**SOFIA PIMENTA DE OLIVEIRA**

# EFFECTS OF MALE QUALITY ON THE REPRODUCTION OF *Tuta absoluta* (LEPIDOPTERA: GELECHIIDAE)

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

Orientador: Eraldo Rodrigues de Lima

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Eraldo Rodrigues de Lima
Orientador

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*"O correr da vida embrulha tudo.*
*A vida é assim: esquenta e esfria,*
*aperta e daí afrouxa,*
*sossega e depois desinquieta.*
*O que ela quer da gente é coragem."*
(Guimarães Rosa)

# ABSTRACT

OLIVEIRA, Sofia Pimenta, M.Sc., Universidade Federal de Viçosa, January, 2020**. Effects of male quality on the reproduction of *Tuta absoluta* (Lepidoptera: Gelechiidae).** Adviser: Eraldo Rodrigues de Lima.

Among several studies, there have been extremely interesting questions related to the reproductive aspects of many species. This occurs basically because reproduction itself, show different effects upon the mating system, for both male and female. Male ejaculate plays a key role in female reproduction and therefore to their offsprings. It is generally assumed that male age, male weight, and male mating status is a potentially important aspect affecting the mating biology of insects and causing many effects on female fitness. Differences related to the contribution of male ejaculate donation for females upon mating have been observed in several Lepidoptera species. In a laboratory experiment we examined the male condition and how it could impact female longevity and reproductive output. The parameters related to the male condition observed, were male pupal weight, male age (2, 4 and 6 days old) and male mating status (1, 2, 3 and 4 matings). We recorded for each female who has mated with these males, parameters such as fecundity, fertility, and longevity. We found that females who have mated to males of different ages, showed a decrease in fertility, fecundity, and longevity as the males get older. Also, the same pattern was observed for male mating status, whereas females' fecundity, fertility, and longevity decreased as the numbers of her previous partner increased. The male pupal weight did not show any effect upon fecundity and female longevity. However, it was significant for female fertility, which increases according to the increase in male pupal weight. These results demonstrated clear evidence of how the male condition impacts female longevity and reproductive aspects, highlighting the critical dependence of *T. abosluta* females upon their male partner condition.

Keywords: Reproduction. Lepidoptera. Male condition.

# RESUMO

OLIVEIRA, Sofia Pimenta, M.Sc., Universidade Federal de Viçosa, janeiro de 2020. **Efeito da qualidade do macho na reprodução de *Tuta absoluta* (Lepidoptera: Gelechiidae).** Orientador: Eraldo Rodrigues de Lima.

Entre vários estudos, surgiram questões extremamente interessantes relacionadas aos aspectos reprodutivos de várias espécies. Isso ocorre basicamente, porque sistemas reprodutivos demonstram efeitos diferentes no sistema de acasalamento, tanto para machos quanto para fêmeas. O ejaculado masculino desempenha um papel fundamental na reprodução feminina e, portanto, para a prole. É geralmente assumido que a idade, o peso e o status de acasalamento masculino são aspectos potencialmente importante que afeta a biologia de acasalamento de insetos e causa muitos efeitos no fitness da fêmea. Diferenças relacionadas à contribuição da doação de ejaculado masculino para fêmeas após o acasalamento foram observados em várias espécies de Lepidoptera. Em um experimento de laboratório, examinamos a condição do macho e como ela poderia afetar a reprodução da fêmea assim como sua longevidade. Os parâmetros relacionados à condição do macho observados foram peso da pupa do macho, idade do macho (2, 4 e 6 dias) e o numero de cópulas do macho (1, 2, 3 e 4 cópulas). Para cada fêmea acasalada com estes machos, foi analisado parâmetros como fecundidade, fertilidade e longevidade. Como resultado, obtivemos que fêmeas que acasalaram com machos de idades diferentes, demonstraram uma diminuição na fecundidade, fertilidade e longevidade a medida que os machos envelhecem. Para os diferentes status de acasalamentos, foi observado nas fêmeas novamente uma diminuição da fecundidade, fertilidade e longevidade a medida que o numero de cópulas do macho aumenta. O peso da pupa do macho não apresentou efeito sobre a fecundidade e longevidade feminina. No entanto, houve um efeito significativo para a fertilidade da fêmea, que aumentou de acordo com o aumento do peso da pupa do macho. Esses resultados demonstraram evidências claras de como a condição do macho afeta a longevidade da fêmea assim como seus aspectos reprodutivos, destacando a dependência crítica das fêmeas de *T. absoluta* em relação a condição macho.

Palavras-chave: Reprodução. Lepidoptera. Condição do macho.

