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AMANDA MARIA MORAES CHARPINEL

THE EFFECTS OF PARTICULATE MATTER AIR POLLUTION ON SURVIVAL OF *HELICONIUS ETHILLA* (GODART, 1819): IMPLICATIONS FOR BUTTERFLY CONSERVATION AT CITIES

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Monografia apresentada ao Departamento de Ciências Biológicas do Centro de Ciências Humanas e Naturais da Universidade Federal do Espírito Santo como requisito parcial para a Obtenção do titulo de Bacharel em Ciências Biológicas.

Orientador: Prof. Dr. Francisco Candido Cardoso Barreto

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SUMÁRIO

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MANUSCRITO

The Effects Of Particulate Matter Air Pollution On Survival Of *Heliconius ethilla* (Godart, 1819): Implications For Butterfly Conservation At Cities

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Abstract Human interventions has been affecting natural ecosystems around the world. Urbanization is associated with general habitat degradation and increased soil and air pollution. Such changes can generate negative effects on some insect species such as growth inhibition, developmental abnormalities, and reduction of reproductive and survival rates. Among the pollutants that can be found in the air, sedimentable particulate matter (PTS) was the focus of this study. It is believed that PTS could promote mechanical obstruction, heavy metal accumulation intoxication, reduction of palatability and stress in different levels for Lepidoptera caterpillars. *Heliconius ethilla narcaea* (Godart, 1819) is a Heliconiinae relatively common in green areas of the city of Vitória, ES (Brazil). After calculating the mean daily rate of PTS deposition in the environment, we created 920 caterpillars in a laboratory, divided into a control and experimental group. The caterpillars were fed with leaves of *Passiflora edulis* Sims f. flavicarpa Deg. In the experimental group the PTS was added on the leaves before being offered to the caterpillars in an increasing concentration according to the age of the caterpillar and the leaf, simulating the gradual deposition of this material in the environment. From the construction and evaluation of life tables we verified the mortality in the control group was 56.6% while in the treated group 62.38%. This result indicates a reduction in the population size of the species and consequent decrease in the chances of long-term survival.

Key words Urbanization · Air pollution · Lepidoptera · Life table · Mortality · Viable populations

Resumo As intervenções humanas têm afetado os ecossistemas naturais em todo o mundo. A urbanização está associada à degradação do habitat e ao aumento da poluição do solo e do ar. Essas alterações podem gerar efeitos negativos em várias espécies de insetos como inibição do crescimento, anormalidades do desenvolvimento e redução das taxas reprodutivas e de sobrevivência. Dos poluentes que são encontrados no ar, o material particulado sedimentável (PTS) foi o foco deste estudo. Acredita-se que o PTS pode promover obstrução mecânica, intoxicação por acumulação de metais pesados, redução de palatabilidade e estresse em lagartas de Lepidoptera. Foi escolhido como modelo a subespécie *Heliconius ethilla narcaea* (Godart, 1819) uma Heliconiinae relativamente comum nas áreas verdes da cidade de Vitória, ES (Brasil). Após calcular a taxa média diária de deposição de PTS no ambiente, procedemos a criação de 920 lagartas em laboratório, divididas em grupo controle e experimental. As lagartas foram alimentadas com folhas de *Passiflora edulis* Sims f. flavicarpa Deg. Ao grupo experimental o PTS foi adicionado sobre as folhas antes de serem oferecidas às lagartas em concentração crescente de acordo com a idade da lagarta e da folha, simulando a deposição gradual desse material no ambiente. A partir da construção e avaliação de tabelas de vida foi verificada que a mortalidade no grupo controle foi de 56.6% enquanto que no grupo tratado 62.38%. Esse resultado indica redução no tamanho populacional da espécie e consequente diminuição das chances de sobrevivência a longo prazo.

Palavras-chave Urbanização · Poluição do ar · Lepidoptera · Tabela de vida · Mortalidade · Populações viáveis

Introduction

Human interventions has been affecting the natural ecosystems all over the world (Qadir & Malik 2009). Modern farming, industrialization, and increased vehicular use have led to high concentrations of pollutants in the environment (Atafar et al. 2010). These pollutants are regularly getting, for example, into air and soil (Lee et al. 2006).

