability estimates, adjusted for replicate, for choice or no-choice of *L. eximia* adults of different sexual groups and stage of ovarian development to control or treatment indicated nongravid and gravid females tend to respond more to the treatments than males in all of the treatments (Table 4). Nongravid females responded 20% more than males to the egg treatment, and gravid females responded 6.0% more than males to the egg treatment. For the second instar treatment, nongravid females responded 84.0% more than males, and gravid females responded 16.0% more to the treatment. Moreover, for the third instar treatment, nongravid females responded approximately 100.0% more than males, and gravid females responded 76.0% more than males to the treatment. The probability estimates, adjusted for replicate, for response of *L. eximia* adults of different sexual groups and stage of ovarian development to control and treatment indicated gravid females responded more than males to control in all assays (Table 5). Gravid females responded 78.0% more than males to the control, and nongravid females responded 7.0% more than males to the egg treatment. For the second instar treatment, gravid females responded 74.0% more than males to the control, whereas nongravid females responded 21.0% less than males to the egg treatment. For the third instar treatment, gravid females responded 80.0% more than males to the control, and nongravid females responded 8.0% more than males to the treatment.

Lucilia eximia attraction to second-instar *C. albiceps* at different densities was marginally significant ($F_{3,16} = 2.5935$; $P = 0.0508$). Estimated probabilities, adjusted for replicate, for the response of gravid females of *L. eximia* to different densities are presented in Table 6. The replicate variance was not significant ($F_{3,16} = 0.2494$; $P = 0.8618$), indicating consistent responses. There was a significant difference in choice (treatment or control side) in the different

Table 6. Estimated probability (SEM) and odds, adjusted for replicate, for oviposition of gravid-females of *L. eximia* to control (resource not colonized treatment) and treatment (resource colonized by different densities of heterospecific larvae[1]) in attraction assays ($N^1 = 4$, $n^2 = 20$)

Larval amount (g)[3]	Estimated *P* (SEM)	Estimated odds (*P*/1-*P*)
1	1.1101 (0.6138)	0.1304
2	1.7809 (1.023)	0.0476
4	0.975 (0.4915)	0.2083
5	0.3666 (0.6055)	0.1812

Estimated probabilities for treatment can be obtained from the following model: $\text{Logit}(\pi) = \beta o + \beta_1 \times X_1 + \beta_2 \times X_2 + \beta_3 \times X_3$, being $\text{Logit}(\pi) = -1.7081 + (-0.3288) \times X_1 + (-1.3364) \times X_2 + 0.1395 \times X_3$, where $X_1 = 1$, $X_2 = 0$, $X_3 = 0$ for 1 g; $X_1 = 0$, $X_2 = 1$, $X_3 = 0$ for 2 g; $X_1 = 0$, $X_2 = 0$, $X_3 = 1$ for 4 g; $X_2 = 0$, $X_3 = 0$ for 5 g. Values calculated can then be computed with $P = \exp(\text{LO})/(1 + \exp(\text{LO}))$. [1]trials; [2]number of flies per trial; [3]approximately 1 g.

Table 7. Mean ± SEM percentage oviposition of *L. eximia* to control (resource not colonized treatment) and treatment (resource colonized by heterospecifics)* in oviposition assays ($N^1 = 5$)

	Mean percentage oviposition ± SEM (n^2)	
Treatment	Control	Treatment
Eggs	71.6 ± 5.8 (1202)	28.4 ± 5.8 (520)
Larvae	76.1 ± 9.7 (1266)	23.9 ± 9.7 (413)

The treatment consists of eggs or second instars of the predator *C. albiceps*.
*100 eggs or larvae of second instar were placed in 50 g of bovine meat; [1]trials; [2]number of flies to respond; [1]trials; [2]number of eggs deposited.