# SUMMARY

## 1. Introduction

Reproductive systems are described and reported for many different species. It takes place mainly on the fundamental transference of sperm to females, which have many different effects on female and offspring fitness (Arnqvist and Nilsson, 2000). In recent years, mating behavior is a tool to examine how ecological circumstances constrain or promote sexual selection and the evolution of different mating systems (Hughes *et al.* 2000).

Lepidoptera species are remarkably used as a model to study the mechanism and effects of reproduction, not only because it is relatively easier to obtain compared to other holometabolous insects, but also because of its ecological and agricultural importance (Gilbert *et al.* 1972). Also, in Lepidoptera, we can directly observe the male contribution to females' reproductive output because they are known as income breeders (Gilbert *et al.* 1972). Income breeders mean that their reproductive output is determined not only by the larval-derived resources but also by resources acquired during the adult stage. These adult resources can be the result of either female feeding or of females having received nutrients from males during mating (Boggs & Gilbert, 1979).

The nutrients received by females upon mating are placed with the sperm in a spermatophore that is formed in the female's reproductive tract during copulation. They contain numerous substances that are produced by reproductive accessory glands, which include sperm, seminal fluid proteins, compounds with an immunostimulant, and antibiotic properties (Gillott, 2003).

When transferred to the female, this complex of materials is likely to cause a variety of fitness implications including physiological and behavioural effects for example by reducing the likelihood of re-mating, stimulating egg production and oviposition, initiating sperm storage and/or release, sperm management and sperm competition, altered morphology of the female reproductive tract and shortening lifespan (Chapman, 2008).

Some studies have shown the effects of male condition on their delivered substances and how it could impact female reproductive output. Male age is directly related to different phases of maturation, and it is generally assumed that age is an important aspect affecting the mating biology of insects and that females use age as a reliable signal of male quality. Therefore by mating with older males, they have more chances to gain indirect benefits (Jones and Elgar, 2004). Within the age, male ejaculate quality is expected to decrease as the male gets older, and the decrease of sperm quality could harm female fitness (Eberhard and Cordero, 1995). Morais *et al.* (2012) showed that females of *Grapholita molesta* (Lepidoptera:

Tortricidae) mated with younger males displayed higher fertility and fecundity. Also, a study done with *Ostrinia nubilalis* (Lepidoptera: Crambidae) demonstrated the decrease of female fecundity, fertility, and longevity by mating with an older male (Milonas & Andow, 2010).

Not only male age can influence reproduction, but also male mating history strongly affects male ejaculate and, consequently, female reproductive output in Lepidoptera (Hughes *et al*., 2000). Hughes *et al.* (2000) demonstrated that after the ejaculate on the first mating, the weight decreased by nearly 30% at the second mating in the *Jalmenus evagoras* (Lepidoptera: Lycaenidae). This decrease had a direct effect on female *J. evagoras* lifespan, which significantly reduce as the number of previous matings of her male partner increased. Torres-Vila and Jennions (2005) demonstrated that in 22 of 25 lepidopteran species, the reproductive output of females mated to virgin males was higher than that of females mated to experienced males.

In Lepidoptera, pupal weight or insect weight is also an essential factor in reproduction. In a range of insects, its explicit that the fecundity and fertility of females are proportional related to their weight and male pupal weight (Calvo and Molina, 2005).

The study insect of this project is the tomato leafminer, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). Females from this species can mate up to six times during their lifespan, with a single mating lasting about 4h to 5h (Hickel *et al*., 1991, Desneux *et al*., 2010, Lee *et al*., 2014). It is one of the most damaging pests of solanaceous crops, especially in South America, its original area, as well as in newly invaded regions. The moth preferentially attacks tomato under both field and greenhouse conditions, showing a high reproductive potential that allows the pest population to increase very quickly (Pereyra and Sanchez, 2006). By doing so, *T. absoluta* exhibits a high capacity to develop in agroecological environments, spreading rapidly in new areas and causing economically relevant damage (Guedes & Picanço, 2012, Siqueira *et al*., 2000).

Among several methods to control this critical pest, the chemical method have been the main control tactic, carried out almost exclusively, with applications in high doses and high frequency of pesticides (Medeiros *et al.* 2006). However, the indiscriminate and excessive use of these substances over a long period contributed to the selection of resistant populations (Siqueira *et al.* 2000, Picanço *et al*., 1998, Guedes & Picanço, 2012). The use of pheromone traps could be an essential tool in the field and greenhouse conditions as a way to decrease the population of this insect. But, as found by Lôbo (2005) in experiments carried out with synthetic sexual pheromone for mass trapping behavioral control of *T. absoluta*, it was observed that despite the high rate of male suppression, losses in production remained above the economic injury level.