In the air, the main sources of air pollution in urban areas are: vehicle exhaust, caused by old cars and heavy vehicles, industrial pollution, road dust, and solid waste incineration. Typical Brazilian urban air pollution measurement includes particulate matter (PM), gas pollutants like ozone (O_3), carbon monoxide (CO), nitrogen oxides (NO_x), and sulfur dioxide (SO_2), and airborne toxic chemicals like hydrocarbons and aldehydes (Nel 2005). According to the 2013 annual report of the quality of the air by the Instituto Estadual de Meio Ambiente e Recursos Hídricos (IEMA) Industries (19.6%), and vehicles (3.9%) are the main causes for PM_{10} emissions. Industrial emissions basically accounted for two-thirds of SO_2 and NO_x emission.

WHO (2006) says "Total Suspended Particles (TSP) also known as particulate matter air pollution are represented by all the particles suspended in the atmosphere with a large particle size range and capable of being sampled. Typically, the aerodynamic diameter of these particles ranges from 0.005 μm to greater than 100 μm. MP_{10} are airborne particles with an aerodynamic diameter of less than 10 μm (including particles of the fine, fine and ultrafine modes) that penetrate the respiratory system and are mainly emitted by mechanical processes in construction activities and by the

resuspension of particles in roadways due to traffic or wind erosion, among others. $MP_{2.5}$ are considered fine particles in suspension that have aerodynamic diameter less than 2.5 μm produced mainly in combustion processes. The $MP_{2.5}$ are stored in the bronchiole, while the other fractions of particles smaller than 10 μm are retained in the nose and nasopharynx, and can later be eliminated from the respiratory system by the defense mechanisms of the human organism (Holgate et al. 1999). There are also ultrafine particles ($MP_{0.1}$) in suspension that have an aerodynamic diameter of less than 0.1 μm, whose effects on human health are not yet well studied".

Studies on the impact of air pollution on health in Latin American countries yield results similar to those in other locations in the world (Romieu et al. 2012). As for health outcomes, respiratory and cardiovascular diseases are the ones most commonly associated with air pollution (World Health Organization 2005). The World Health Organization (WHO) released new estimates on March 24, 2014, on the impact of air pollution on health. These new estimates were based on mortality data for 2012 in the world and new evidence on the health risk posed by exposure to air pollution. The WHO reported that about 3.7 million deaths occurred in 2012 associated with outdoor air pollution: heart ischemia (40%), heart attack (40%), chronic lung obstruction (11%), lung cancer (6%), Respiratory infections in children (3%). While indoor air pollution was associated with 4.3 million deaths from heart attack (34%), cardiac ischemia (26%), chronic pulmonary obstruction (22%), respiratory infections in children (12%) and Lung (6%).

Air pollution has been positively associated with several adverse outcomes in public health for different age groups. Many studies have indicated that aged people as well as the children are more sensitive to the acute and chronic adverse effects of air pollution (Dockery et al. 1994; Saldiva et al. 1995; Bascom et al. 1996; Braga et al. 1999; Lin et al. 1999). In São Paulo, for example, air pollution exposure has been associated with mortality due to respiratory diseases among children under 5 years of age (Conceição et al. 2001). Braga et al. (1999) and Lin and colleagues (1999) found a strong association between air pollution and hospital admissions due to respiratory problems for children and adolescents younger than 13 years. Analyses stratified by age group showed that the strongest effects occur among infants (Braga et al. 2001). Other studies have shown that the effects of air pollutants can start as early as during pregnancy. Ritz and colleagues investigated the birth outcomes due to air pollution in California, USA, and found a positive association between air pollution and both birth defects (Ritz et al. 2002) and preterm birth (Ritz et al. 2000). Wang and colleagues (1997) and Bobak and Leon (1999) reported associations between air pollutants and low birth weight. Also, Pereira and co-workers (1998) demonstrated a positive association between intrauterine death and air pollution.

Urbanization is associated with habitat degradation, including decreased plant species diversity, reduced water quality, and increased air and soil pollution (McKinney 2009). Studies of the effects of urbanization on biodiversity have focused primarily on vertebrates, including reptiles (See Germaine & Wakeling 2001), amphibians (See Clark et al. 2008), mammals (See Riley et al. 2003), and birds (See Miller et al. 2003; See Lee et al. 2004). Less attention has been paid to the effects of habitat loss and fragmentation on terrestrial invertebrates (Gibb & Hochuli 2002; Tscharntke et al. 2002).

