Biocontrol for dog strangling vine (*Vincetoxicum rossicum*): Longevity and egg maturation of *Hypena opulenta* (Lepidoptera: Erebidae)

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Abstract

Vincetoxicum rossicum is an invasive species that has been negatively impacting forests in Ontario, and Quebec over the past 40 years. This non-native species from the Ukraine is a highly competitive species capable of choking out understory plants and trees in Canadian forests. In North America this species can create monocultures, therefore decreasing species biodiversity. Both chemical and mechanical control have been used in the past to manage *V. rossicum*, however, these methods of control are not practical forms of management, especially where the spread of *V. rossicum* is wide spread. For this reason, using a classical biological control agent is the most logical next step for management, as biological control is a long term and sustainable management tool. To find a suitable agent researches went back to the Ukraine to find herbivores for *V. rossicum*. *Hypena opulenta*, a moth that feeds on *V. rossicum* was found, and extensive testing has shown that this moth is host specific for this invasive species. Feeding caused by *H. opulenta* can lead to plant mortality of *Vincetoxicum spp.* Hence the need to explore the fitness of this moth to ensure its establishment in Canadian forests. Measurements of success and fitness for insects can be determined using longevity and fecundity, as these analyses are used to better understand reproduction. For starters this study found that adult females live for 14 days, while males live for 10 days at 23°C (room temperature) and 75% relative humidity. Females also tended to weight more than males, and pupal weight can is a predictive measure for longevity. Measurements of pupal width or length can also predict longevity. It is also possible to make approximations for adult longevity using measurements of length and width of empty pupal casings, therefore making it possible to use these measurements for field populations as an indicator for their fitness. Pupal weight cannot predict fecundity or egg size for *H. opulenta*, however, it was discovered that females develop their eggs over time and need a preoviposition period of 24 hours for their eggs to grow in both size and number. Therefore, releases can be timed as to when eggs will be fully developed.

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1. Introduction

Invasive Species

Invasive species have been wreaking havoc on many of Canadas natural ecosystems, especially the most vulnerable habitats that contain rare species. Ontario currently has the highest number of invasive species plaguing its natural habitats, and this is a result of the provinces high volume of imported goods (Nienhuis and Wilson, 2018). As a result, the province has been invaded by a slew of non-native species raging from animals and plants to diseases coming from across the globe. These non-native species have been altering Ontario's ecosystems to accelerate their invasion, which can in turn allow for an ease of entrance for other non-native species (Jeschke et al, 2014).

Forest ecosystems, in particular, are vulnerable to the threats posed by invasive species. This is due to naturogenic or anthropogenic disturbances that they face allowing for non-native species to invade these newly disturbed areas (Nienhuis and Wilson, 2018). This can result in the loss of ecological, social, or economical services that forests provide. Due to losses that invasive species can pose to forested systems ensures that this threat is one the leading causes for concern to Ontario's forest industries (Nienhuis and Wilson, 2018).

There is a long list of invasive species that currently has a negative impact on Ontario's forests, for example dog strangling vine, and purple loosestrife are just 2 of the 29 terrestrial invasive plants that can be found across Southern Ontario (Ontario's Invading Species Awareness Program, 2018). These species are outcompeting native plants, and are therefore displacing native flora and fauna (Nienhuis and Wilson, 2018). This is especially concerning for rare species that could become extinct as a result of the monocultures that are created by invasive species (Hejda, Pysek, and Jarosik, 2009).

History of *Vincetoxicum rossicum*

Vincetoxicum rossicum (Kleopow) BarBar, more commonly known as dog strangling vine (DSV) or pale swallow-wort, is a perennial vine from the Ukraine that was introduced to North America in the 1800's as an ornamental plant (Milbrath, 2008). Within the last 30 to 40 years *V. rossicum* has become established as an invasive species in several states in the USA as well as parts of Canada, in Ontario and Quebec (Young and Weed, 2014). The introduction of *V. rossicum* to North America has led to a loss of biodiversity in forested habitats, grasslands, as well as agricultural fields due to its highly competitive nature (Milbrath, 2014; Weed et al, 2010). This noxious weed creates dense monocultures, thereby displacing native understory trees and vegetation that house the local fauna.

The propensity of *V. rossicum* to displace native vegetation is especially concerning in sensitive ecosystems such as the rare alvar communities that can be found in Ontario, as they are home to a variety of rare or endangered bird and arthropod species (Douglas et al, 2009; Milbrath, 2008). Another concern to Ontario forests is the spread of this noxious weed to the Carolinian forests, where there are at least 125 threatened or endangered plant species that could be permanently displaced by *V. rossicum* (Casagrande et al, 2011). The competitive nature of *V. rossicum* is also threatening the regeneration of seedlings in woodlots, nurseries, and plantations therefore jeopardizing the income of forest managers (Anderson, 2012).

Invasive species are highly competitive in nature, by outcompeting native plants for light, nutrients, water and space (Anderson, 2012). *Vincetoxicum rossicum* is highly competitive for several reasons; 1) It is a twining vine that can physically choke out small trees and understory vegetation. These twining vines also casts shade which is not suitable for shade intolerant species (Milbrath, 2008); 2) *Vincetoxicum rossicum* have a large root biomass which allows for a it to absorb the majority of nutrients in the soil thereby preventing native species from gaining access to these resources (Gibson et al, 2015); 3) This species is highly prolific, producing a large quantity of wind dispersed seeds, or spreading via root propagation (Douglas et al, 2004; Weed and Casagrande, 2010); 4) There are no predator's native to North America that feed on *V. rossicum* (Douglas et al, 2004); and 5) This species has adapted to a variety of light and soil conditions through phenotypic plasticity (Weed et al, 2010).

In addition to the traits outlined above, *V. rossicum* has been suspected of altering the soil through allelopathic chemicals, however it has been highly debated as to whether or not this is indeed true (Weed et al, 2004; Gibson et al, 2015). If this species did carry allelopathic traits it would have to the ability to alter the soils chemical properties to better suit its own needs, thus inhibiting other plants from growing in the vicinity, as well as preventing native seeds from germinating in the surrounding soils (Gibson et al, 2015).

Methods of Control

Mechanical Control

The two most common methods for managing invasive plants like *V. rossicum* are mechanical or chemical control. Mechanical control often employs the use of volunteers or workers to dig, mow, or tarp invasive plants. These methods can be effective in the short-term and in areas where the spread of the species is relatively small (Milbrath et al, 2017b), however, this form of management must be repeated for several years to be effective and can be quite costly on a large scale when it is not a feasible solution for volunteers to handle the large infestation. For these reasons mechanical control is not a practical form of management for *V. rossicum*.

Chemical Control

On the other hand, chemical control, which employs the use of herbicides to kill unwanted plants, has been found to be a more effective method of control for invasive species over mechanical control however there are drawbacks to the use of herbicides. Chemical control is a short-term form of management and can be restrictive as licences are often required for the use of herbicides. In addition to these draw backs the process of spraying herbicides must be repeated for several years, which makes it very expensive to treat invasive species where the spread is quite vast (Milbrath et al, 2017b; Weed et al, 2010).

Biological Control

The use of mechanical or chemical control are short-term management tools to combat *V. rossicum,* and this is just one of the reasons as to why researchers investigated classical biological control to reduce this weed. Biological control is a long-term and sustainable form of control for invasive species and has been used to combat targeted species for hundreds of years

(MacQuarrie et al, 2016). It is important to note that the use of biological control agents as a form of control for *V. rossicum* cannot eradicate this weed in its entirety, however, it will be used to recreate an equilibrium between the invasive species and its predators (Warne, 2016).

As there are no herbivores for *V. rossicum* in North America, scientists went to the Ukraine where this invasive plant is originally from. In Ukraine researchers observed the native herbivores of this weed that reduced either above ground or below ground biomass of *V. rossicum.* (Weed et al, 2010). The surveyors found four potential agents for the biological control of *V. rossicum*. They found three leaf feeders *Abrostola asclepiadis, Chrysolina aurichalcea asclepiadis,* and *Hypena opulenta,* a root feeder *Eumolpus asclepiadeus*, and a seed feeder *Euphranta connexa* (Gassmann et al, 2011). *Chrysolina aurichalcea asclepiadis* and *E. asclepiadeus* were found to feed on plants native to North America and was ruled out as a biological control agent for *V. rossicum* (CABI, 2018). The leaf feeders *H. opulenta* and *A. asclepiadis* were found to be host specific for *Vincetoxicum spp.,* however tests still need to be done on *E. connexa* to determine the host specificity for this potential agent (CABI, 2018).

Hypena opulenta is of particular interest for use as biological control agent for the forest industry as this species was found to feed primarily on *V. rossicum* in forested sites (Weed et al, 2010). Due to the extensive damage that swallowworts have caused to forested habitats, it is imperative to better understand the biology of *H. opulenta* so that it may become established in Ontario and Quebec's forests.

Milbrath (2008) conducted a study on the effects of artificial defoliation on swallowworts to determine how effective defoliating insects will be at controlling this species. This study found that defoliation in shaded areas, such as a forest understory, caused mortality of the plant. Other studies by Weed and Casagrande (2010) have found that although larval feeding caused by *H. opulenta* greatly reduces the aboveground biomass, there is no impact on belowground biomass. The reduction of aboveground biomass did however have an impact in the flower production, as it reduced the plants capability to produce flowers, seedpods, and therefore the number of seeds (Weed and Casagrande, 2010; Doubleday and Cappuccino, 2011). Weed and Casagrande (2010) found that a single generation of feeding by *H. opulenta* did not cause mortality, but other studies

have found that several rounds of larval feeding could be fatal to *V. rossicum* (Milbrath and Biazzo, 2016).

Success for Biological Control

For a biological control agent to be successful it needs to follow the guidelines of best practices; 1) the agent must be efficient and safe for use and undergo rigorous testing in quarantine (Mason et al, 2017; Warne, 2016); 2) the agent(s) being used must be host specific for the target species that it is being used against (MacQuarrie et al, 2016); 3) there must be follow-up monitoring of the non-target impacts it may have, and how it interacts with their surroundings (MacQuarrie et al, 2016); 4) the agent cannot be a contaminant and it must be heavily screened to ensure its safe for use (MacQuarrie et al, 2016); 5) there needs to be multi-levels of approval for its use as an agent (Mason et al, 2017); and 6) there needs to be communication with the public (Mason et al, 2017).

There are several examples of successful applications of biological control agents against invasive plants in North America. Success is often defined as the establishment and long-term impact that an agent has in the ecosystems in which it is being released (MacQuarrie, 2016). One such example of success is purple loosestrife (*Lythrum salicaria*), which employs the use of four biological control agents to reduce the spread of this non-native plant (Warne, 2016). Three of the agents used against *L. salicaria* are leaf feeders *Neogalerucella* calmariensis L.*, *Neogalerucella* pusilla Duftschmidt*, and *Nanophyes marmoratus*, and the other agent is a root feeder, *Hylobius transversovittatus.* The use of biological control as a form of management has resulted in the reduction of approximately 90% of the biomass for *L. salicaria* in Ontario (Warne, 2016). There has been success for the use of insect agents against invasive plants in many different ecosystems in Canada however, none of these agents have been used in forested ecosystems, therefore the agents being used on *V. rossicum* may be the first insect agents in Canada to be used as a biological control for an invasive plant in a forest (MacQuarrie, 2016).

Biology of *Hypena opulenta*

To ensure that *Hypena opulenta* is safe to use as a biological control in North America tests were conducted to determine the host specificity of this moth. A plant list of 82 species, including native and economically important introduced species, were used for the host specificity tests (Hazlehurst et al, 2012). This study found that *H. opulenta* was only able to complete the full larval development on *Vincetoxicum* species and is therefore host specific to *Vincetoxicum rossicum* (Hazlehurst et al, 2012). A petition was created for the release of *H. opulenta* into open-fields in 2011 (Casagrande et al, 2011), and moths were first released in Ontario, Canada in 2013 (Young and weed, 2014).

What is known about the life cycle of *H. opulenta* is that it takes roughly 19 days (at 20ºC) for the larvae to develop and then enter the pupal stage (Weed and Casagrande, 2010). The whole process from egg hatching to moth emergence takes about 36 days at 20ºC (Weed and Casagrande, 2010). The adult moths live for an average of 17 days and females lay approximately 400 eggs during their entire reproductive life span (Weed and Casagrande, 2010). In its native range this moth can go through two generations per year, laying eggs and defoliating *V. rossicum* before undergoing pupal diapause for the winter (Young and Weed, 2014). Despite what is known about their life cycle there is no information currently available with regards to the success and fitness of this moth.