Many reasons could be associated with such limited efficacy control. One of the proximate reasons might be related to the lack of knowledge on *T. absoluta* reproductive performance concerning male condition, once males of this species can have a high reproductive capacity. Therefore, the present study aimed to fill some gaps in *T. absoluta* reproductive aspects. We hypothesized that *T. absoluta* female that will mate with an older virgin male will show a lower fecundity, fertility, and longevity compared to the female that will mate with younger virgin males. Also, we hypothesized that as male mating increases through successive mating, female reproductive aspects and longevity are expected to decrease.

## 2. Material and Methods

### 2.1 Insect Rearing

The colonies of *Tuta absoluta* were obtained from an existing colony, maintained at the Laboratório de Manejo Integrado de Pragas da Universidade Federal de Viçosa, Minas Gerais (Brasil).

The colonies were kept in a rearing room with a temperature of 25 ± 2ºC, 12 h photophase and 70 ± 5% relative humidity. Eggs were placed in wooden cages covered with organza canvas (30x20x30cm), and the hatched larvae were provided with fresh tomato leaves (cv. Santa Clara) previously placed in a glass jar of water whenever necessary new leaves were inserted into the cage.

After the larval phase, the pupae were handly removed from tomato leaves and placed in Petri dishes and inserted into new cages. Emerging adults were fed 10% sucrose and provided with tomato leaves for egg-laying. Leaf stems were cut and placed through a hole in the transparent glass jar of a 500-ml recipient containing water serving as an oviposition site.

The oviposition was removed every 24h and new leaves placed in the adult cage. Sugar was periodically changed every three days. The eggs were again put into a wooden cage to continue the insect life cycle (Proffit *et al*., 2011).

### 2.2 Plant Material

Tomato plants (*Solanum lycopersicum*, cv. Santa Clara) were used in insect rearing and experiments. The plants were obtained from sowing in plastic trays containing a substrate for vegetables. The seedlings, with two final leaves, were transplanted to 3L pots containing MecPlant® soil conditioner. Macronutrients were provided every seven days using NPK (10:10:10) as per manufacturer specifications. The plants were grown in a greenhouse with a temperature of 25 ± 2ºC and relative humidity of 70 ± 5% (Bogorni *et al*., 2006).

### 2.3 Sex differentiation and Weight Standardization

Both *T. absoluta* female and male pupae were individualized, and sex differentiation was done based on genital pore location according to the descriptions and drawings of Coelho & França (1987) using a stereomicroscope Leica EZ4®. After sex differentiation, both male and female pupal masses were weighed using an analytical balance Shimadzu® AUW220D. Female pupal mass was standardized by previously weighed 55 female pupae to estimate the weight range used in the experiments. By calculating the standard deviation of each female weight collected, the female weight range used in the experiments was = 2.5 ± 0.5 mg

Male pupal masses were weighed and collected but were not standardized, and each of the male pupal mass was used in the statistical analyses. Pupal weight was made according to the methods performed by Muller *et al*., 2016.

All the female and male pupal weight were accessed, and each of the pupae sexes was labeled with numbers with their respective weights (ex: female 1, female 2, male 1, male 2 and so on) and placed individually into a 70mL plastic tube in a room with 25 ± 2ºC,70 ± 5% RH and 12L:12D photoperiod.

### 2.4 Influence of male age in the female reproductive parameters

To test whether female reproductive parameters such as longevity and reproductive output were influenced by male age, virgin males from different ages (2, 4, and 6-days old) were mated to virgin females of 2-3 days old. After weighting male and female pupae were individually placed into a 70mL plastic tube covered with organza fabric on top.

It was placed outside of each tube a number of which corresponds to both the male pupae, female pupae, and pupal weight, respectively, in order to maintain the organization of the couples being formed during the experiment (ex: male one mated female 5).

To separate males of different ages, the tubes containing the male pupae were placed in a separate room from the female pupae (25 ± 2ºC, 70 ± 5% RH and 12L:12D photoperiod). Upon adult emergence, the males were separated according to their respective ages. Thus, adult male who has reached 2, 4, and 6 days old were placed in another room, where the females were kept (25 ± 2ºC, 70 ± 5% RH and 12L:12D photoperiod) to mate virgin females of 2-3 days old. After the copulation, females were removed from containers and kept isolated, with access to water with 10% sucrose, a tomato leaflet for oviposition.

A total of n=69 females was used in this experiment, of which n=25 were for females that mated with 2 day old males, n=23 were for a female that mated with 4 days old males and n=21 for a female that mated with 6-day old males.

