

# Life History and Mating Behavior of *Rhagoletis solanophaga* (Diptera: Tephritidae), a Non-Diapausing Species with Highly Variable Mating Duration

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**Abstract** As an initial contribution to understanding the adaptive value of behavioral and life-history strategies, the life cycle and mating behavior of an unstudied species of tephritid fruit fly in the genus *Rhagoletis* are characterized for the first time. Over a 9-month fruiting period, a small proportion of *Solanum appendiculatum* Dunal (< 10 %) was found to be infested with a single larva of *Rhagoletis solanophaga* (Hernández & Frías). The average duration of *R. solanophaga* lifecycle (c.a. 140 days from egg laying to death of adults) exceeded the three month fruitless period. Additionally, *R. solanophaga* is capable of exploiting Solanaceous plants in at least two genera. These features could have selected for a non-diapausing species of *Rhagoletis*, a genus where most species are univoltine. Nevertheless, some individuals in the population became dormant. As other members of the genus, *R. solanophaga* exhibited a resource defense mating system with forced copulations and multiple mating. Both males and females could be highly promiscuous and individual mating success exhibited a wide range of outcomes. Regardless of mating success, mated females stored similar amounts of sperm in two spherical spermathecae. Long copulations were observed, perhaps functioning as a form of mate guarding with probable disadvantages for females. We outline hypotheses and opportunities for future comparative studies examining sperm competition and mate guarding.

**Keywords** Mate guarding · sperm competition · copulation duration · paternity assurance

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## Introduction

Understanding of the adaptive value of behavior and life history strategies requires both knowledge of phylogenetic relationships of the organisms that possess them and the nature of the environment where behavioral and demographic traits evolve (Prokopy and Papaj 2000). In the case of specialist frugivorous insects, host plant fruiting phenology is a key determinant of life history evolution (Tauber et al. 1986, Delinger 2002, Mattsson et al. 2015) and has been postulated to influence the evolution of particular mating systems (Headrick and Goeden 1994).

The genus *Rhagoletis* is composed of more than 60 valid species (Smith and Bush, 2000), most species in the group are univoltine, specialized, and exhibit a resource defense mating system where males guard fruit against intruding males and copulate with females seeking egg laying sites without exhibiting courtship (Prokopy and Papaj 2000). With the exception of species in the walnut infesting *suavis* group (Lalonde and Mangel 1994, Nufio et al. 2000), females generally lay a single egg per fruit and deposit a host marking pheromone that deters other females from laying eggs in occupied fruit (Prokopy and Papaj 2000). Larval intraspecific competition is usually strong, in most cases a single larva develops from infested fruit with the first larva eliminating successive competitors (Averill and Prokopy 1987). All studied species in the group mate multiply and females do not appear to enter a mating refractory period (Opp and Prokopy 2000). According to findings for *Rhagoletis pomonella* (Walsh) (the only species in which this has been published) sperm is stored in three spermathecae and the second male to copulate fertilizes the majority of the eggs (Opp et al. 1990). After mating, in some species of *Rhagoletis* associated to walnuts, males have been observed to engage in oviposition site guarding (Prokopy and Papaj 2000), and in some cases, males appeared to engage in mate guarding (Opp et al. 1996). In general, larval development in fruit takes 1–3 weeks after egg laying followed by exit and burying into the soil to pupate. Most species of *Rhagoletis* become dormant and emerge as adults during the following fruiting season of their maternal host plant (Boller and Prokopy 1976). Some exceptions to these rules include flies infesting plants in the Solanaceae.

There are 18 species of *Rhagoletis* infesting fruit of plants in the Solanaceae, arranged in four species groups (Hernandez-Ortíz and Frías 1999, Ramirez et al. 2008). Three of these species groups (*nova*, *psalida*, and *ferruginea*) are of exclusive South American distribution, while the *striatella* group includes species occurring in the Caribbean, South America, Central America, and North America. With the exception of one species in the *striatella* species group, neotropical *Rhagoletis* constitute a separate monophyletic group than palearctic and North American species (Ramirez et al. 2008). There is some biological information for some species of South American *Rhagoletis* due to their economic importance. These infest tomato *Lycopersicon esculentum* Mill. and sweet cucumber *Solanum muricatum* Aiton (Smyth 1960, Foote 1981, Frías et al. 2008). All other species are largely unstudied and in some cases even their host plant affiliation is unknown.