Insects have strong relationship with ecology and are popularly used as bioindicators since long time (Davis et al. 2001). Acute and chronic effects of air pollution on some insects are frequently reported in the form of growth inhibition, developmental abnormalities, reduced reproduction, and decreased hatchability (Sildanchandra & Crane 2000). Air pollution has been associated with both primary (direct) and secondary (indirect) effects on insect populations. For example, sulfur reduced the efficiency of pollinating bees (Przybylski 1968), on mulberry leaves, oil-soaked lesions induced by sulfur dioxide were associated with reduced feeding rate, inactivity, non-uniform growth, delayed cocooning, and cuticular softening of silkworm larvae (Kuribayashi 1971) and reduced flight activity and brood-rearing activity occurred in bees exposed to $S0_2$ (Hillman 1972), but little information is available on the modes of action and toxicology of sulfur compounds in insects. In addition to the major classes of pollution affecting insects, there are some references suggesting that both arsenic (Mueler & Worseck 1970) and nitrogen oxides (Sierpinski 1970; Sierpinski 1972) may have direct primary effects. Additionally, there is a potential for air pollutants, particularly 0_3, to negatively influence the searching behaviour of parasitoids (Gate, Mcneill & Ashmore 1995), but effects of air pollution on insect populations are poorly understood.

Urban areas are known as "green spaces" or "open spaces". Whitmore et al. (2002) define urban open spaces as 'any vegetated areas (green areas) including nature reserves, private and public gardens, sport and recreational grounds, roadsides, rail verges and transmission line servitudes, cultivated, derelict and undeveloped land'. However, the effects of the urbanization on butterflies and other insects have scarcely been appraised in detail (New & Sands 2002). According to Ehler's (1978) the term "Urban environments" means a wide shared connection, reflecting the enormous variety of

ecological situations at cities, towns and their surrounding áreas that collectively encompass numerous continua of disturbance and change.

More than 17000 species of butterfly are found worldwide and they are important bioindicators (Kumar 2014). Insects are typically the overwhelmingly dominant invertebrate faunal group and extensively used in biomonitoring and bioassessment programs throughout the world (Izam et al. 2015). Butterflies have ecological fidelity and are sensitive to environmental changes and quality. According to Chen et al. (2005) these insects have been successfully used as bioindicators for environmental pollution and heavy metals contaminations near industrial states and even within urban areas.

Ramírez-Restrepo and MacGregor-Fors (2017) shows that the reviewed studies report negative effects of urbanization on butterflies. MacGregor-Fors et al. (2015) shows that butterflies were the most sensitive wildlife group to urbanization. Matteson et al. (2013) found lower diversity (species richness and abundance) of flower-visiting insects, including butterflies, in heavily developed neighborhoods in comparison with urban green areas. Butterflies respond differently to urbanization depending on their area of distribution and taxonomic identity for example (Soga and Koike 2012a, 2012b). Specialist butterflies decreases with increasing urbanization (Bergerot et al. 2011, 2012; Soga and Koike 2012a, 2013) and urbanization can lead to local extinctions of infrequent, nonabundant, specialist butterfly species (Soga & Koike 2012a).

To date, important efforts have been made at some cities and their surroundings for conserving endangered butterfly species (Murphy & Weiss 1988; Daniels 2009). As has been shown in previous studies or initiatives (Snep et al. 2006; Kadlec et al. 2008), butterfly conservation in urban areas is a feasible task, since many species are able to thrive in urban areas. Hopefully, creative urban planning and management, such as habitat design and planting of native host and nectar-rich plants could enhance and improve urban habitats for butterflies. All actions must be monitored and need to be based in previous knowledge on the biology and ecology of the target species in order to be successful (Kremen et al. 1994).

The reasons for using insect species, butterflies included, as indicator are: (1) use of several different taxa of different habitat gives more robust results, (2) a quantitative indicator value needs to be associated with the bioindicators, (3) there is similarity between different landscape features, (4) there is comparison of community, (5) these taxa can be reliably identified, sampled, and quantified, and (6) more than one family surely indicate species richness of an order (Niu et al. 2002). They give a rapid and sensitive response to accumulation of heavy metals (Cervera et al. 2004). In addition, since they have dynamic reproductive cycles, thus responding rapidly to changes in the vegetation and climate, they are conspicuous and therefore easily observed and sampled at any time of the year and have a well-known taxonomy (Brown 1991; Freitas et al. 2003). They serve as model in research on population ecology and behavior (Dessuy & Morais 2007).