It is vital to understand the fitness of biological controls as it defines how adept a population is at producing viable offspring (Roitberg et al, 2001). Fitness can be measured by observing size, development rate of an individual, as well as the survival of an individual's descendants (Roitberg et al, 2001). Therefore, it is imperative to study these measures to better understand the fitness of *H. opulenta*. This paper will go into more detail with regards to size and longevity of individuals as well as egg maturation, as there is evidence suggesting that there is a relationship between egg size and offspring fitness in Lepidoptera (Torres-Vila, and Rodriguez-Molina, 2002). In their study, Torres-Vila and Rodriguez-Molina (2002) found that there were higher rates of survival for the European grapevine moth's (*Lobesia botrana)* larvae when the eggs they produced were greater in size. Larger egg size for Lepidoptera has also been linked to lower rates

of larval mortality, larger mouth parts as well as increased larval dispersal (Torres-Villa and Rodriguez-Molina, 2002).

Objectives

The purpose for this study was to better understand the fecundity and longevity of *Hypena opulenta* to make predictions on the fitness of this moth as a biocontrol for *V. rossicum*. This study specifically asked; 1) What the longevity for Hypena opulenta is, and whether it is possible to make predictions of their adult life span based on their pupal weight; 2) Do females emerge with their full egg count OR do they develop eggs over time in the ovarioles? If the latter is true at what rate does this occur? Is it possible to predict fecundity or egg size based on pupal weight; and 3) Does the size of the eggs change over time in the ovarioles? Is there a relationship between egg size and the number of eggs produced?

2. Methods

General Rearing

To begin the rearing process, newly emerged moths were placed into 55 x 40 x 22 cm oviposition cages (Fig. 1). There was a limit of 4 females and 4 males per cage. To provide sustenance for the moths, strips of cheesecloth soaked in a honey water solution were hung from the tops of the cages. The cages were kept at 20ºC with a relative humidity of 80% at a photoperiod of 16:8 (L:D) h. Each cage contained a potted plant of *V. rossicum* for the moths to lay their eggs on. This plant was replaced every 1 to 2 days to ensure that the moths had enough space to lay their eggs. The plants that had eggs laid on them were placed in new oviposition cages, that did not contain moths, to allow for the eggs to hatch. Once the larvae reached the second instar, they were removed from the oviposition cages and placed into smaller 35 x 20 x 12 cm rearing boxes (Fig. 2). A layer of moist paper towel was laid down on the surface of the box to increase the humidity in the boxes, as well as to collect frass. Chicken wire was placed

above the paper towel to separate the foliage from the frass. Each box contained approximately 20-30 larvae to ensure that the larvae were not over crowding one another. Larvae were monitored daily to clean cages or add fresh foliage as needed. Once in the pupal stage, individuals were placed on moistened cotton balls in separate 250 ml cups. Each pupa was given a unique ID and the date of pupation, sex, weight and measurements of length and width were recorded. The measurements of length and width were taken using a configured Dino-lite digital microscope (Fig. 3**).** Date of adult emergence was also recorded, as well as measurements (length and width) of empty pupal casings (Fig. 3).

Longevity

Due to complications with high mortality in the rearing processes, as well as papal diapause that was unanticipated there were fewer individuals available for this experiment than previously anticipated. Due to these setbacks, only 22 males and 18 females were used for the adult longevity experiment.

Date of emergence was recorded for each individual, and they were provided with a honey water solution on a strip of cheesecloth hanging from the lid for sustenance. Moths were kept in 250 ml cups at room temperature (23ºC), and a 16:8 (L:D) h photoperiod. The cheesecloth was lightly sprayed daily to ensure that adults had access to the honey water solution, as well as ensuring that humidity in the cups remained high. The cheesecloth was replaced every week to prevent mold from accumulating. Moths were check daily for mortality, and the date of death was recorded.

Fecundity

To understand how the fecundity of female *H. opulenta* changes over time after emergence, ovariole dissections were conducted on adult females 24 hours, 3 days, and six days after emergence. For this experiment 30 newly emerged and unfertilized female moths were used. 10 individuals were used for each treatment (24 hours, 3 days, 6 days) and kept at room temperature (approximately 23ºC). For the first treatment, 24 hours, individuals that emerged within a 24-hour time period were placed in the freezer to later be dissected. The individuals being used for

the second (3 days) and third (6 days) treatments were kept in the 250 ml cups with cheesecloth soaked in honey water for the allotted 3 or 6 days. After the 3 or 6 days the individuals were placed in the freezer for dissection.

Abdomens of the females were removed using forceps and transferred to a saline solution of 0.9 g of NaCl in 100 ml of distilled water. The ovarioles were separated from the abdomen using forceps, and the unfertilized mature eggs were counted under a dissecting microscope (Fig. 4). 10 eggs were then separated and measured under a Dino-lite digital microscope (Fig. 5). Female moths would lay eggs in the cup despite being unfertilized. These eggs were also counted and recorded to determine the full egg count.

Statistical Analysis

To better understand the fecundity of female *H. opulenta* over time, the number of eggs produced were compared to the time after emergence and the size of eggs using ANOVA (analysis of variance). Kruskal-Wallace tests (if the data was non-parametric) and ANOVAs (if the data was parametric) were used to compare egg sizes at the different time treatments, as well as pupal weight, and predicting fecundity.

The length and width of pupa was compared to the weight of each sex separately using ANOVA. ANOVA was also used to determine if the weight of a pupa could be determined from pupal casings (length or width). To determine the differences in weight for males and females the Kruskal-Wallace test was used. MBLM (median based linear models) and ANOVA was used to compare pupal weight and longevity of both sexes, and ANOVA was used to determine which sex lived longer. In order to determine whether weight could predict adult longevity, ANOVA was used. For all parametric data, Tukey's HSD was used to comparing means, and Post Hoc was used for non-parametric data. All analyses were conducted with the help of Dr. M. Lukas Seehausen using R Studio.

3.Results

Longevity

Females were found to live longer than males (P= 0.048; F=4.174; Df=1) (Fig. 7). On average the female moths lived for 14 days, while males on average lived for 10 days. The female pupae of *H. opulenta* also tended to weight more than the male pupae (Fig. 8), however, it was found that there is no significant difference between male and female pupal weight as the P value was 0.0662 (Df=1; Chi-squared=3.374). Adult longevity was found to increase as pupal weight increased (P=0.0004) (Fig. 9). With regards to the females there is a significant relationship between pupal weight and longevity (P=0.0016) (Fig. 10. Males on the other hand did not have a relationship between their pupal weight and longevity (P=0.226) (Fig. 11). There is a relationship between pupal weight and pupal length (R-squared=0.885), as well as pupal weight and pupal width (R-squared=0.8678). Both the former and latter statements are true for both sexes (Fig. 12). Pupal weight has a relationship with both the length (R-squared=0.6486) (Fig. 13) and width (R-squared=0.692) (Fig. 14) of empty pupal casings.

Fecundity

Egg development

Females of *H. opulenta* do not emerge with their full egg count, however, there is evidence (P<0.0001; F=32.72; Df=2) to suggest that they develop eggs over time as the total egg count increased over time (Fig. 15). Within the first 24 hours after emergence adult females have an average of 86 eggs, increasing by 75% over the next 3 days (with an average of 156 eggs) and 113% over 6 days (with an average of 190 eggs).

Both the amount of eggs in the ovarioles increased over time (P<0.0001; F=23; Df=2) (Fig. 16) and the amount of unfertilized eggs laid (P=0.0007; F=13.23; Df=1). No eggs were laid for any of the moths during the first 24 hours, and egg count in the cups was significantly higher in the 6-day treatments than the 3-day treatments (P=0.0007; F=13.23; Df=1) (Fig.17). After a period of 3-days the moths laid an average of 3 eggs, with 10 eggs being the highest amount of eggs

laid. Within the 6-day period after emergence moths laid an average of 10 eggs, and one individual laid 57 eggs. The data shows that there is no relationship between the number of eggs produced over time and the weight of pupae (P=0.7169; F=0.1328; Df=1) (Fig. 18).

Egg size

The size of unfertilized eggs after 24 hours was 0.54mm on average and was 0.63mm on average for both 3- and 6- days after emergence. Egg size was found to increase between the first day of emergence and after a 3-day period (P=0.0003; Df=2; Chi-Squared=16.028) but there was no difference in the average size of eggs between 3- and 6-days (Fig. 19). Looking at Fig. 20, the egg load, which is the relationship between the egg size and the number of eggs, was significantly lower for 24 hours (P=0.0029; F=17.831; Df=1) than for 3- and 6-days. There was no significant difference in egg load between the 3- and 6-day treatments (P=0.9030; F=0.0158; Df=1, and P=0.1986; F=1.9649; Df=1 respectively) (Fig. 20). However, there is a correlation between the size of eggs and the total amount of eggs in the ovariole over time (P=0.0001; F=49.14; Df=1), as shown in Fig. 21, as the total number of eggs increase the size of eggs also increases. The size of *H. opulenta* eggs cannot be predicted by pupal weight (Fig. 22) for 24 hours (P=0.1027; V-value=44; Df=1) and 3-days after emergence (P=0.1055; V-value=44; Df=1) as weight does not have an effect on these treatments, however, there is a correlation between pupal weight and egg size for 6-days after emergence (P=0.0371; V-value=48; Df=1).

4. Discussion

The purpose of this study was to better understand key aspects of *H. opulenta*s' biology so that this moth can be used as an effective biological control for *V. rossicum*. Specifically, this study sought to understand the adult longevity and fecundity of adult females, in an effort to determine whether it is possible to make any predictions on the fitness of this moth for future field releases.

Longevity

On average adult females lived for 14 days, while their male counterparts lived for 10 days, and therefore females tended to live longer than males. With regards to field releases of *V. rossicum*, the females of *H. opulenta* only have 2 weeks to lay hundreds of eggs to defoliate this invasive plant. The adults used in this study did not live as long as the adults in Weed and Casagrande (2010) study, which found that *H. opulenta* adults live for 17 on average. The premature death this study may have been be the result of unfavorable environmental conditions such as a lower than optimal humidity, or a limited access to sustenance. Other factors that may have reduced the lifespan of adult *H. opulenta* is the possibility of airborne disease, or even genetics. Unfortunately, the factors causing this premature death of adults will not be able to be determined for the purposes of this study. Replicates of this study may need to be done with a new genetic stock in a sterile environment to either support or negate these findings.

The average weight of female pupa was higher than that of male pupa, and although there is a trend (Fig. 17), this study found that there was no significant relationship between sex and pupal weight. This trend could have been a result of the selection of pupae used in this study as there were only 22 males and 18 females used in this study. In order to fully flush out whether there is a relationship between pupal weight and sex for *H. opulenta* more data will need to be acquired. It would be beneficial to have a larger sample size in future to determine if females do generally weigh more than males. However, previous literature on other species of Lepidoptera has suggested that females have a tendency to be larger than males (Stillwell et al, 2014). Lepidoptera females tend to weight more as they require more fat for fecundity, or possibly this

size difference could be the result of differing qualities or quantity in food (Stillwell et al, 2014).

Even though it is not possible to confirm whether females tend to weight more than males from this study, it can be confirmed that adult longevity can be predicted by pupal weight as adults lived longer as pupal weight increased. When isolating the results for females it is possible predict their longevity based on their pupal weight, however, the same cannot be said for the males used in this study. This is likely due to the selection of pupae, which may have skewed the data as the females selected tended to weight more than the males.

In addition to the relationship between longevity and pupal weight, it is possible to predict pupal weight using either pupal length of width. Seeing as pupal weight can predict adult longevity is it reasonable to assume that it is also possible to predict longevity based on pupal length or width. There is also a weak correlation between pupal weight and the pupal width or length of empty pupal casings, thus it is possible to approximate the adult longevity of *H. opulenta* using empty pupal casing measurements. Measuring either pupae or empty pupal casings where *H. opulenta* has been released could be used to determine the longevity of adults in the field to estimate how long females are laying eggs for.