Table 8. Estimated probability (SEM) and odds, adjusted for replicate, related to *L. eximia* oviposition on substrate with heterospecific eggs or second instars of *C. albiceps* ($N^1 = 5$, $n^2 = 40$)

Treatment	Estimated *P*	Estimated odds (*P*/1-*P*)
Eggs	0.0091	0.4326
Larvae*	0.0032	0.3756

Estimated probabilities for treatment can be obtained from the following model: $\text{Logit}(\pi) = \beta o + \beta_1 \times X_1$, being $\text{Logit}(\pi) = \text{e-}0.9790 + 0.1411 \times X_1$, Where $X_1 = 1$ and $X_2 = 0$ for RPCE, $X_1 = 0$ and $X_2 = 0$ for RPCL. Values calculated can then be computed with $P = \exp(\text{LO})/(1 + \exp(\text{LO}))$; *100 eggs or larvae of second instar were placed in 50 g of minced beef. RPCE = resource colonized with eggs; RPCL = resource colonized with larvae; [1]trials; [2]number of flies per trial.

densities ($F_{3,16} = 3.2369$; $P = 0.0212$), with 90.0% for the control when paired with a resource colonized with 1 g of larvae, 96.0% for the control when paired with a resource colonized with 2 g of larvae, 82.0% for the control when paired with a resource colonized with 4 g of larvae, and 77.0% for the control when paired with a resource colonized with 5 g of larvae.

According to the probability estimate values, blow flies responded more to resource colonized by 4 g of larvae treatment assay than other treatment assays, with 82.0% of gravid females having chosen the control side. However, repellence to the resource colonized with 5 g of larvae was lower (with 77.0% for control side) than to the resource colonized with 2 g of larvae (96.0% for control side).

Oviposition Response of L. eximia

Lucilia eximia oviposition was influenced by the presence–absence of *C. albiceps* eggs or larvae. Oviposition was significantly predicted by the presence or absence of the competitor ($F_{1,10} = 13.3531$; $P = 0.0003$). The flies preferred to oviposit on the resource without larvae or eggs rather than on the resource colonized (Tables 7 and 8).

The probability estimate values, adjusted for replicate, for oviposition behavior are presented in Table 5. Replicate variance was significantly different ($F_{9,10} = 9.0080$; $P < 0.0001$), with Replicates 2, 3, and 4 for eggs showing significance ($F_{1,10} = 4.8526$, $P = 0.0276$; $F_{1,10} = 6.6934$, $P = 0.0097$; $F_{1,10} = 9.0385$, $P = 0.0026$, respectively), with 60.7%, 94.2%, and 69.2%, respectively, having preference for the NCR. When comparing level of response to liver with eggs or larvae, flies had a greater probability of laying eggs on a resource with eggs (55.7%) rather than larvae (44.3%).

Discussion

Sex and stage of ovarian development play a crucial role in regulating adult insect attraction and/or colonization of a resource. In the present study, gravid *L. eximia* were less attracted to, and colonized less, resources with different life stages of *C. albiceps* present. Clearly, this species avoids such resources for a good reason. As previously mentioned, *C. albiceps* larvae predate on all instars of native species from Brazil, such as *L. eximia* and *Co. macellaria* (Faria and Godoy 2001, Silva et al. 2003, Rosa et al. 2006, Faria and Godoy 2001).

Male and nongravid female *L. eximia*, on the other hand, were attracted to the resource colonized by *C. albiceps*. Considering these flies rely on the resource for securing a mate or locating a protein source for producing eggs, no interactions with the predatory larvae are expected to occur (Martín-Vega and Baz 2013). Thus, no selection has appeared to take place for them to avoid such resources. They most likely rely on the carcasses as an aggregation point by males and virgin females, to avoid extensive energy spent during mate searching and may also serve to increase the chances of successful breeding (Mohr and Tomberlin 2014). In contrast to our findings, a recent study carried out by Brodie et al. (2014) demonstrated no difference in attraction by gravid and nongravid female *L. sericata* and *Phormia regina (*Meigen) (Diptera: Calliphoridae) to resources with conspecifics and gravid heterospecifics. They determined gravid females were attracted to a resource previously attended by nongravid and gravid females. However, these species do not experience predation by one on the other.