Air-pollution has frequently been suggested as a cause of the decline of some butterfly species: a suggestion based mainly on lowered species richness close to industrial areas in Europe. There have been frequent calls, in vain, for research on the direct effect of air-pollution on Lepidoptera, recent research being confined to the indirect role via climate change (Corke 1999). Today, still, no experiments have been conducted and attention has focused mainly on the possible effects of the indirect results of pollution (climate change) on butterfly ecology (Dennis 1993).

Since urbanization, wich is associated with habitat degradation and increased air pollution, (McKinney 2009), because insects are popularly used as bioindicators (Davis et al. 2001) including the group Lepidoptera (Kumar 2014), since negative effects on butterflies are caused by urbanization (MacGregor-Fors et al. 2017) and can lead to the reduction of some butterfly species (Corke 1999), because butterflies are the most sensitive wildlife group to urbanization (MacGregor-Fors et al. 2015) due to what Sildanchandra & Crane (2000) said about the effects of air pollution on some insects, which are growth inhibition, developmental abnormalities, reduced reproduction, reduced survival and decreased hatchability, and since the effects of the urbanization on butterflies have scarcely been appraised in detail (Corke 1999) including few studies about the effects of urbanization (New & Sands 2002), few studies about the effects of air pollution on butterfly also being conducted (Dennis 1993) and no studies about the effects of the particulate matter air pollution on the group of Lepidoptera, this study, which is conducted with a species of Heliconiinae relatively common in urban green areas of the city of Vitória, ES (Brazil) called *Heliconius ethilla* is a pioneed experiment on the investigation of the effects of the particulate matter air pollution on this model urban species of butterfly.

Our objective is to investigate the effects that the particulate matter air pollution has on the survival of *H. ethilla* and what are the effects. Our hypothesis is that the greater the concentration of particulate matter air pollution worse is the effect on the survival of *H. ethilla*.

Material and Methods

The collection of *H. ethilla* bionomic data were developed in the Entomological and butterfly house in the Laboratory of the Environmental Education Center of ArcelorMittal Tubarão, located in the municipality of Serra, Espírito Santo, Brazil.
To determine the viability and the time of development of the eggs until the adult emergence, eggs were collected and kept in air-conditioned chambers (B.O.D.) at 25 ± 0,3 ° C and in photoperiod of 12 hours until adult emergence, if there was one. After monitoring with duration of 6 months using the standard PO¬UTL¬LTAR-00-0003 of the Laboratory of Utilities of the CST referring to the gravimetric analysis of sedimentable particulate material was applied we calculated the mean daily rate of PTS deposition in the environment. We created 920 caterpillars in a laboratory, divided into a control and experimental group. The caterpillars were fed with leaves of *Passiflora edulis* Sims f. flavicarpa Deg. In the experimental group the PTS was added on the leaves before being offered to the caterpillars in an increasing concentration according to the age of the caterpillar and the leaf, simulating the gradual deposition of this material in the environment.

The life tables and graphs of the life cycle were elaborated with the data of mortality and survival of the individuals of the control and experimental groups according to Rockwood (2006), Bellows Jr et al. (1992) and Gotelli (2009), where x indicates the stages of the life cycle and it has a phase of egg (Fig. 1), five larval stages designated from L1 to L5 (Phase L5 in Fig. 2), pupa (Fig. 3) and adult (Fig. 4); S(x) is the number of individuals at the beginning of each stage; l(x) indicates the proportion of the population surviving to stage x; p(x) is the probability of surviving phase and q(x) the probability of death. Additionaly, the proportion of the population that lived until phase x which is L(x), the proportion of the population that lived until phase x and in all subsequente phases which is T(x) and life expectancy e(x) were also calculated. Based on the mortality and survival profiles of the life table, the life cycle charts were done.