## 2.5 Influence of male mating status in the female longevity and reproductive output

To test whether female longevity and reproductive output are influenced by male mating status, virgin females of 2-3 days old were offered to copulate with virgin males, to males that had previously mated twice, three times and four times in their lifetime.

Thus, to separate males of different mating status, the tubes containing the male pupae were placed in a separate room from the female pupae (25 ± 2ºC, 70 ± 5% RH and 12L:12D photoperiod) and upon adult emergence, a certain amount of the virgins male were offered to copulate with a virgin female and after copulation ended the reproductive output and longevity parameters of those females was counted.

To complete males second mating, another group of virgin males was also offered to groups of virgins female, but after the first male copulation, these same group of the males who had previously mated once was offered a different group of virgins female, which after the second copulation had ended the reproductive output and longevity parameters of those females was counted. To reach males third mating, a different group of virgin males was offered to copulate with different new groups of virgin female three times, of which at the third mating the reproductive output and longevity parameters of those females were counted. The same was done for the male that had mated four times. Each time, these groups of males were offered a new group of virgin females until these males had completed 4 matings. In the fourth mating, the reproductive output and longevity parameters of those females were counted. The pupae were weighed as descibred above. The females that copulated were isolated in plastic tubes (70mL) containing cotton with 10% sucrose and a tomato leaflet serving as an oviposition site inserted into a 1.5mL glass vial full of water.

Males and females were placed in different rooms with 25 ± 2ºC, 70 ± 5% RH and 12L:12D photoperiod. A total of n=62 females was used in this experiment, of which n=23 were for females that mated with a virgin male, n=12 were for a female that mated with two times mated males, n=14 for a female that mated with three times mated males, n=13 for females that mated with four times mated males. This experiment was done accordingly to Hughes *et al*., (2000).

### 2.6 Fecundity effects of male age and mating status

To determine the effect of male age and mating status on female fecundity, egg production were measured daily for females exposed to virgin males from different ages (2, 4, and 6-days old) and male that had previously mated (1, 2, 3, and 4 times). After mating in each of the categories, the females were isolated in 70 mL containers containing 10% sucrose solution, and a tomato leaflet deep in water for oviposition. Egg counts were made along with tomato leaflet replacement every day until the females were ten days after eclosion. This experiment was carried out in a room with a 25 ± 2ºC, 70 ± 5% RH, and 12L:12D photoperiod. All the procedure was done following Morais *et al*., (2012).

### 2.6.1 Fertility effects of male age and mating status

To determine the effect of male age and mating status on female fertility, egg fertility (% hatching) was counted. The leaflets collected previously were placed in Petri dishes under a humid filter paper. After four days, the number of larvae hatched was counted. Eggs that remained white, with no signs of embryo development after eight days or eggs that showed evident signs of dehydration or malformation, were considered unviable.

### 2.6.2 Female longevity under male age and mating status

To demonstrate female longevity under male age and mating status influence, the same females used in fecundity test who previously mated with males of different ages (2, 4 and 6 days old) and previous mating status (1, 2, 3 and 4) were left until the days they died. Longevity was measured as the number of days until the female died. This experiment was carried out in a room with 25 ± 2ºC, 70 ± 5% RH and 12L:12D photoperiod.

### 2.6.3 Statistical Analyses

All the statistical procedures were performed in R version 3.5.3 and R Studio version 1.2.5001 (R Core Team 2017). We studied the relationship between the fecundity, fertility, and longevity of females (dependent variables) and the explanatory variables (age and male pupal weight for virgin males; and mating status and male pupal weight for mated males). Data on fecundity and fertility were checked for Poisson and negative binomial distributions (i.e., discrete distributions for count data) through visual inspection of theoretical and empirical distributions (function 'fitdist', package fitdistrplus, Delignette-Muller and Dutang, 2015). Since the negative binomial distribution was the most suitable, generalized linear models (GLM) with negative binomial distribution and log-link function (function 'glm.nb', package MASS) were fitted. For the variable fertility, GLMs with binomial distribution (appropriate for proportional data) and logit-link function were fitted using number of eggs as weight. The final models were checked for patterns in the residuals plotted against fitted values to confirm the error distribution and the model suitability.

## 3. Results

### 3.1 Virgin male fecundity

The male age (df=1.64. *z*= -4.552. P<0.001) significantly affected female fecundity, causing a 3-fold reduction in the number of eggs laid per female (Fig 1) . Conversely, male pupal weight (df= 1,64, *z*= 1.495, P= 0.06735) did not affect female fecundity.