Fecundity

Egg Development

This study confirmed that females of *H. opulenta* do not emerge with their full egg count, instead they develop their eggs over time requiring a preoviposition period of at least 24 hours. Further investigation is required to understand whether the preoviposition period of this moth is only 24 hours or longer. Seeing as this study only compared fecundity for 1- and 3-days it is still unknown if the preoviposition lasts for more than 24 hours. Moreover, this study only observed individuals that had emerged within a full 24-hour period and were not tracked on an hourly basis, therefore it is still unknown whether there are differences in fecundity within a full 24 hours.

It is not possible to predict the number of eggs produced at any time after emergence from pupal weight, as there is no relationship between egg count and pupal weight for any of the treatments in this study. However, a study conducted by Haulioja and Neuvonen (1985) found that the number of eggs laid was correlated with the pupal weight for other Lepidoptera, and therefore it is possible to predict the amount of eggs a female produced in their full reproductive life span by their pupal weight. In order to confirm if this is the case for *H. opulenta* future studies would need to observe the total number of eggs laid for their entire adult lifespan in conjunction with their pupal weight, rather than just observing the total number of eggs in the ovariole at different times after emergence. If it is possible to predict female fecundity using pupal weight, it would also be possible to make predictions of fecundity using pupal measurements, such as the length or width of empty pupal casings. This method could be used to predict the fecundity from field populations, in order to assess the fitness of those populations, and could therefore be used to inform future release strategies. For example, if the fitness of a field population was declining, those populations could be subsidized by releasing new individuals.

Another more complicated index that can be used to measure fecundity is the ovigeny index (OI). This index predicts the life-time egg production of insects by calculated by the quantity of eggs produced after adult emergence divided by their lifetime egg count (Jervis et al, 2005). The OI can be used to predict lifetime egg production based on the life-traits of adult Lepidoptera, such as; longevity, fat reserves versus the number of eggs in the ovarioles, the ability to reabsorb eggs, body size, and nuptial gifts of sperm or nutrients provided by the male (Jervis, Boggs, and Ferns, 2005). This study looked at a few of the life-traits required to determine the OI of *H. opulenta*, such as adult longevity, body size, and the number of eggs produced, however it did not investigate the fat reserves of this moth, its ability to reabsorb eggs, or nuptial gifts which are all required to calculate OI. In order to do so future studies would need to compare of the fat reserves versus the egg count in the abdomen at emergence, as well as this moth's ability to reabsorb eggs, and the impact that nuptial gifts have on female reproduction in accordance with longevity, body size, and egg production. The OI would be a beneficial predictive measurement for *H. opulenta* as this moth is being used as a biological control for *V. rossicum,* and therefore it would be advantageous to have an accurate means to predict the amount of eggs produced, as this study was not successful at predicting fecundity.

Egg Size

It was important to study egg size of *H. opulenta* as egg size is one of the main reproductive traits for understanding life-histories of Lepidoptera (Torres-Vila and Rodriguez-Molina, 2002). This study found that egg size did change over time after emergence as there was a noticeable increase in egg size after 24 hours however, there was no change between 3- and 6-days. This is likely since *H. opulenta* needs at least 24 hours to pre-oviposit, and after that preoviposition period eggs have adequate time to fully develop. There was no relationship between pupal weight and egg size for 24 hours or 3 days after emergence. There was, however, a relationship between pupal weight and egg size 6 days after the moths emerged. One might assume that if there was no relationship between pupal weight and the egg size for time after emergence at 24-hours and 3-days that 6-days would also have no relationship, however this was not the case for this experiment. The inconsistency in the results may be because one of the individuals used for this experiment was considerably lighter than the other pupae, and this individual may have skewed the data. To ensure consistent results future tests would require the use of individuals that weighted between 0.05 and 0.08g to provide more concrete evidence to support or oppose whether pupal weight can indeed predict egg size.

This experiment only observed the changes of egg size over the first 6 days after adult emergence, but studies show that egg size decreases as Lepidoptera age (Torres-Vila and Rodriguez-Molina, 2002), which would make it vital that egg size was observed for the entire life-span of adult *H. opulenta* to determine when their eggs were largest in order to calculate what the optimal time for releases as larger eggs are associated with increased larval fitness. Larger eggs have been linked to fitness and survival of larvae in other Lepidoptera as larger eggs produce bigger larvae (Torres-Villa and Rodriguez-Molina, 2002). Bigger larvae typically have lower rates of mortality, are able to disperse further than smaller larvae, and have larger mouth parts (Torres-Villa and Rodriguez-Molina, 2002). These are all important factors for *H. opulenta* as a biological control agent for *V. rossicum* as these indicators of fitness will aid in the establishment of this moth in North America.

Lastly there is evidence that shows a correlation between the size of eggs and the number of eggs in the ovariole, as this study found that as the number of eggs increased in the ovariole the size of

the eggs also increases. This is likely since both factors are increasing as time is proceeding, and the eggs have more time to develop in the ovarioles. It would be beneficial to compare in future research, whether nuptial gifts have an impact on egg size or the amount of eggs produced in the ovariole in order to determine the affect that nuptial gifts have on fecundity.

5. Recommendations

Mating should occur before releases of *H. opulenta* takes place to ensure that future releases are successful. However, mating should occur after 24 hours as the females need this time for preoviposition. A preoviposition period will allow for eggs of *H. opulenta* to fully develop, and it will give the eggs time to increase in size in the ovarioles. As this study only observed egg size for 1, 3, and 6 days, it did not measure the egg development for 2 days, for this reason, future studies can be conducted to compare how egg development changes over a 72-hour period to determine whether it is more effective to release adults after 2 or 3 days. In addition to studying the preoviposition period it would be advantageous to investigate how egg size changes as adults mature, as other studies have found that egg size decreases as Lepidoptera matures (Torres-Vila and Rodriguez-Molina, 2002). This could be used to decide at what stage the eggs of *H. opulenta* are largest for successful releases.

This information would be vital for the fitness of *H. opulenta* as larger eggs have been associated with lower rates of mortality in larvae of other Lepidoptera (Torres-Villa and Rodriguez-Molina, 2002). To fully understand the impacts that egg size has on survival of *H. opulenta* a test should be done to determine whether larger eggs are in fact associated with lower rates of mortality, and larger larvae. This would have to be done on fertilized eggs, unlike this paper which only compared the development of unfertilized eggs.

The moths that are being used for future releases should be selected based on pupal weight, as heavier pupae live longer, and have more time to lay eggs and give their offspring a fighting chance to feed on *V. rossicum*. Further research would be needed to fully understand the

relationship between pupal weight and total egg count, as other studies have found that pupal weight has an impact on fecundity in other Lepidoptera (Haulioja and Neuvonen, 1985). If pupal weight was found to have an impact on fecundity, it would also be suggested that larger pupae should be used for field releases in an effort for *H. opulenta* to establish their population.

These applications of this experiment can be repeated for other insects that are being used as biological control agents for invasive species in Canadas forests and natural environments, such as the parasitoids that are being used to control Emerald Ash Borer, *Ectropis crepuscul* a moth which defoliates on purple loosestrife, or even other potential agents for *H. opulenta*.

6. Conclusions

It is possible to predict the longevity of *H. opulenta* using its pupal weight, length or width. Females lived longer than males due their tendency to weight more. Therefore, heavier pupae should be used for field releases as they will live longer and will have more time to lay eggs as *H. opulenta* does develops eggs over time rather than emerging with their full egg content. Longevity can also be determined using measurements of width and weight of empty pupal casings, this application could be used for field releases to make assessments on the fitness of field populations. Neither fecundity nor egg size could not be predicted for 24 hours, 3 days or 6 days using pupal weight, however, it was revealed that females require a preoviposition period of 24 hours, as both their egg size and fecundity increased 24 hours after emergence.

Literature Cited

Anderson, H. (2012). Invasive dog-strangling vine (*Vincetoxicum rossicum*) best management practices in Ontario. *Ontario Invasive Plant Council*. From https://www.ontarioinvasiveplants.ca/wp-content/uploads/2016/06/OIPC_BMP_DogStranglingVine.pdf

CABI. (2018). Controlling swallow-worts the sustainable way. From https://www.cabi.org/projects/project/46591

Casagrande, R., Weed, R., Hazlehurst, A., Tewksbury, L., Gassmann, A., and Bourchier, R. (2011). A Pettition for Experimental Open-field Release of *Hypena opulenta* a Potential Biological Control Agent of Swallow-worts (*Vincetoxicum nigrum* and *V. rossicum*) in North America. Retrieved from: https://web.uri.edu/biocontrol/files/2012/11/Hypena-Release-Petition-Final-version-Nov-16-2011.pdf

Doubleday, L., and Cappuccino, N. (2011). Simulated Herbivory Reduces Seed Production in *Vincetoxicum rossicum. Botany, 89,* 235-242.

Douglass, C., Weston, L., and DiTommaso. (2004). Black and Pale Swallow-wort (*Vincetoxicum nigrum* and *V. rossicum*): The Biology and Ecology of Two Perennial, Exotic and Invasive Vines. *Management of Invasive Weeds, 5,* 261-277.

Gibson, D., Vaughan, R., and Milbrath, L. (2015). Invasive Swallow-worts: An Allelopathic Role for – (-) Antofine Remains Unclear. *Journal of Chemical Ecology, 41*, 202-211.

Gassmann, A., Weed, A., Tewksbury, L., Leroux, A., Smith, S., Dejonge, R., Bourchier, R., and Casagrande. (2011). Evaluating the Potential for Biological Control of Swallow-Worts (*Vincetoxicum nigrum* and *V. rossicum*) in Eastern North America. *International Symposium on Biological Control of Weeds.*

Haukioja, E., and Neuvonen, S. (1985). The Relationship Between Size and Reproductive Potential in Male and Female *Epirrita autumnata* (Lep., Geometridae). *Ecology Entomology, 10,* 267-270.

Hazlehurst, A., Weed, A., Tewksbury, L., and Casagrande, R. (2012). Host Specificity of *Hypena opulenta*: A Potential Biological Control Agent of *Vincetoxicum* in North America. *Environmental Entomology, 41,* 841-848.

Hejda, M., Pysek, P., and Jarosik, V. (2009). Impact of Invasive Species on the Species Richness, Diversity, and Composition of Invaded Communities. *Journal of Ecology, 97,* 393-403.

Jervis, M., Boggs, C., and Ferns, P. (2005). Egg Maturation Strategy and Survival Trade-offs: A Synthesis Focusing on Lepidoptera. *Ecological Entomology, 30*, 359-375.

Jeschke, J., Bacher, S., Blackburn, T., Dick, J., Essl, F., … Kumschick, S. (2014). Defining the Impact of Non-Native Species. *Conservation Biology, 28,* 1188-1194.

MacQuarrie, C., Lyons, D., Seehausen, M., and Smith, S. (2016). A history of biological control in Canadian forests, 1882-2014. The *Canadian Entomologist, 148*, S239-S269.

Mason, P., Cock, M., Barratt, B., Klapwijk, J., Lentere, J., Brodeur, J., Hoelmer, K., and Heimpel, G. (2017). Best practices for the use and exchange of invertebrate biological control genetic resources relevant for food agriculture. *Biocontrol, 63*, 149-154.

Milbrath, L. (2008). Growth and Reproduction of Invasive *Vincetoxicum rossicum* and *V. nigrum* Under Artificial Defoliation and Different Light Environments. *Botany, 86*, 1279-1290.

Milbrath, L., and Biazzo, J. (2016). Impact of the Defoliating Moth *Hypena opulenta* on Invasive Swallow-worts (*Vincetoxicum* species) Under Different Light Environments. *Biological Control, 97,* 1-12.

Milbrath, L., Davis, A., and Biazzo, J. (2017a). Demography of Invasive Black and Pale Swallow-wort Populations in New York. *Northeastern Naturalist, 24*, 37-53.

Milbrath, L., Davis, A., and Biazzo, J. (2017b). Identifying Critical Life Stage Transitions for Biological Control of Long-live Perennial *Vincetoxicum* species. *Journal of Applied Ecology, 55,* 1465-1475.

Nienhuis, S., and Wilson, R. (2018). Invasive Species in Ontario: The Threat, the Strategy, and the Law. *The Forestry Chronicle, 94,* 97-102.

Ontario Invading Species Awareness Program. (2018). Invasive plants; Terrestrial plants. From http://www.invadingspecies.com/plants/

Roitberg, B., Boivin, G., and Vet, L. (2001). Fitness, parasitoids, and biological control: An opinion. *The Canadian Etomologist, 133*, 429-438.

Stillwell, R., Daws, A., and Davidowitz, G. (2014). The Ontogeny of Sexual Size Dimorphism of a Moth: When Do Males and Females Grow Apart? *PLoS ONE, 9*.