Formulas that were used to build the life tables:

$$l(x) = \frac{N_{x+1}}{N_x} \qquad p(x) = 1 - q(x) \qquad q(x) = 1 - p(x)$$

$$L(x) = \frac{l_x + l_{x+1}}{2} \qquad T(x) = L_x + L_{x+1} + L_{x+2} \ldots . L_{x+n} \qquad e(x) = \frac{T_x}{l_x}$$

Results

According to the results observed, the p(x) and q(x), which represent respectively probability of survival to the next phase and probability of deat on the population, until phase L3 all the individuals survived in the experimental group (Table 1 and Fig. 5) while for the control group the survival was 26%, 91% and 95% (Table 2 and Fig. 6). In experimental group in the phase L4 and L5, 62.4% and 61.9% of the individuals survived to the next phase respectively (Table 1 and Fig. 5) while in the control group it accounts respectively for 94% and 96% (Table 2 and Fig. 6). The percentage of the individuals that survived the pupal phase in the experimental group was 59.4% (Table 1 and Fig. 5) while in the control group it was 67.1% (Table 2 and Fig. 6). The control group had 73.9% of mortality in the egg phase while in the experimental group it was 0% (Table 1 and 2). About the larval phases, there were no mortality in the egg, L1, L2 and L3 phases (Table 1) while in the control group there was mortality in all the larval phases, which were respectively 9.2%, 5.2% and 5.9% (Table 2).

The means for each phase development, in crescent order, in the treatment were: 3.28 days (SD± 0.98) for the eggs, 3.27 for the phase L1(SD± 1.03), 2.63 for the phase L2 (SD± 0.91), 3.40 for the phase L3 (SD± 0.95), 3.82 for the phase L4 (SD± 1.05), 5.55 for the phase L5 (SD± 1.19), and 11.64 for the pupal phase (SD± 1.15) (Table 3). Comparing to the control group, the eggs took 3.95 days (SD± 1.04) to go to the next phase, L1 took 2.31 days (SD± 0.68), L2 took 2.38 days (SD± 0.98), L3 took 2.75 days (SD± 1.00), L4 took 3.17 days (SD± 1.02), L5 took 4.44 days (SD± 1.10) and the pupa took 10.27 days (SD± 1.09) complete its development (Table 4). In general, for the duration of each phase the experimental group took between zero and three days more than the control group. About the duration of all of the larval phases, the control group took a mean of 15.05 days while the experimental group took 18.67 days. The complete cycle for the treatment group was of 33.59 days (SD ± 3.17) (Table 3) while the complete cycle of the control group was 29.27 days (SD± 2.17) (Table 4). In general the difference of development between the control and experimental group considering

the complete life cycle was aroud 4 to 8 days, in which the experimental group took aroud 4 to 8 days more to complete the cycle.

With the construction and evaluation of life tables it was verified that, in general, the mortality in the control group was 56.6% while in the treated group 62.38%. There were less deaths in lower concentration of PTS while increasing number of deaths followed the increasing concentration of PTS (Fig. 7). Additionaly, from all the individuals in the experimental group, only 18.84% developed until the phase of imago. The control group had 2.3x more individuals surviving until the imago phase, which accounts for 43.4%. Additionally, the analysis of the data shows that the weight of the pupa decreases significantly with the increasing concentration of PTS (Fig. 8). There was a significant negative effect of the PTS on the weight of the pupa and mortality of the individuals (parameter a p-value = 7.31 e^{-09}, parameter b p-value = 3.58e^{-05} and parameter c p-value = 0.00507) (Table 5).

Discussion

Probably, the food stress caused by the concentration of particulate matter air pollution caused the reduced growth rate and longer time of the development. According to Bauerfeind and Fischer (2005) the larval food stress significantly reduced the Lepidoptera larval growth rate, pupal and adult mass, whereas the larval development time of the Lepidoptera was prolonged. They suggest that Lepidoptera larvae facing food stress compensate for temporarily reduced nutrient intake by extending the larval period. That result is believed to be universal and generally predicted by life-history models (Berrigan and Charnov 1994, Gotthard and Nylin 1995, Arendt 1997, Blanckenhorn 1999).

In addition, Bauerfeind and Fischer (2005) also observed that larval food limitation had not only negative effects on the growth rate and longer time of development but also reduction in the fecundity and reproductive rate, which were basically mediated through a reduction in body size. Although we do not use fecundity or reproductive rate in our study and that based on the observation of these authors and comparing with what we have in our study with *H. ethilla* we could expect a reduction in the fecundity and natality rate due to the reduction in the body size, probably because the smaller the body the smaller the space to produce eggs and due to what Sildanchandra & Crane (2000) said about the increasing appearance of developmental abnormalities and decreased hatchability. We could expect than negative effects on the survival of the species in long term, specially in a scenario where air pollution is getting, over time, stronger (concentration of particulate air matter pollution and other components of air pollution).