### 3.2 Virgin male fertility

Both male age (df= 1.64. *z*= -7.421. P<0.001) and male pupal weight (df= 1.63, *z*= 5.493. P<0.001) affected the number of emerged larvae. The fertility of *T. absoluta* females is predicted to decrease from 0.77 to 0.59 when male age increases from 2 to 6 days (Fig. 2A). Conversely, male pupal weight had a positive effect on fertility. The fertility of *T. absoluta* females is predicted to increase from 0.60 to 0.84 when pupal weight is incremented from 1.52 to 3.51 mg (Fig. 2B).

### 3.3 Female longevity

The male age (df= 1.63, *z*= -4.069. P<0.001) had a significant negative effect on female longevity. Conversely, male pupal weight (df=1.64, *z*= -1.747. P=0.06311) was not related to female longevity. Females mating with older males (6-days old) lived 2.4 fold less compared to those mating younger males (2-days old) (Fig. 3).

### 3.4 Mated male fecundity

The male mating status (df=1.63, *z*= -3.795. P<0.001) had a significant negative effect on female fecundity. Conversely, male pupal weight (df= 1.63. *z*= 1.056. P= 0.06735) was not related to female fecundity. Females mating more experienced males (4 matings) laid 58% less eggs compared to those mating less experienced males (1 mating) (Fig. 4).

### 3.5 Mated male fertility

Both male mating status (df=1.60, *z*= -9.888. P<0.001) and pupal weight (df=1.59, *z*= 3.885. P= 0.003263) affected the number of emerged larvae. Females mating more experienced males (4 matings) presented a reduction of 31% in fertility, compared to those mating less experienced males (1 mating) (Fig. 5A). Conversely, male pupal weight had a positive effect on fertility. The fertility of *T. absoluta* females is predicted to increase from 0.64 to 0.78 when pupal weight is incremented from 1.53 to 3.47 mg (Fig. 5B).

### 3.6 Female longevity

The male mating status (df= 1.59. *z*= -3.706. P= 0.0001439) had a significant negative effect on female longevity. Conversely, male pupal weight (df= 1.60. *z*= -1.781. P= 0.074912) was not related to female longevity. A decrease in female longevity from 13.15 to 5.80 days occurs when the male mating status increases from 1 to 4 matings (Fig. 6)

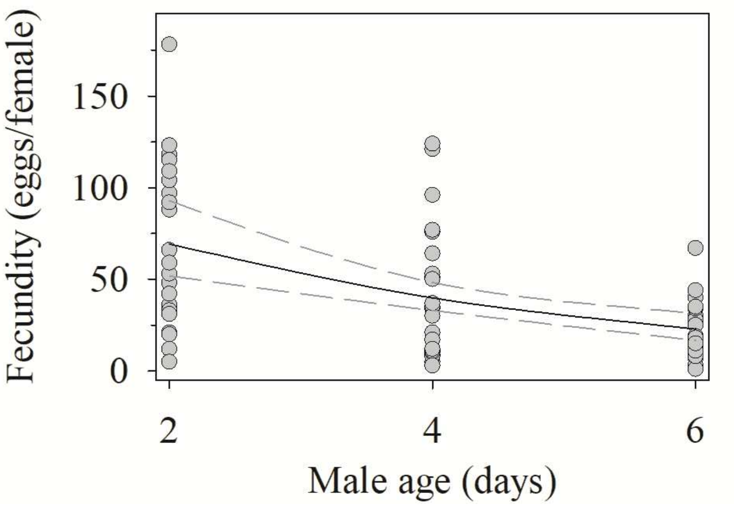

**Fig 1.** Fecundity (number of eggs laid during 10 days) of *Tuta absoluta* females mating with males of different ages (2, 4, and 6-days old). Generalized linear models assessing the importance of virgin male age on female fecundity. Dashed lines represent $CI_{95\%}$.