In general, oviposition behavior of adult blow flies has received modest attention. Oviposition is a crucial biological process for flies because it determines the potential population size for successive generations (Ullyett 1950, Smith 1986). And, choice of oviposition patches and dispersion of eggs by adult insects can vary among host species or substrate type, among individuals within a host population, or among individuals of a particular host population (Dukas et al. 2001, Holland et al. 2004). Results from our study demonstrate that the presence of predators, such as *C. albiceps*offspring, on a resource could reduce attraction, and subsequent oviposition, by *L. eximia*. As seen in these experiments, adults laid more eggs on control resources than those containing second-instar *C. albiceps*. This behavior of avoidance may allow for *L. eximia* persisting in an environment despite the presence of facultative predators like *C. albiceps*. However, Andrade et al. (2002) determined larval aggregation level of *C. albiceps* increased on resources previously colonized

by native species, indicating an arms race with regards to species detection and avoidance between these competing species.

Microbes play a significant role in the decomposition processes of carrion, usually producing volatile organic compounds (VOCs) that may serve as cues regulating attraction or repellence of arthropods to these resources (Tomberlin et al. 2012, Ma et al. 2012). Recently, Liu et al. (2016) examined the impact of different doses of dimethyldisulfide, phenylacetic acid, indole, and isobutylamine (compounds associated with vertebrate carrion decomposition) on fly behavior, such as attraction. They determined that behavioral responses of adult flies were dependent on concentrations of the referred compounds and fly physiological state (gravidity and sex). These results could partially explain the responses observed in our experiments, as the bacterium, *Proteus mirabilis*, which is associated with larval *Lucilia* sp. (Thomas et al. 1999, Mohd-Masri et al. 2005), is known to produce these compounds (Ma et al. 2012). Thus, *P. mirabilis* might be absent in the tissue colonized by *C. albiceps*, resulting in different VOCs being produced. Chaudhury et al. (2010) determined the adult female primary screwworm, *Cochliomyia hominivorax* (Coquerel) (Diptera: Calliphoridae), was selectively attracted to, and oviposited on, resources containing different bacteria. They concluded that volatiles produced by these bacteria governed the behavioral responses observed by the flies.

In contrast, different densities of *C. albiceps* larvae on a resource did not impact attraction exhibited by *L. eximia*. Other studies based on population dynamics of *L. eximia* found little impact of *C. albiceps* on fecundity and size (tibia and wing length) of resulting *L. eximia*, regardless of the presence of *C. albiceps* on carrion (Gião and Godoy 2006). Similarly, Linhares (1981) showed that populations of *L. eximia* are not strongly influenced by high densities of *C. albiceps*.

Although *L. eximia* larval survivorship can be drastically reduced due to predation by *C. albiceps* larvae (Reis et al. 1999, Rosa et al. 2006), previous studies suggest *L. eximia*, unlike *Co. macellaria*, might be capable of maintaining a more stable population (Moura et al. 1997, Silva et al. 2003). Silva et al. (2003) indicated through a mathematical model that *L. eximia* is capable of maintaining a more stable population size than other calliphorid species when facing environmental disturbances (i.e., possibly the introduction of a predator). *Lucilia eximia* populations could persist by maintaining population sizes much greater than zero, as it exhibits an eigenvalue smaller than 1 ($\lambda = -0.513$) and, therefore, has a one-point equilibrium. This might partly be due to the ability of *L. eximia* gravid adults to detect and avoid resources colonized by a predator and thus increase the likelihood of larval survival, as seen in our study.

Results from this study indicate the presence of *C. albiceps* on a resource in Brazil alters the behavior of the native blow fly, *L. eximia*. In fact, this study demonstrated *L. eximia* expressed reduced attraction and colonization of such resources. This information is crucial for the field of forensic entomology. Historically, succession data have often been used to estimate a postmortem interval (PMI; Goff et al. 1988, Perez et al. 2014). However, if succession patterns shift due to the presence of this invasive species, application of historical data, which were generated in its absence, could be misleading when estimating an associated PMI.

Although our results are informative with regards to the impact of *C. albiceps* on the attraction and colonization of vertebrate carrion by *L. eximia*, fieldwork is still needed to determine if such behavioral responses occur under natural conditions. As stated by Catts (1992), data generated from studies on ecology and behavior of native and invasive blow flies could be used to better understand the processes of vertebrate decomposition and could lead to novel methods for estimating the PMI of human remains.

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