Additionaly, the reduction of the weight of the pupa with the increasing concentration of the PTS suggests that the adults would probably have a reduced size and it can also be expected a reduced fecundity. Comparing these effects observed by Bauerfeind and Fischer (2005) and based on our observations on the survival rate and reduction in the pupal weight and especulations about the fecundity and natality rates it could be expected that *H. ethilla* would suffer the same effects observed by Bauerfeind and Fischer (2005) and by Sildanchandra & Crane (2000). Consequently, our results and especulations allied with the observations of these other authors could indicate a reduction in the population size of the species and consequent decrease in the chances of long-term survival due to the increase in mortality at different stages of life and probable reduction in the reproduction and natality rates. Mulder et al. (2005) show that the butterflies and their host plant species have low tolerance to pollution and that there is a correlation between the sensitivity of the butterflies and their hosts. Additionaly, these same authors enphasised that there could be coexistence of indirect effects of the pollutants on the adult buterflies and a direct effect of xenobiotics on the larvae. That's what *H. ethilla* could be faccing.

Conclusion

With this study we can conclude that the PTS had significant negative effects on the population of the species model which showed reduction in body size and lower survival. Although we do not use fecundity and reproductive rate in our study, based on the observation of other authors and comparing with what we have in our study with *H. ethilla* we could expect a reduction in the fecundity and natality rate. It can also be concluded that in the long term *H. ethilla* could be facing extinction specially due to a predictable scenario were historically human interventions are getting increasingly intense in the environment. This study is pioneer in studying the effects of PTS in a butterfly species and what could happen in the future with increasing air pollutants emmitions, in this particular case the PTS emmition. Tt is importante though to conduct more studies with also other model species of urban buterflies so that we can be ready to face the chalenges that are to come. Conservation actions will be

extremely necessary to protect the species, not only of Butterflies but also any other species that have any degree of low tolerance to the urbanization and its consequences in the environment.

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Appendix

Fig. 1 Egg of *H. ethilla narcaea* Godart, 1819 (Dell' Erba et al. 2005) Bar = 0.3mm

Fig 2 Fifth instar larvae (L5) of *Heliconius ethilla narcaea* Godart, 1819 (Silva & Moreira in preparation) Bar = 2.5 mm

Fig 3 Pupa of *Heliconius ethilla narcaea* Godart, 1819 (Silva & Moreira in preparation) Bar = 2 mm

Fig. 4 Dorsal and ventral views respectively of an adult of *H. ethilla narcaea* Godart, 1819 (Silva & Moreira in preparation) Bar = 1 cm

Table 1. Life Table of *H. ethilla* in the treatment group in 25 ± 0,3°C and photoperiod of 12 hours (x=stage of life; S(x)=individuals in each instar or age class; l(x)=probability of survival; p(x)=probability of survival to the next phase; q(x)=probability of death; L(x)= proportion of the population that lived until phase x; T(x)=proportion of the population that lived until phase x and in all subsequente phases; e(x)=life expectancy

x	S(x)	l(x)	p(x)	q(x)	L(x)	T(x)	e(x)
Egg	69	1.000	1.000	-	1.000	4.284	4.284
L1	69	1.000	1.000	-	1.000	3.284	3.284
L2	69	1.000	1.000	-	1.000	2.284	2.284
L3	69	1.000	1.000	-	0.689	1.284	1.284
L4	26	0.377	0.624	0.376	0.312	0.595	1.578
L5	17	0.246	0.619	0.381	0.203	0.283	1.150
Pupa	11	0.159	0.594	0.406	0.080	0.080	0.503

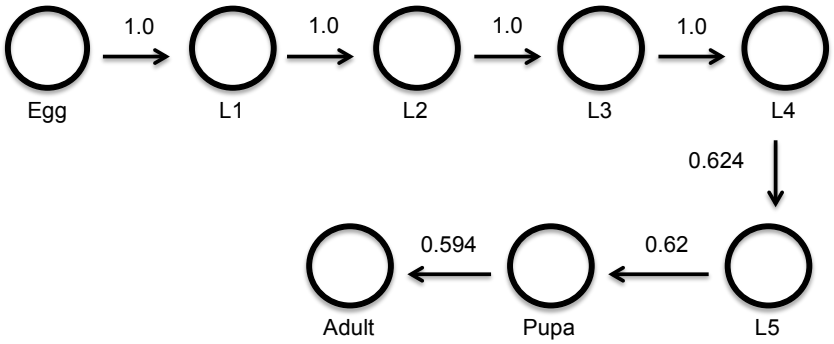

Fig. 5 Graph of the life cicle of the treatment group from egg to adult of *H. ethilla*