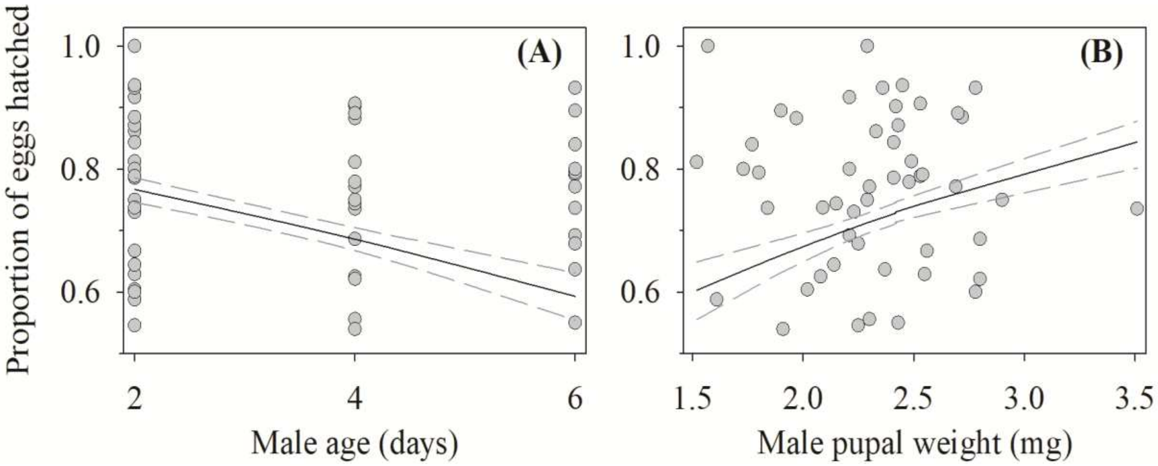

**Fig 2.** Fertility (proportion of eggs hatched) of *Tuta absoluta* females regressed against **(A)** male age (2, 4, and 6-days old) and **(B)** male pupal weight. Generalized linear models assessing the importance of predictors (male pupal weight and age of virgin males) on fertility. Dashed lines represent $CI_{95\%}$.

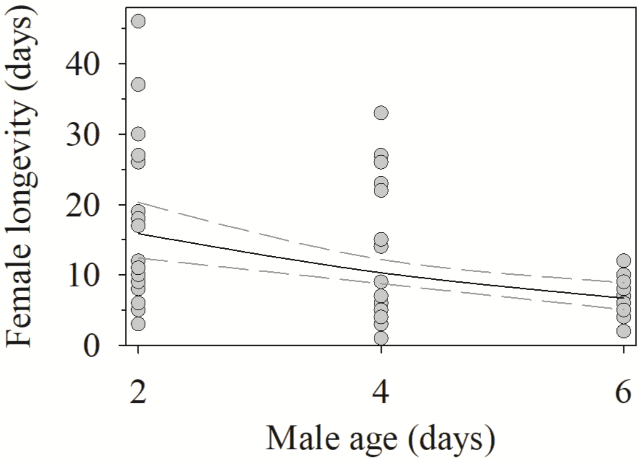

**Fig 3.** Longevity (number of days until death) of *Tuta absoluta* females mating with males of different ages (2, 4, and 6-days old). Generalized linear models assessing the importance of virgin male age on female longevity. Dashed lines represent $CI_{95\%}$.

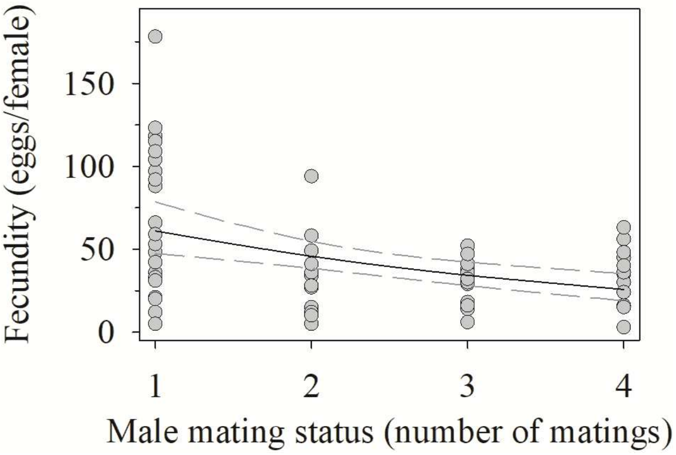

**Fig 4.** Fecundity (number of eggs laid) of *Tuta absoluta* females mating with males of different mating status (1, 2, 3, and 4 matings). Generalized linear models assessing the importance of male mating status on female fecundity. Dashed lines represent $CI_{95\%}$.