Torres-Villa, L., and Rodriguez-Molina, M. (2002). Egg Size Variation and its Relationship with Larval Performance in the Lepidoptera: The Case of the European Grapevine Moth *Lobesia botrana, 99*, 272-283.

Warne, A. (2016). Purple loosestrife (*Lythrum salicaria*) best management practices in Ontario. *Ontario Invasive Plant Council.* From http://www.ontarioinvasiveplants.ca/wp-content/uploads/2016/07/Purple-Loosestrife-BMP-April-2016-final.pdf

Weed, A., and Casagrande, R. (2010). Biology and Larval Feeding Impact of *Hypena opulenta* (Christoph) (Lepidoptera: Noctuidae): A Potential Biological Control Agent for *Vincetoxicum nigrum* and *V. rossicum. Biological Control, 53,* 214-222.

Weed, A., Gassmann, A., Leroux, A., and Casagrande, R. (2010). Performance of Potential European Biological Control Agents of Vincetoxicum spp. With Notes on Their Distribution. *Journal of Applied Entomology, 135*, 700-713.

Young, J., and Weed, A. (2014). *Hypena opulenta* (Erebidae): A European Species for the Biological Control of Invasive Swallow-Worts (*Vincetoxicum* spp.) in North America. *Journal of the Lepidopterists' Society, 68,* 162-166.

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Figures

Fig. 1. Oviposition cages, 55 x 40 x 22 cm.

Fig. 2. Rearing boxes, 35 x 20 x 12 cm. B. and C. depict how the rearing cages are set up; damp paper towel lining the bottom of the cage, with chicken wire separating the foliage from the paper towel.

Fig. 3. Measurements (length and width) of *Hypena opulenta* pupa.

Fig. 4. Ovarioles of *Hypena opulenta* under a dissecting microscope.
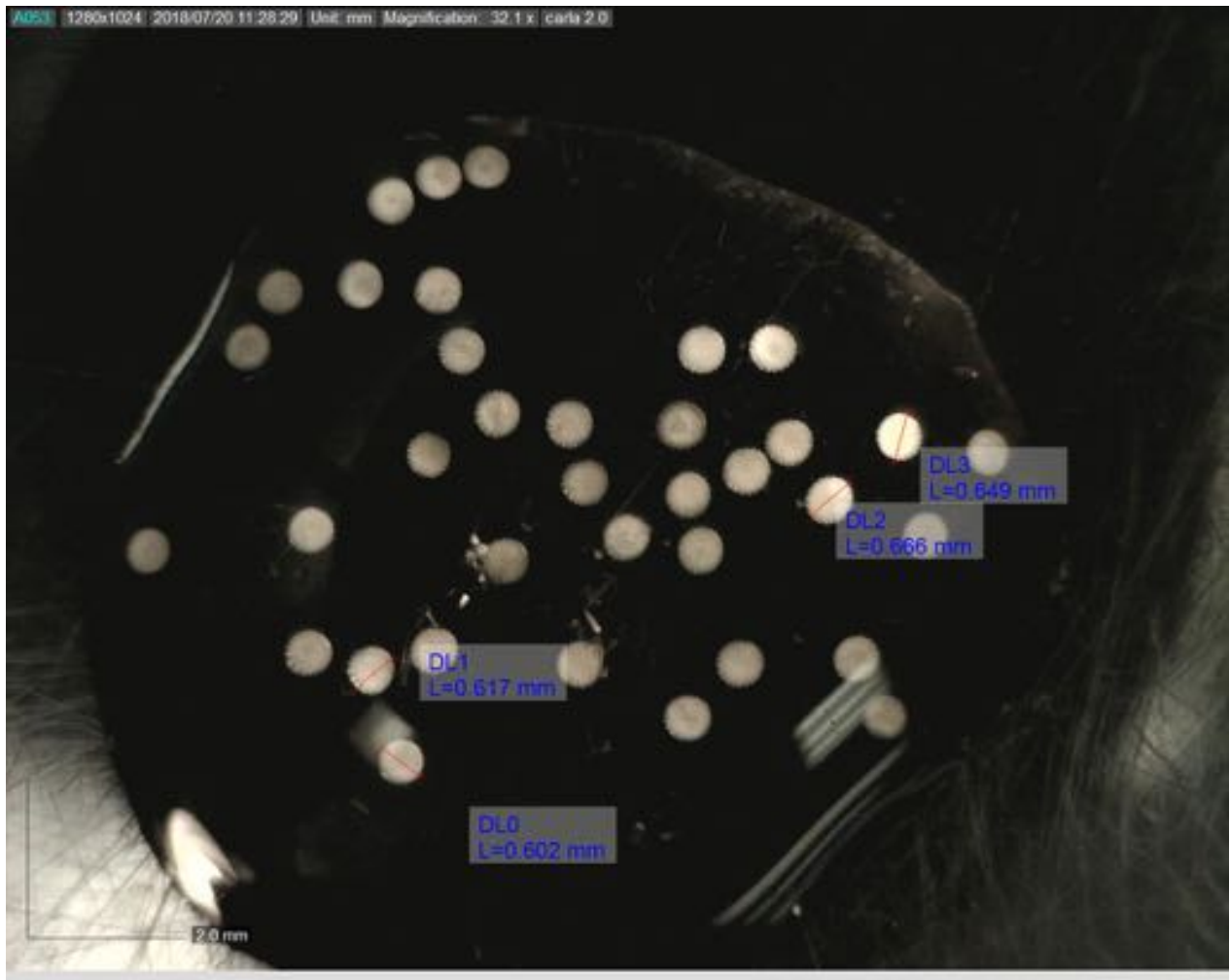
Fig. 5. Unfertilized eggs of *Hypena opulenta* under the Dino-lite microscope.
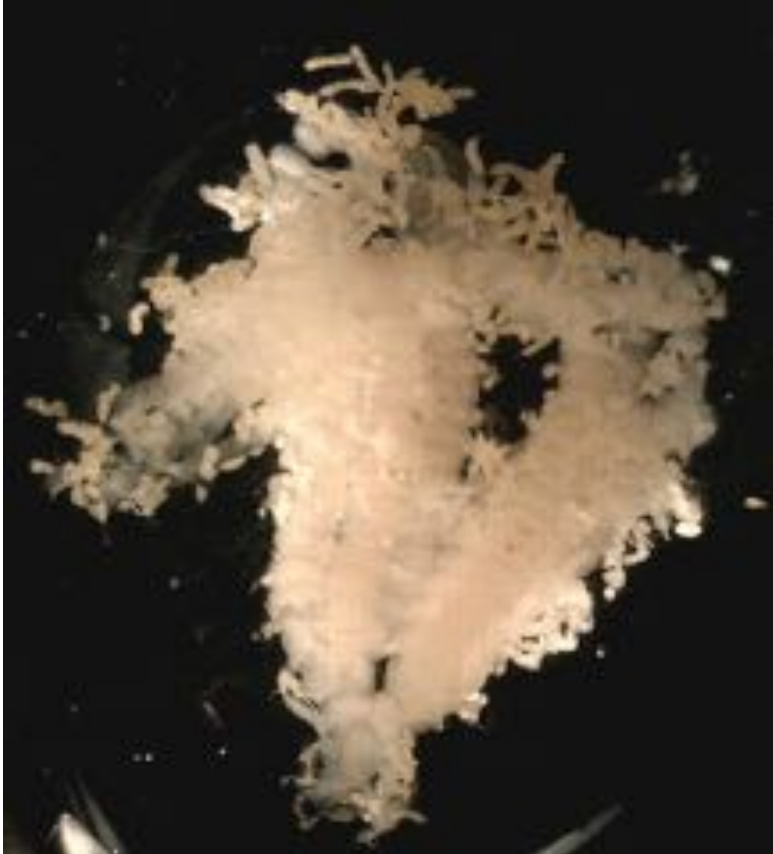
Fig. 6. Ovarioles of *Hypena opulenta* after 24 hours (A.) and after 3 days (B.). (There is no picture for ovarioles after 6 days
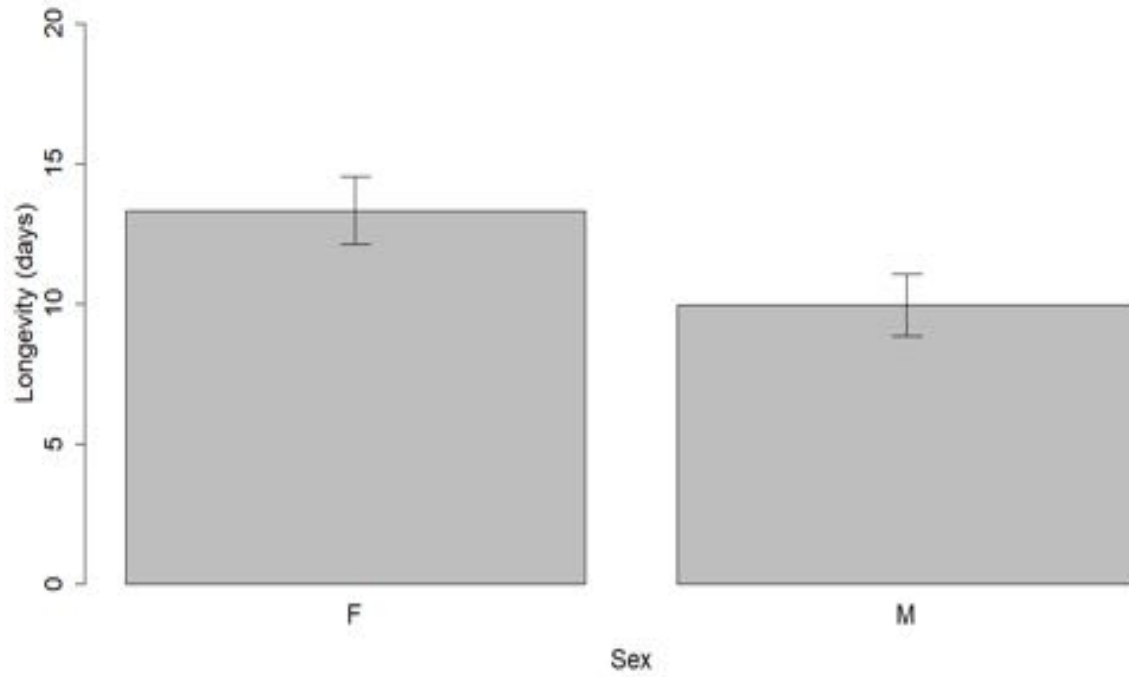
Fig. 7. Comparing the longevity (in days) of male and female moths (P= 0.048; F=4.174; Df=1). There is no uncertainty and there is a significant difference between the longevity of males and females.
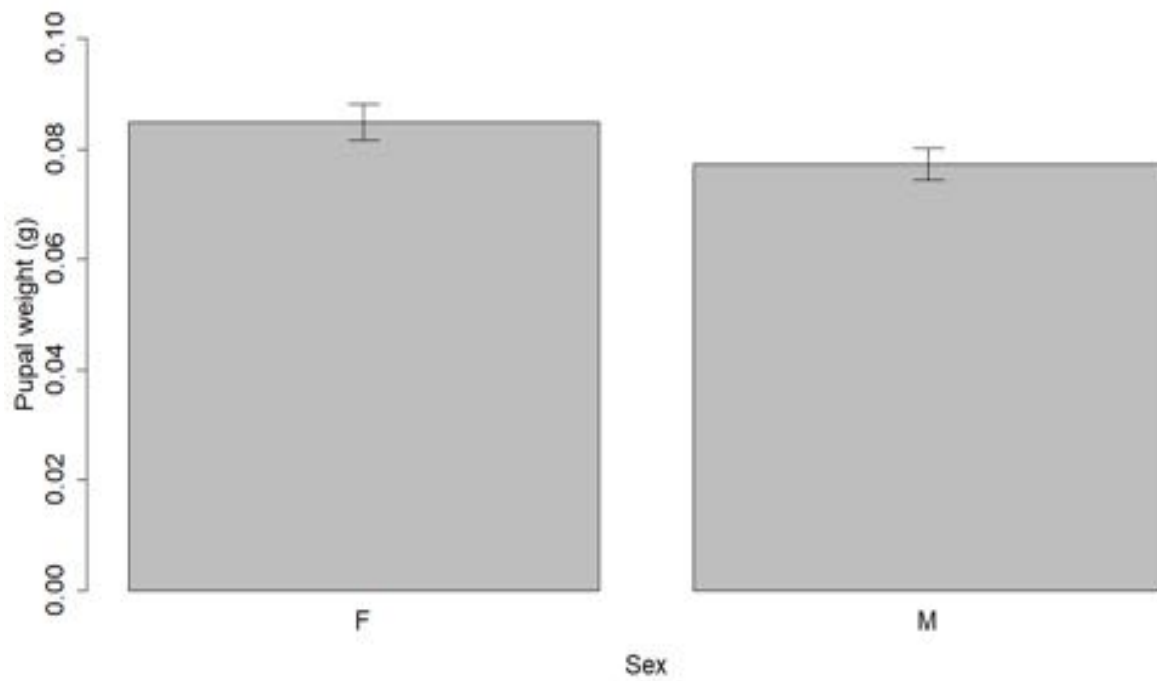
Fig. 8. Comparison of pupal weight (grams) for males and females (P=0.0662; Df=1; Chi-squared=3.374). There is no uncertainty and no significance difference between the pupal weight of male and females.
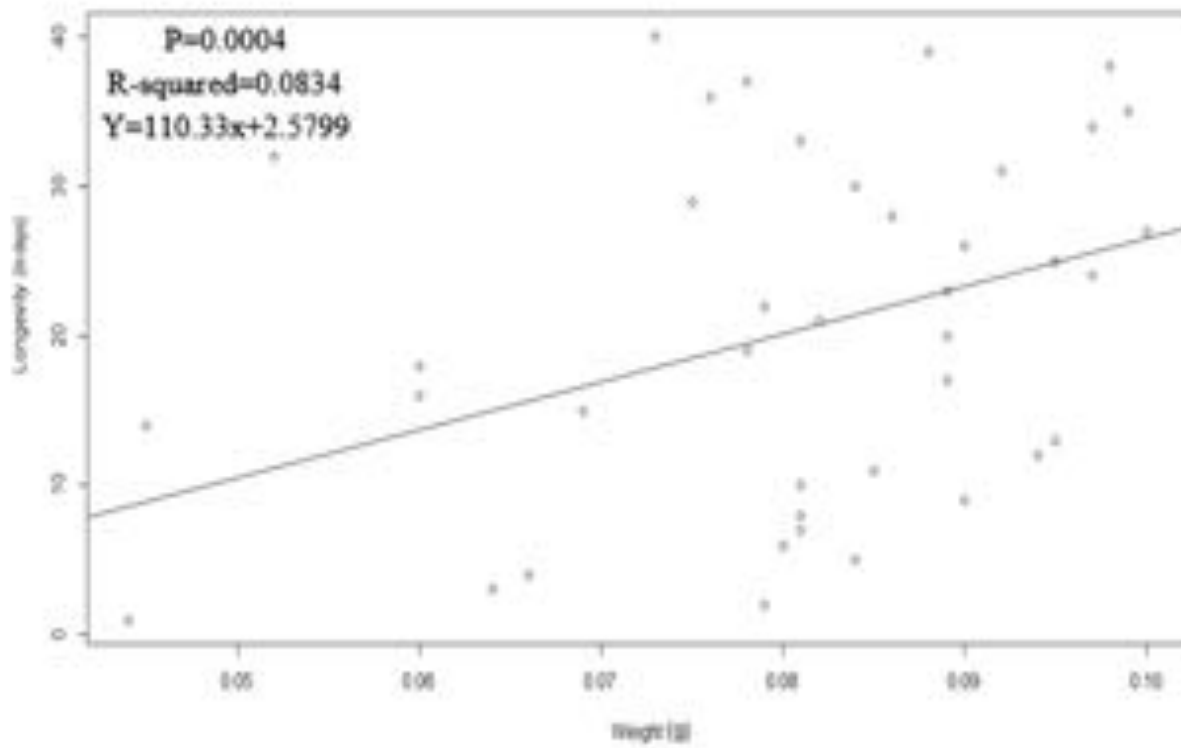
Fig. 9. Longevity (in days) for both sexes compared to pupal weight (grams).
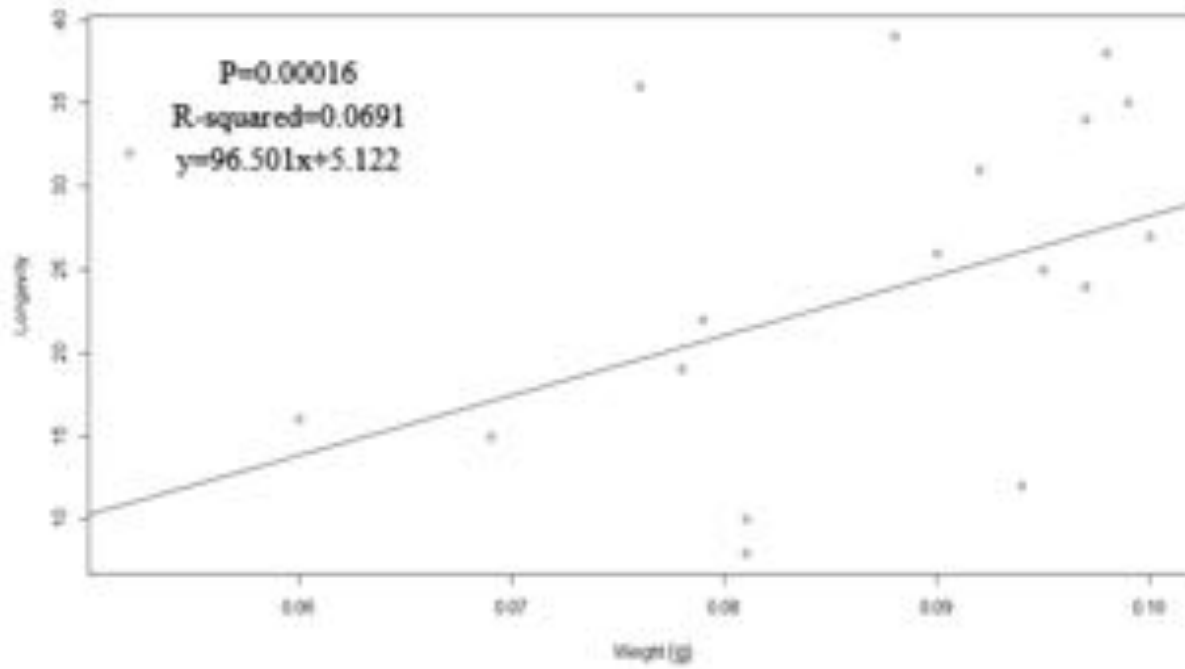
Fig. 10. The longevity (in days) of females versus pupal weight (grams).