Table 2. Life Table of *H. ethilla* in the control group in 25 ± 0,3°C and photoperiod of 12 hours (x=stage of life; S(x)=individuals in each instar or age class; l(x)=probability of survival; p(x)=probability of survival to the next phase; q(x)=probability of death; L(x)= proportion of the population that lived until phase x; T(x)=proportion of the population that lived until phase x and in all subsequente phases; e(x)=life expectancy

x	S(x)	l(x)	p(x)	q(x)	L(x)	T(x)	e(x)
Ovo	3255	1.000	0.261	0.739	0.631	1.864	1.864
L1	850	0.261	0.908	0.092	0.249	1.233	4.724
L2	772	0.237	0.948	0.052	0.231	0.984	4.152
L3	732	0.225	0.941	0.059	0.218	0.753	3.347
L4	689	0.212	0.961	0.039	0.208	0.535	2.524
L5	662	0.203	0.831	0.169	0.186	0.327	1.611
Pupa	550	0.169	0.671	0.329	0.141	0.141	0.834

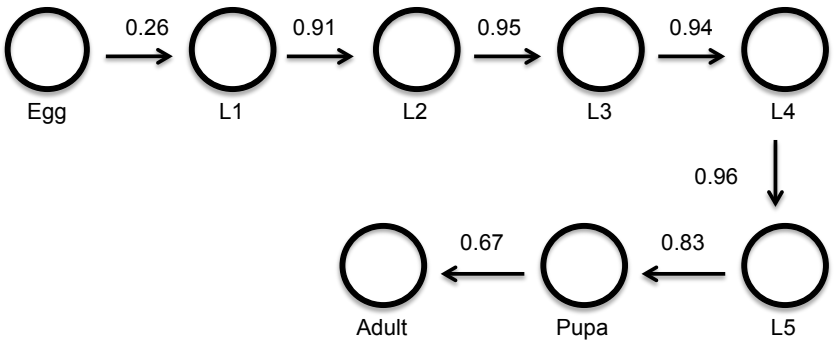

Fig. 6 Graph of the life cicle of the control group from egg to adult of *H. ethilla*

Table 3. Time of development in days (H) of the life cycle of *H. ethilla* in each phase from the treatment group in 25 ± 0.3°C and photoperiod of 12 hours

Phase	H ± SD*
Egg	3.28 ± 0.98
L1	3.27 ± 1.03
L2	2.63 ± 0.91
L3	3.40 ± 0.95
L4	3.82 ± 1.05
L5	5.55 ± 1.19
Pupa	11.64 ± 1.15
Complete Cycle	33.59 ± 3.17

*Standard Deviation

Table 4. Time of development in days (H) of the life cycle of *H. ethilla* in each phase from the control group in 25 ± 0.3°C and photoperiod of 12 hours

Phase	H ± SD*
Egg	3.95 ± 1.04
L1	2.31 ± 0.68
L2	2.38 ± 0.98
L3	2.75 ± 1.00
L4	3.17 ± 1.02
L5	4.44 ± 1.10
Pupa	10.27 ± 1.09
Complete Cycle	29.27 ± 2.17