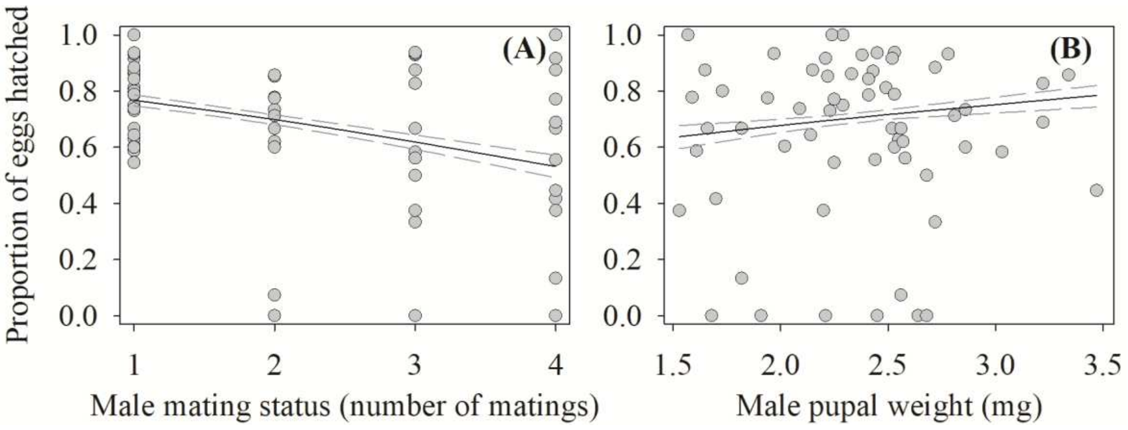

**Fig 5.** Fertility (proportion of eggs hatched) of *Tuta absoluta* females as a function of **(A)** male mating status (1, 2, 3, and 4 matings) and **(B)** male pupal weight. Generalized linear models assessing the importance of predictors (male pupal weight and male mating status) on female fertility. Dashed lines represent $CI_{95\%}$.

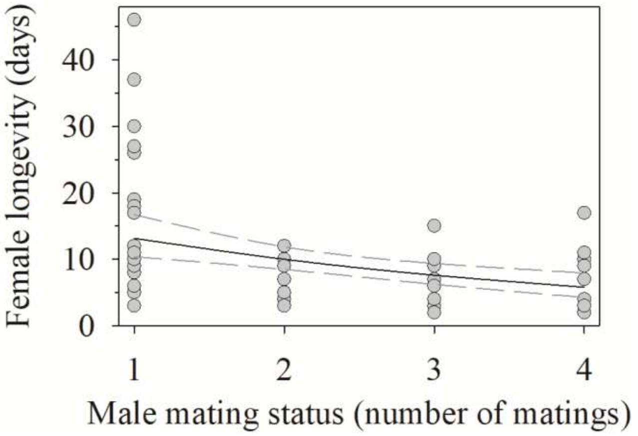

**Fig 6.** Longevity (number of days until death) of *Tuta absoluta* females as a function of male mating status (1, 2, 3, and 4 matings). Generalized linear models assessing the importance of male mating status on female longevity. Dashed lines represent $CI_{95\%}$.

## 4. Discussion

Our results demonstrate that mating with younger males is advantageous for the reproductive success and longevity of *T. absoluta* females, as females that mated with older compared to younger showed (1) decreased in fecundity (2) reduction in hatching success of eggs and (3) a shorter lifespan.

These findings are in agreement with many studies of other insect species which have shown that mating with an older male may decrease female fitness overall, including reproductive success and lifespan (Kotiaho & Simmons 2003; Martin & Hosken 2004; Paukku & Kotiaho 2005). Milonas and Andow (2010) found that for *O. nubilalis* (Lepidoptera: Crambidae) female fitness was affected by the male age at mating. Therefore, females that mated with younger virgin males had higher fecundity and longer life spans. Also, females that mated with older males had not only reduced fecundity but also decreased longevity (Milonas and Andow, 2010).

With this, age is often presumed to affect mating biology, mostly because in many invertebrates, and especially in holometabolous insects, physiological condition (body mass, fat content) diminishes with age (Karlsson 1994; Kemp 2002; Fischer *et al*. 2008). However, this is not always the case. Other studies have shown that for *Dermestes maculatus* (Coleoptera: Dermestidae) older males showed high female fertility and egg production compared to younger males. Such effect happened because of the differences in the quantity of sperm transferred between males, which younger males transferring significantly lower numbers of sperm to a female than older males (Jones & Elgar, 2004).

In Lepidoptera, while mating, males transfer nutricious substances to females, such as the male accessory gland secretions that are placed with the sperm in a spermatophore that is formed in the female reproductive tract during copulation. There are several reports showing that male spermatophore contains a variety of materials, which include proteins, hydrocarbons, triglycerides, sterols, and phospholipids (Bissoondath & Wiklund 1995; Karlsson 1995). These products may substantially contribute to female longevity and/or reproductive effort (Boggs & Gilbert 1979; Wedell 1996; Karlsson 1998).

Some males are competent in producing a large spermatophore, therefore, being considered of superior physiological state. Males of the superior physiological state generally obtain a higher proportion of matings (Thornhill Alcock 1983). However, although larger spermatophores and their materials may function to increase female fecundity (Cook & Wedell

1996; Marcotte *et al*. 2005; Wedell & Ritchie 2004), sperm number and possibly also sperm quality (Kehl *et al*., 2015) it is expected to decrease with age.