P=0.226
R-squared= 0.0368
Y=73.731x+4.2605
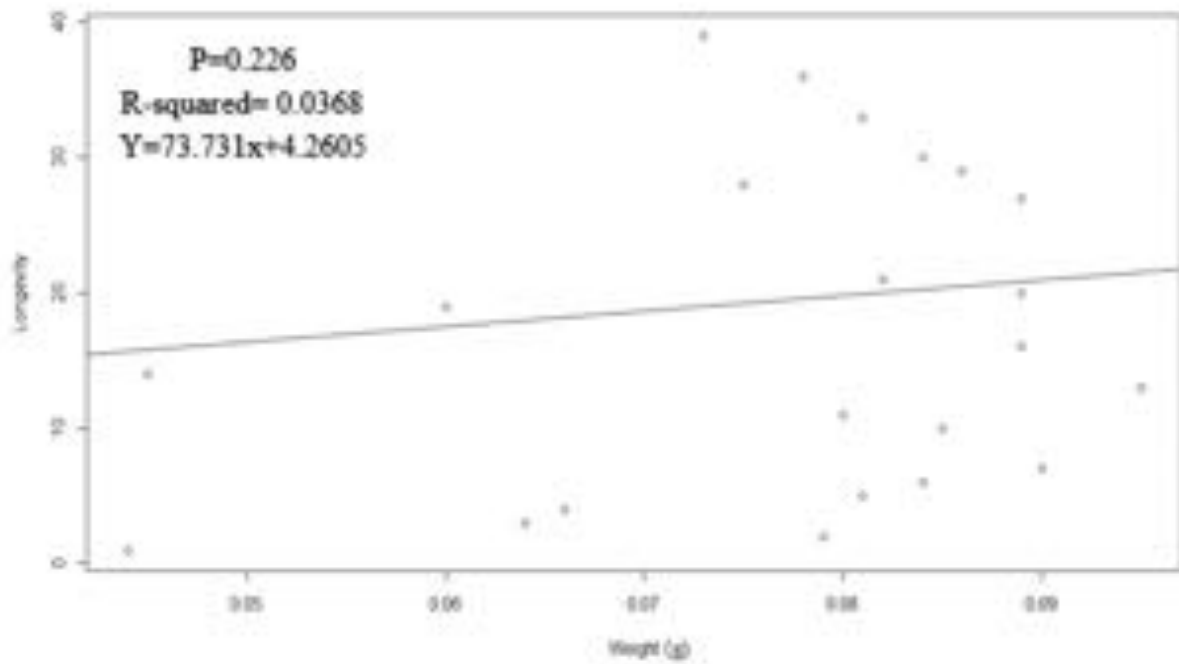
Fig. 11. The longevity (in days) of males versus pupal weight (grams).
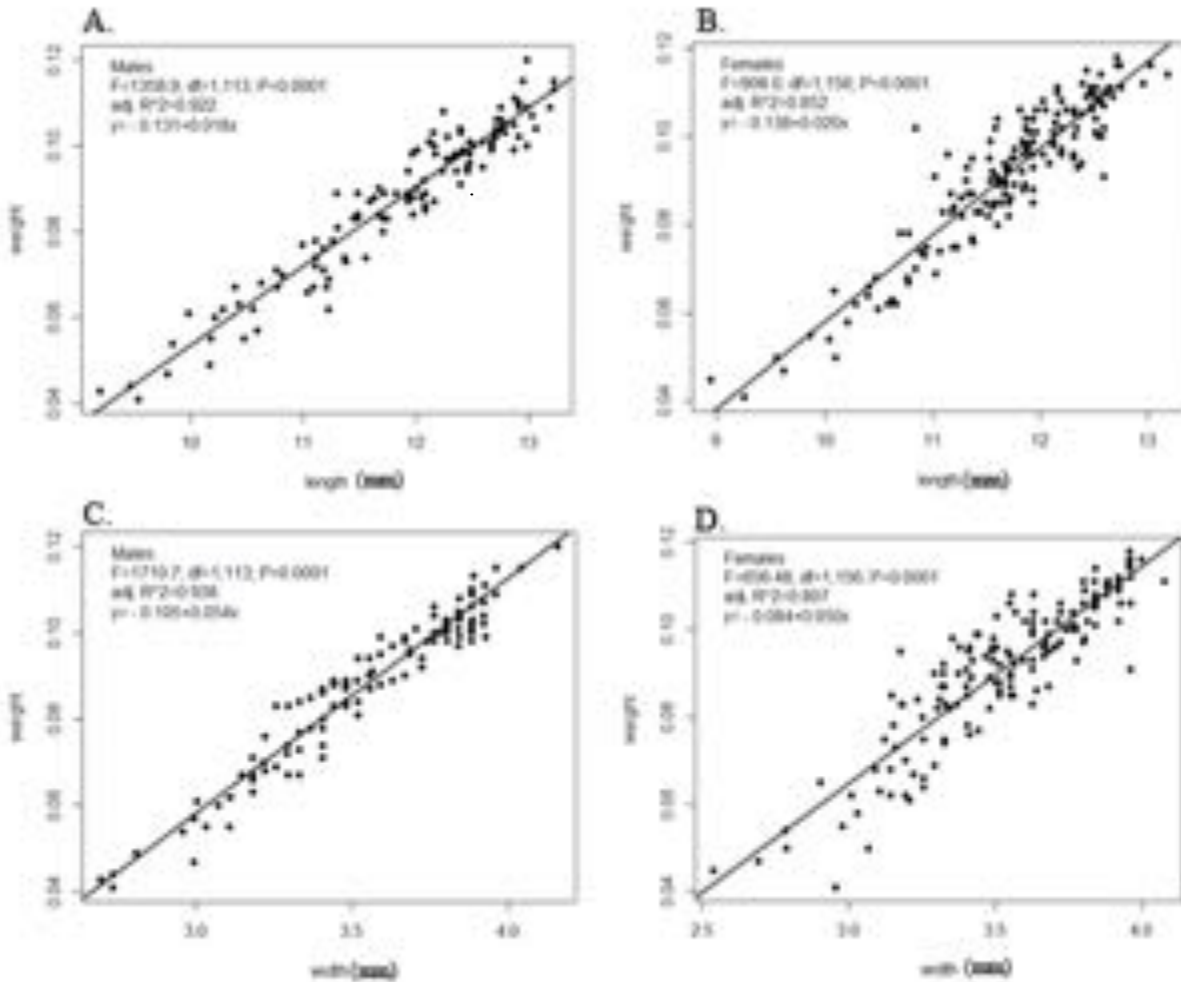
Fig. 12. Measurements of pupae compared to pupal weight; comparing the pupal weight (g) to pupal length (mm) for males (A.), comparing the pupal weight to pupal length for females (B), comparing the pupal weight (g) to pupal width (mm) for males (C.), and comparing the pupal weight (g) to pupal width (mm) for females (A.),
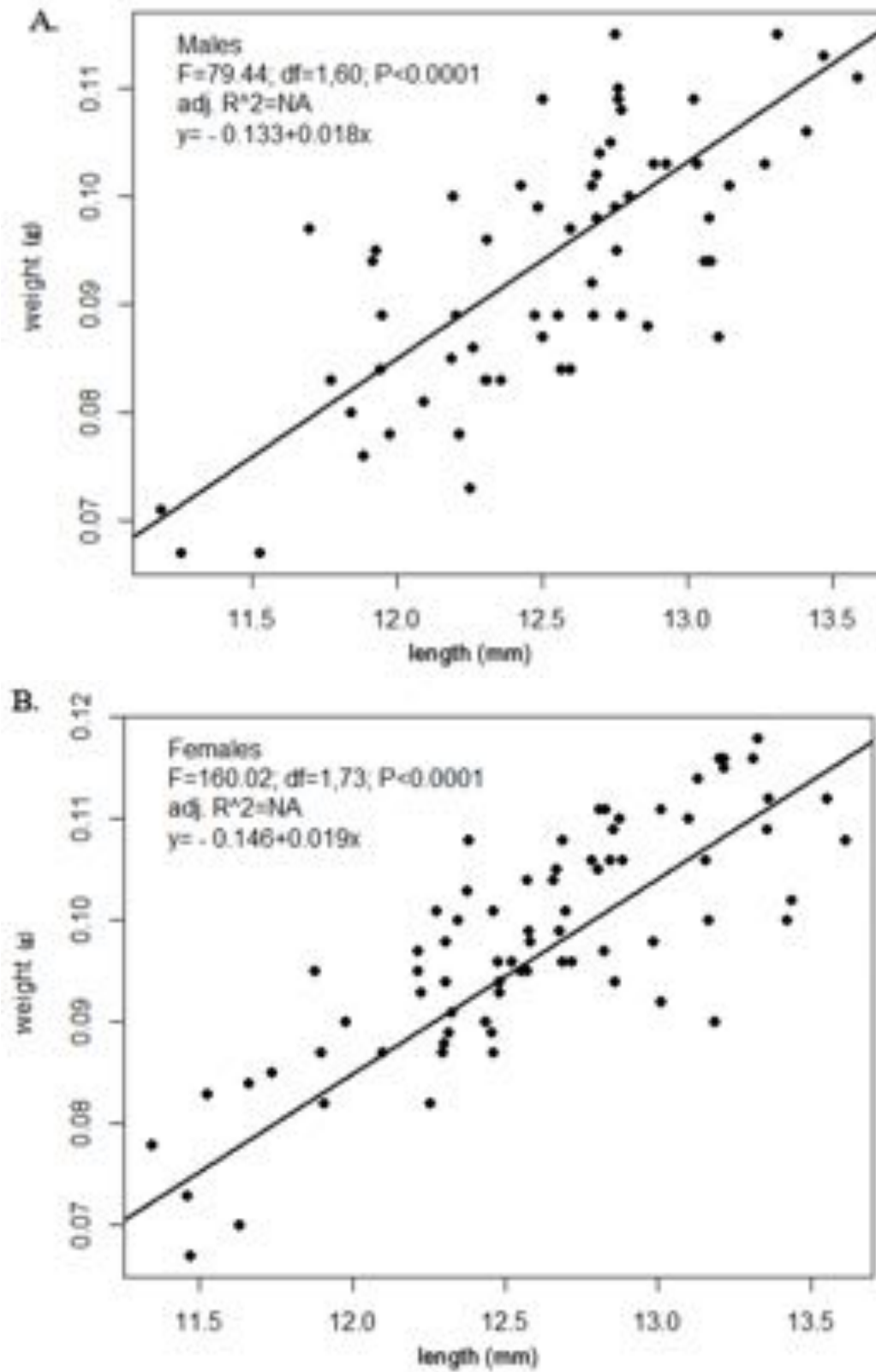
Fig. 13. Empty pupal casings length measurements compared to pupal weight for males (A.) and females (B.).