*Standard Deviation

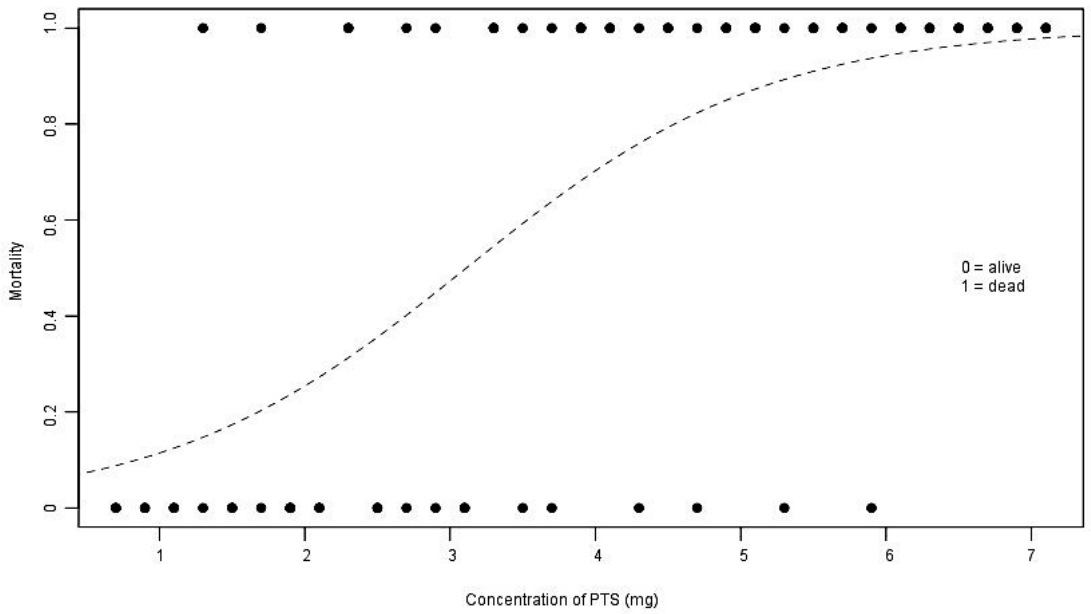

Fig. 7 Graph of the mortality of *H. ethilla* with the increasing concentration of PTS

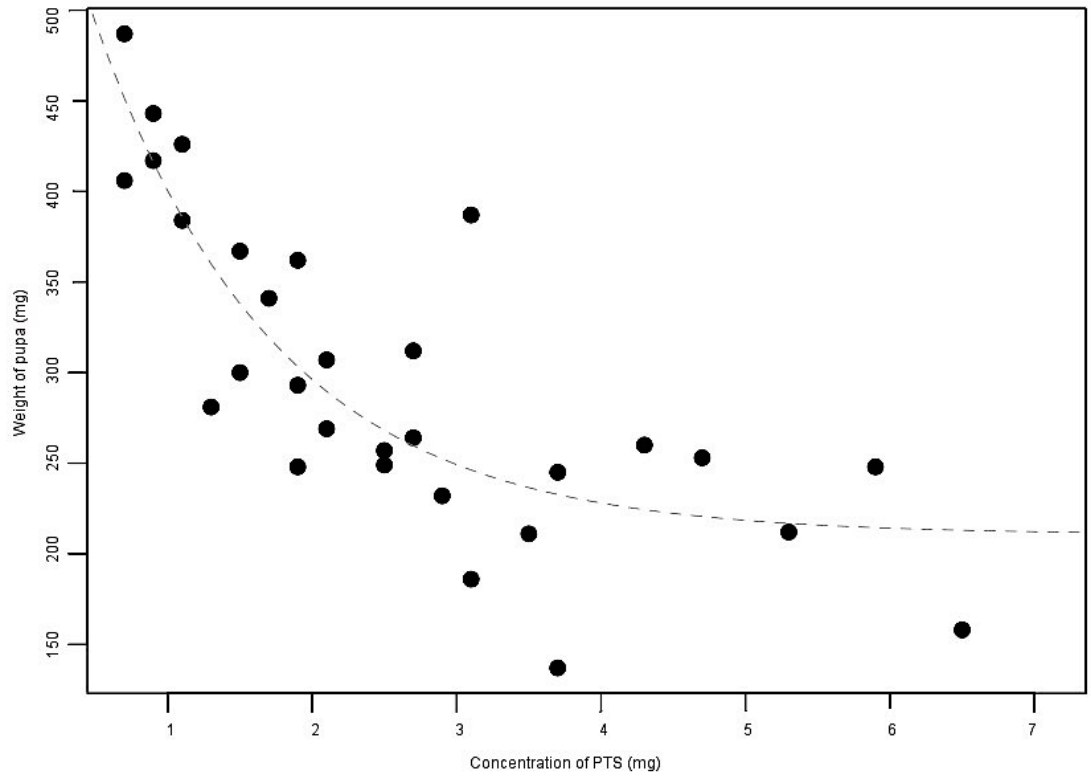

Fig. 8 Graph of the weight of ths pupas of *H. ethilla* with the increasing concentration of PTS

Table 5. Results of the negative exponencial function of three parameters (a, b and c) adjusted to the data. Each parameter has an estimative, standard error, t-value and p-value

Parameter	Estimative	Standard Error	t-value	p-value
a	210.6135	25.5130	8.255	7.31e-09
b	-420.4281	85.1168	-4.939	3.58e-05
c	0.7961	0.2609	3.051	0.00507