Some studies have shown that antioxidant defenses decreased with age in male *Bicyclus anynana* (Lepidoptera: Nymphalidae) and older males may have reduced sperm quality (Aitken *et al*. 2014; Kehl *et al*. 2015). Those reductions should have negative effects in reproductive success and female longevity. This explanation could play a key role in our results as well, once we have demonstrated that females who mated with an older male had a significantly decrease in reproductive parameters and longevity.

With this parameters described above, it is most likely to expected that if a female who shows losses of fitness while mating with an older male, this female should develop some adaptations to, therefore, discriminate a male based on its age. However, if the lack of such a discriminating mechanism happens, it could suggest that the direct benefits do not drive mating preferences for the male age (Milonas and Andow, 2010). Thus, as we have seen strong age-related effects on female fitness in this system, we expect that in a natural condition, *T. absoluta* females will choose younger males over older males to mate.

Our findings also show that the reproductive success of *T. absoluta* females is affected by male mating history. A reduction in fecundity, hatching success of eggs and lifespan was observed in females that mated with males that had prior successive matings in comparison with females that mated with virgin males. Besides the cumulative evidence of the effect of male mating history our results support the hypothesis that males experience a depletion of spermatophore precursors, nutrients and size after each mating (Torres-Vila and Jennions, 2005).

Oberhauser (1997) noted that most species in which spermatophore depletion has been shown to affect fecundity and fertility are polyandrous. *Tuta absoluta* females has been described as having a high polyandry level according to Wedell *et al*. (2002). Such assumption is directly related to the level of dependence of females on male-derived nutrients. Lee *et al*. (2014) have demonstrated a clear relationship of *T. absoluta* female on male-derived nutrients, basically because most of the females that had multiple matings with virgin males compared to those who have only mated once showed a higher reproductive output and longevity. This demonstrated a positive effect of polyandry in this species and also that polyandry increases reproductive output, whereas polygyny decreases female reproductive output, as shown in our study.

Lôbo (2005) found that for *T. absoluta* males, there is a decrease of 40% in the volume of spermatophore produced by males from the first to the second mating. The same pattern was showed in other studies in which the spermatophore volume had a direct effect on female fertility. For instance, Jiménez-Pérez and Wang (2004) demonstrated that the spermatophore volume of *Cnephasia jactatana* (Lepidoptera: Tortricidae) males transferred in successive copulations decreased 45% reflecting a decrease in female reproductive output of this species.

Seeing the importance of male-derived nutrients in female fitness, we have also found a negative influence of successive male mating in *Tuta absoluta* female longevity. A similar relationship between female longevity and male mating history has been found in *Colias eurytheme* (Lepidoptera: Pieridae) (Rutowski *et al*. 1987). It has been described that the mechanism by which female lifespan is affected by multiple matings of males may be related to the amount of male-derived nutrients transferred through the spermatophore while mating. Therefore, small spermatophores from multiple mated males might decrease male nutritional donations, which in turn affect females' longevity negatively.

Male Lepidoptera produces two types of sperm, the functional eupyrene sperm with a nucleus, which is able to fertilizing the eggs and the non-functional apyrene sperm, which lacks a nucleus (Friedlander 1997). The ratio of eupyrene sperm may decrease in successive matings, which may be related to the reduced fertility of females mated with successive mated males (Kehl *et al*., 2015).

With this discussion, based on male age and mating status, we can conclude that our data on female reproductive output suggest that either older and previously mated males produce ejaculates of more inferior quality compared with young virgin males. As described before, spermatophore from young virgin male may be more rich in resources being of critical importance to females (South & Lewis 2011, 2012). Thus, the higher reproductive output found for females having mated with young virgin males may be caused by using resources transferred by the males upon mating, which may be less abundant in older and previously mated males.

Finally, we have found no significant correlation of male pupal weight with total eggs laid and also with life span of females. However, male pupal weight had a positive effect on female fertility. This might suggests that heavier pupae may lead to emergence of larger moths and that females chasing for a mating partner may show better reproductive output by mating with heavier males. Also, heavier males produce a bigger spermatophore compared to small ones, and it could therefore play a role in the positive reproductive output in females (Bissoondath *et al*., 1995, Fenemore *et al*. 2010).

In conclusion, one remaining question is whether *T. absoluta* females show a preference for one of the features discussed here (male age, mating status, or weight) while searching for mates. Further research is, therefore, needed to investigate *T. absoluta* mating preferences towards the male condition. The present work brings an essential perspective of the impact of *T. absoluta* males' status on female's reproductive output. Therefore, such knowledge positively contributes to future researches and may improve the development of control strategies of this pest.

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