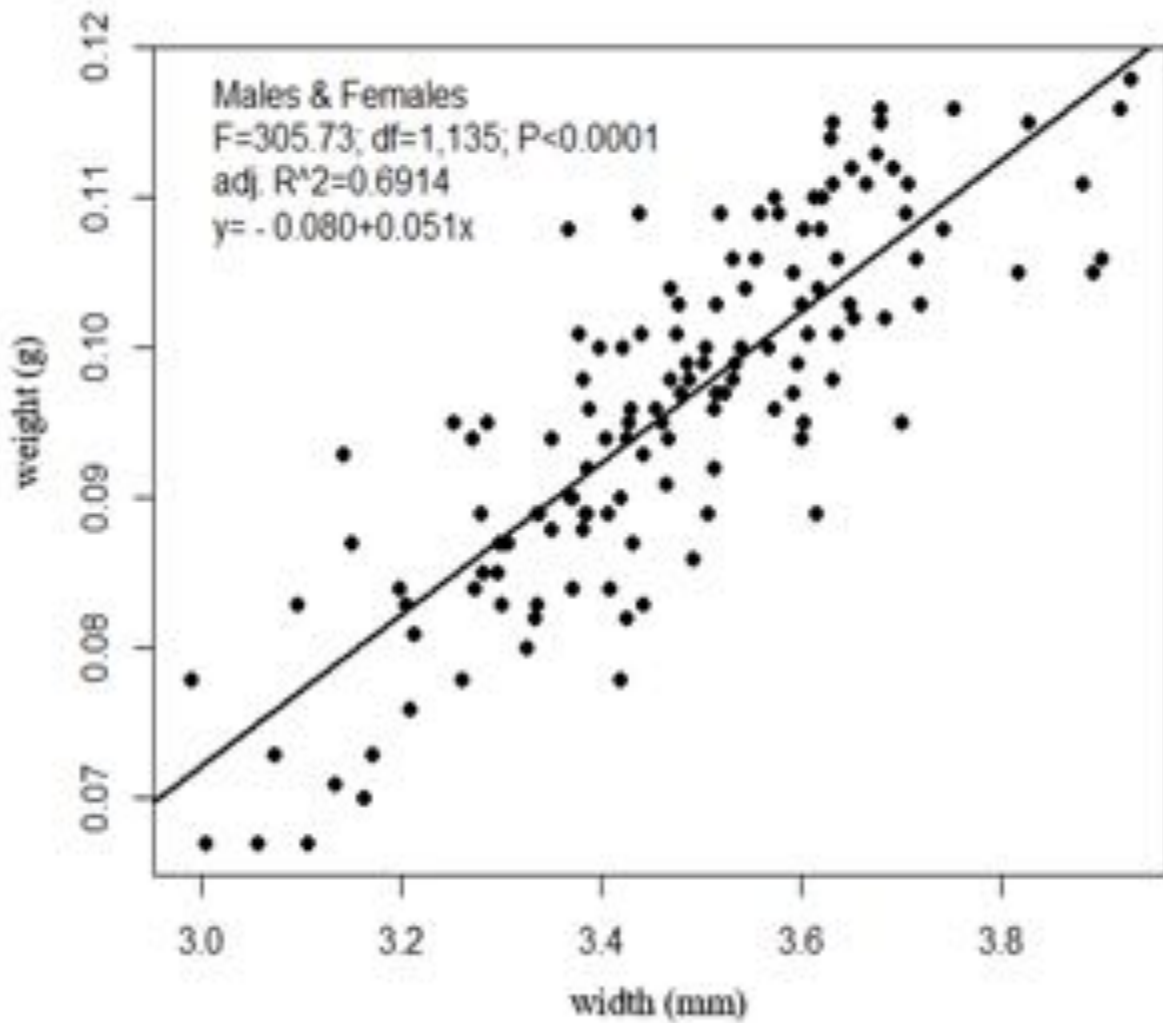
Fig. 14. Pupal weight (g) compared to the width (mm) of empty pupal casing for both males and females.
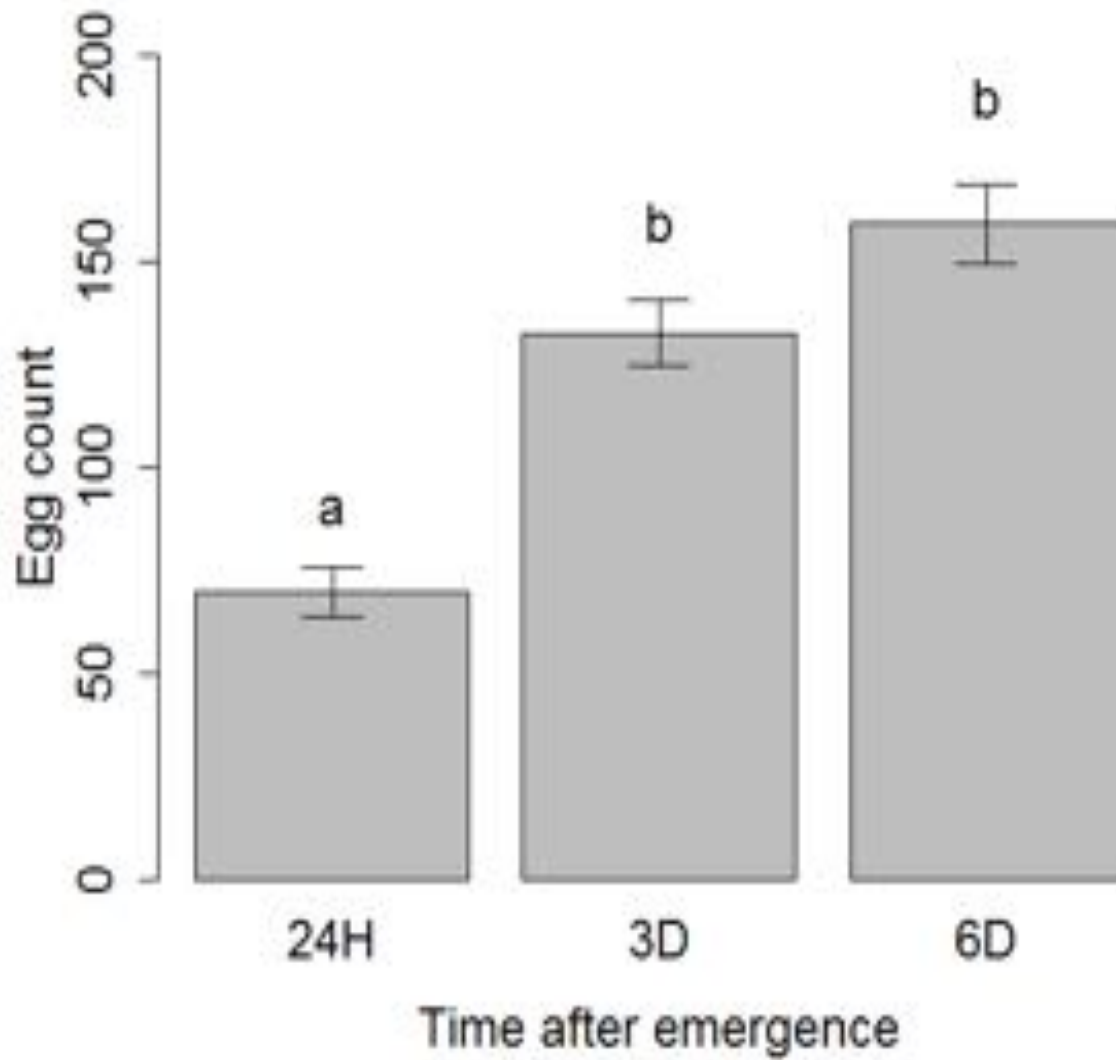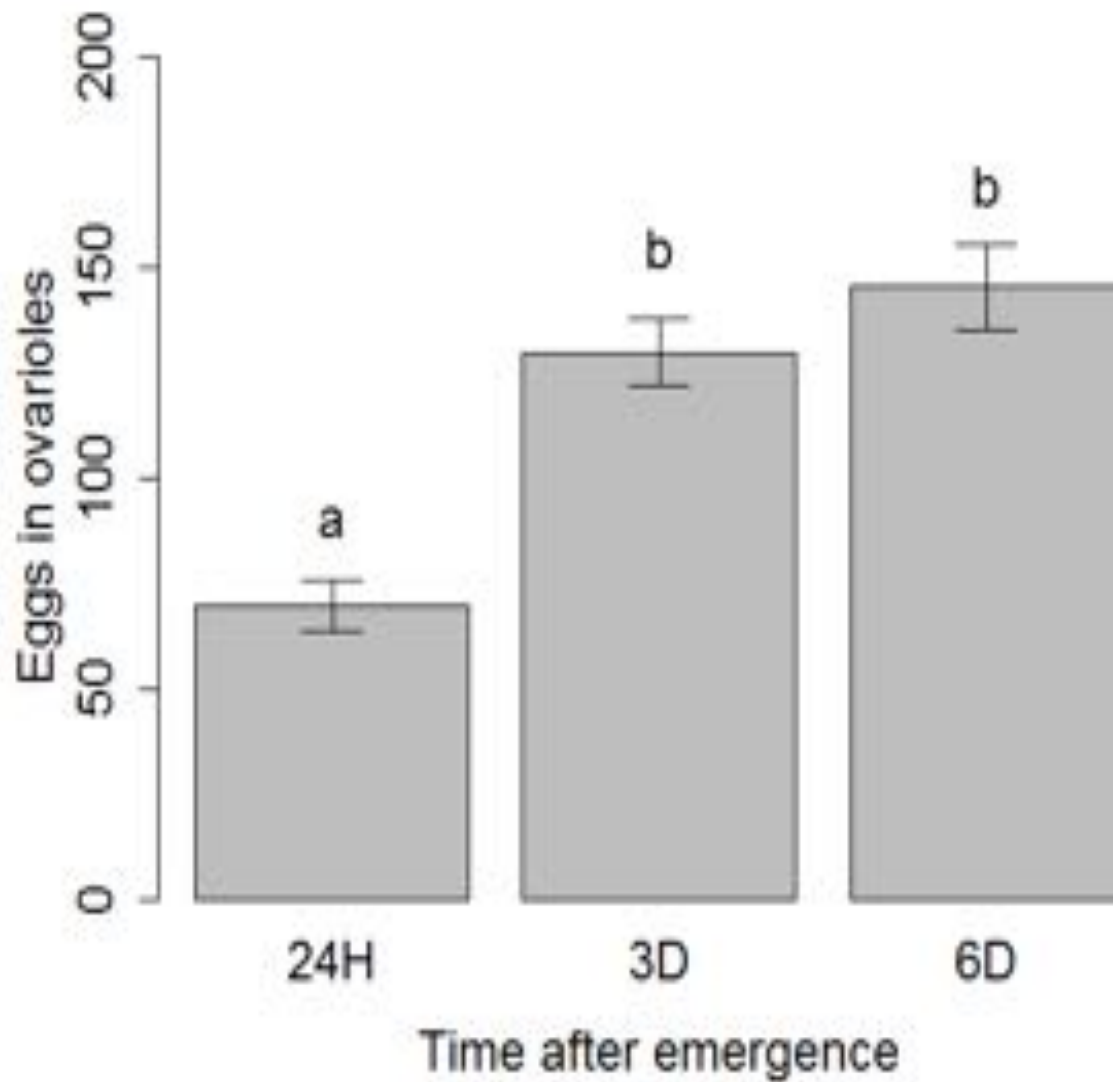
Fig. 15. A comparrison of the total egg count of *H. opulenta* under the different treatments (24 hours, 3 days, 6 days) for time after emergence ($P<0.0001$; $F=32.72$; $Df=2$). The error bars show that there little uncertanty, and that there is no significant difference for the number of eggs between 3 days (b) and 6 days (b) after emergence. There is a significant difference between the number of eggs at 24 hours (a) and the other treatments.

Fig. 16. A comparrison of the total egg count in ovarioles to the different treatments (24 hours, 3 days, 6 days) for time after emergence (P=0.0007;F=13.23;DF=1). The error bars shows that there is little uncertaity. There is a significant difference between the number of eggs in the ovariole for 24 hours (a) and the other two treatment, however there is no significant difference between the 3 days and 6 days (b) after emergence.
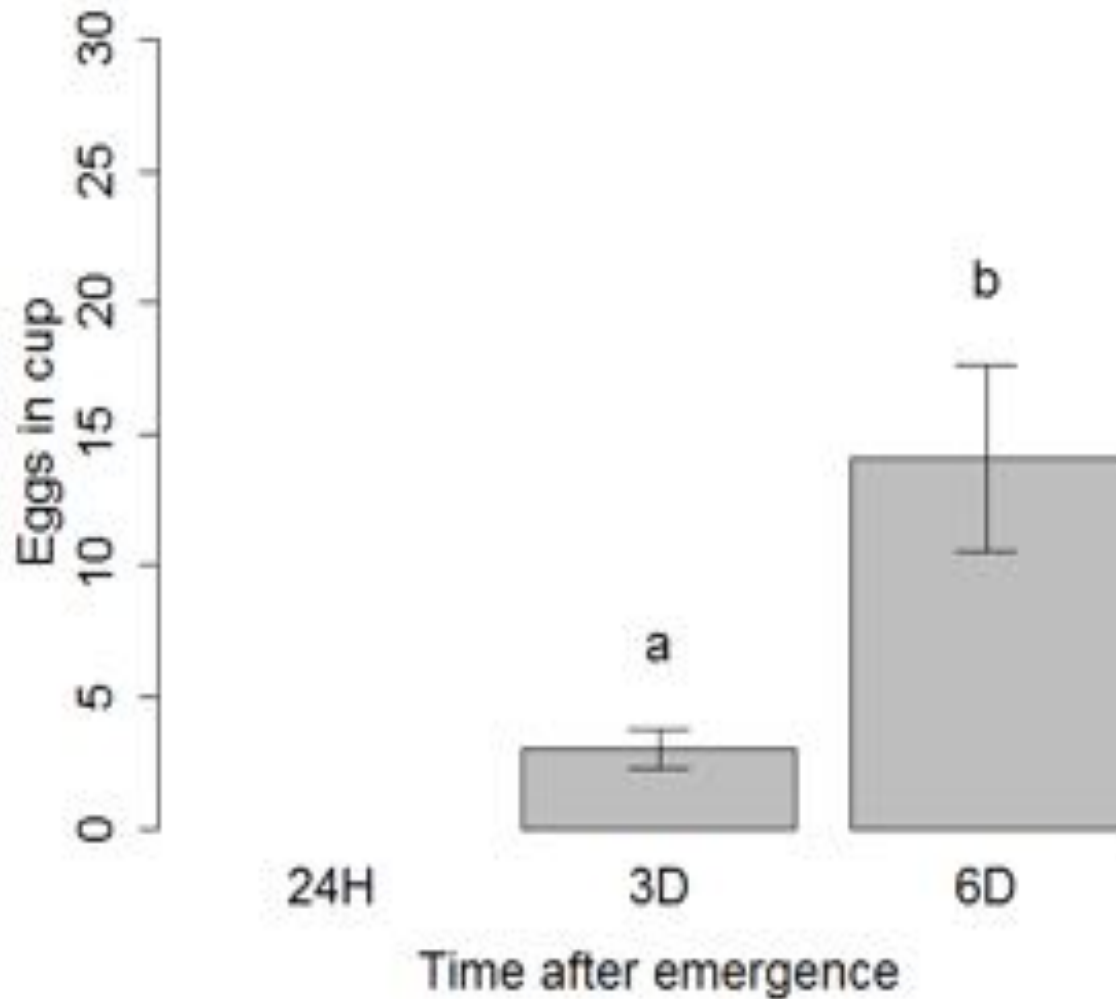
Fig. 17. A comparrison of the total eggs laid in cups for the different treatments (24 hours, 3 days, 6 days) for time after emergence (P<0.0001; F=23; Df=2). There were no eggs laid at 24 hours after emergence. There in significant difference between 3 days (a) and 6 days (b) after emergence for the amount of eggs laid, however there is some uncertainty for 6 days as the error bar is quite large.
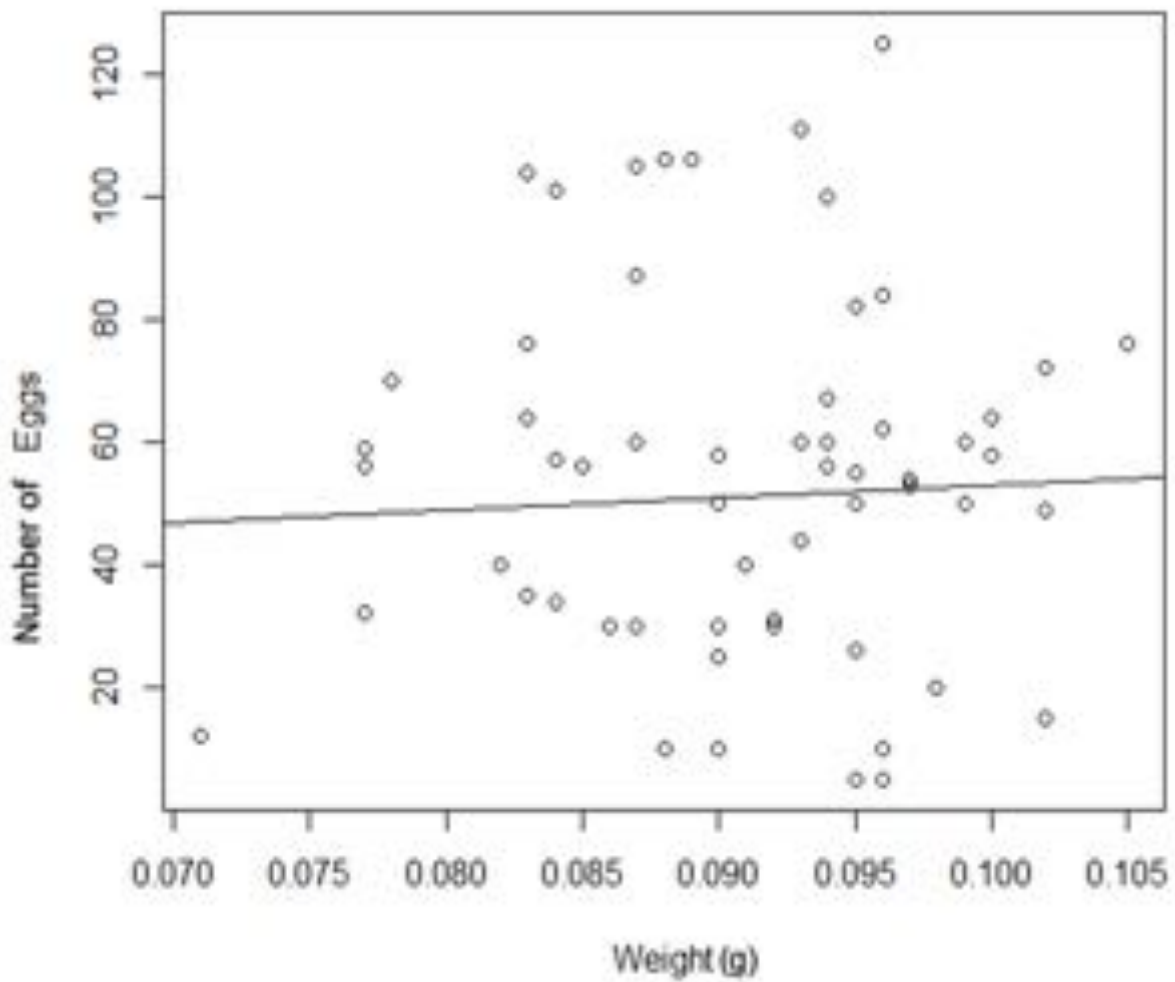
Fig. 18. The number of eggs after emergence (24 hours) compared with pupal weight (grams) (P=0.7169; F=0.1328; Df=1).
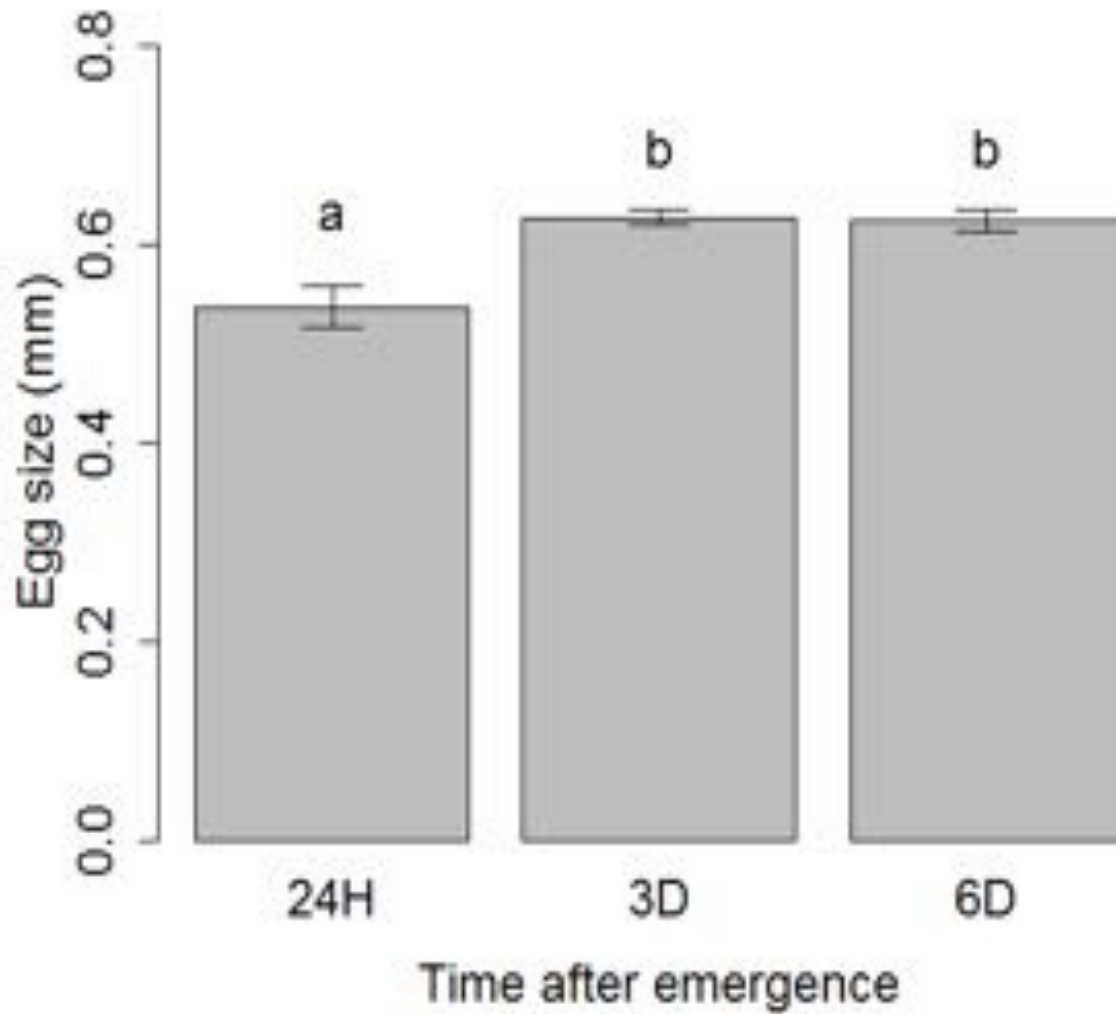
Fig. 19. The size of the egg (mm) compared to the time after emergence for the three treatments (24 hours, 3 days, 6 days). (P=0.0003; Df=2; Chi-Squared=16.028) There is little uncertainty as shown by the error bars, and there is a significant change in egg size between 24 hours (a) the other two treatments, 3- and 6 days (b).
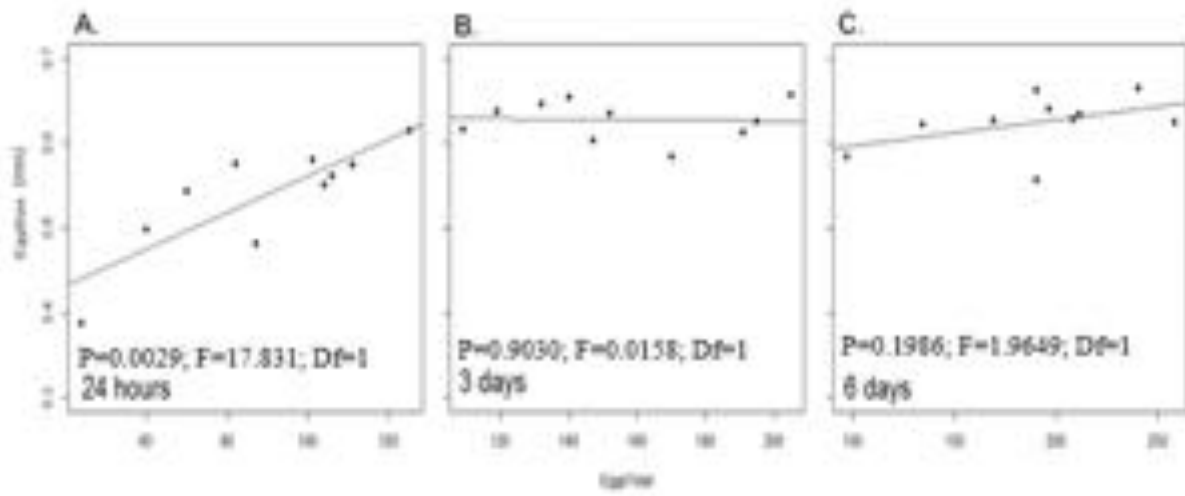
Fig. 20. Egg size (mm) compared to the total number of eggs over the three treatments (A.;24H, B.; 3 days, and C.; 6 days).
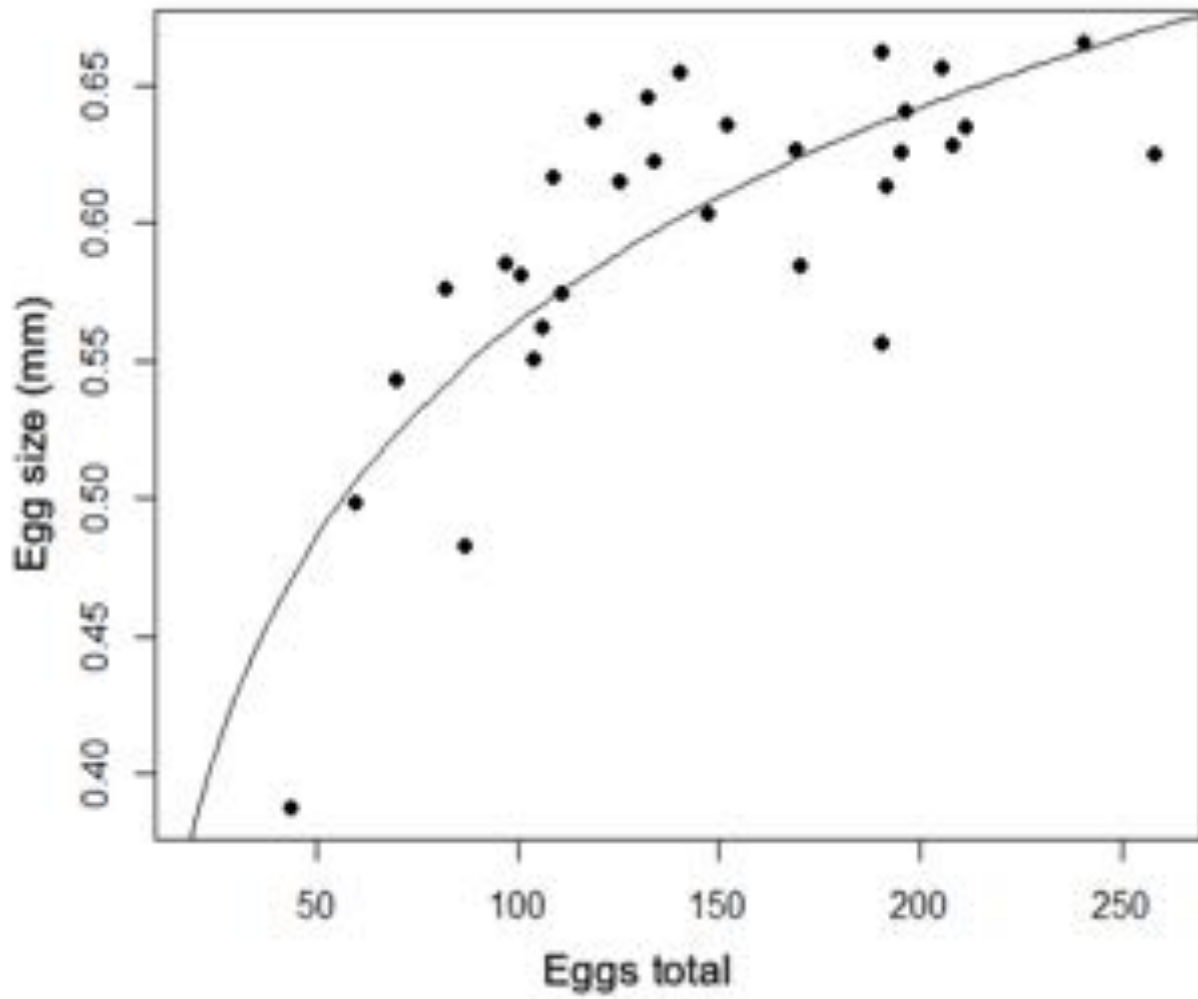
Fig. 21. A curve of the egg size (mm) versus the total number of eggs in the ovariole (P=0.0001; F=49.14; Df=1).
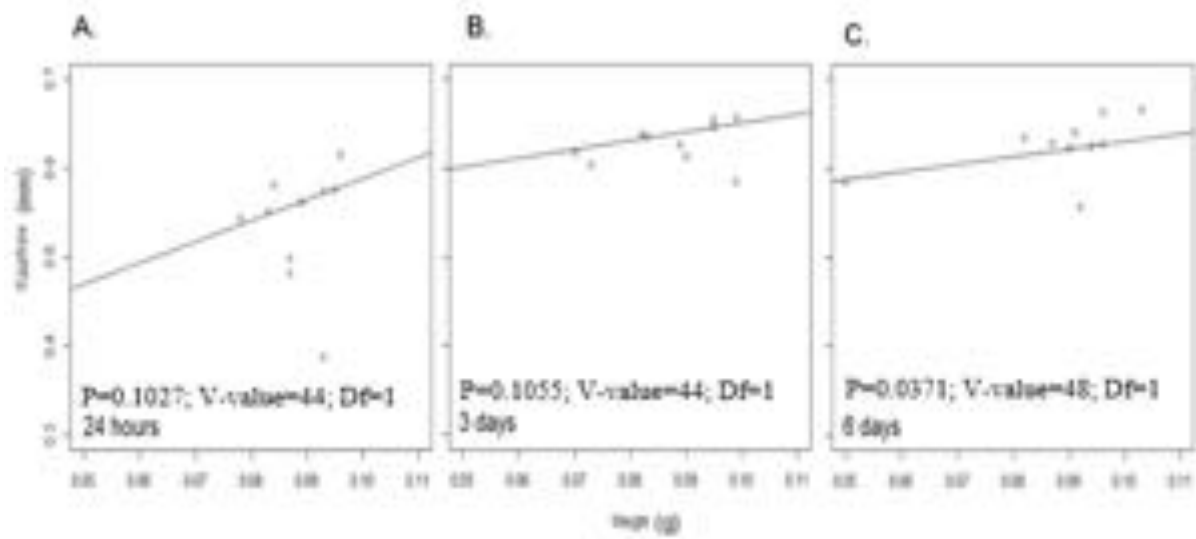
Fig. 22. Egg size (mm) compared to pupal weight (grams) for the three treatments (A.;24H, B.; 3 days, and C.; 6 days).