With respect to life history evolution, *Rhagoletis*, tend to exploit hosts with seasonal discrete fruiting periods separated by adverse environmental conditions. Such species cope with periods of host scarcity by becoming dormant at the pupal stage (Boller and Prokopy 1976). In the case of species exploiting Solanaceae, *R. lycopersella* (Smyth), *R. nova* (Schiner), *R. conversa* (Brethes) and *Rhagoletis solanophaga* (Hernandez &

Frías) a large proportion of the population (>50 %) has been reported to be at least bivoltine or multivoltine and forgo diapause (Smyth 1960, Frías 1986, 2001, Frías et al. 1991, Hernández-Ortíz and Frías 1999). There is little information on life cycle duration and seasonal host plant phenology for these species, *R. nova* in Chile appears to bridge between *S. muricatum* fruiting periods by exploiting the introduced weed *Solanum nigrum* L. (Frías 1986). It is therefore difficult to determine the nature of life history strategies adopted by solanum infesting *Rhagoletis*.

In the case of *R. solanophaga*, larvae develop in fruit of *Solanum appendiculatum* Dunal, a vine growing on trees of cloud forest habitats with a short relatively dry season and mild winters (Hernández-Ortíz and Frías 1999) most individuals in the population do not enter diapause and there is no information on duration of most life stages, adult longevity, or mating behavior. Interestingly, *R. solanophaga* is sympatric with *Rhagoletis turpiniae* Hernández and *Rhagoletis zoqui* Bush, two species in the *cingulata*, and *suavis* species groups that exploit host plants with discrete fruiting periods and become dormant during periods of host scarcity (Rull et al. 2016a, b). These three species live under identical abiotic conditions, yet host use patterns appear to exert selection on life history traits yielding different outcomes. Generating demographic information of *R. solanophaga* is therefore the basis for future comparative studies.

Here, we surveyed fruiting patterns and infestation by *R. solanophaga* on *S. appendiculatum* monthly over a year and performed a series of demographic and behavioral observations on *R. solanophaga* recovered from such survey. Our goal was to contribute in understanding the evolution of life history strategies of a species of non-diapausing *Rhagoletis* and characterize its mating system.

## Materials and Methods

### Natural Infestation Levels

Fruit was recovered on and under vines of *S. appendiculatum* growing on the grounds of the Instituto de Ecología A.C., the Clavijero Botanical Garden, the Santuario de Bosque de Niebla (19°30'96" N 96°56'38" W), and Las Animas in Xalapa, Veracruz México (19°31'31" N 96°53'13" W)(Fig. 1). Additionally, a collection of fallen fruit under vines of *Lysianthes sideroxiloides* (Schltdl.) Bitter in the Santuario de bosque de niebla, in October 2013, yielded 8 pupae out of which a female *R. solanophaga* emerged. From 36 to 82 fruit were recovered monthly over a year (from December 2012 to November 2013). The climate in the collection area can be classified as Cfb according to Köppen and Geiger, with a rainy season from May to October, and annual rainfall totaling 1587 mm (Climate-Data.org 2015 <http://es.climate-data.org/location/5707/>). Collected fruit was taken to the laboratory and placed in perforated plastic trays over a larger tray lined with moist vermiculite to recover pupae. Trays were kept in a fruit processing room under shelter with no temperature control and inspected at 2–3 day intervals. The time from collection of infested fruit to pupation was recorded. In order to estimate infestation rates, 962 individual fruit were placed in 4.0 cm diameter × 1.5 cm depth Petri Dishes and observed to record the resulting number of pupae, 117 pupae were obtained. Recovered pupae were placed at 24 °C, 65 % R.H., 13/11 L/D cycle and the time elapsed from fruit recovery to adult eclosion was recorded.



**Fig. 1** Male *Rhagoletis solanophaga* guarding fruit of *Solanum appendiculatum* (Dunal), a vine growing on cloud forest trees in central Mexican highlands

### Life Cycle Duration

The duration of development of immature stages, from egg-laying to fruit exit and pupation, was estimated during behavioral observations for non-infested fruit exposed to 4 and 5 pairs of sexually mature adults. The time from pupation to adult eclosion was established as described above. Longevity of adults was estimated by placing newly emerged individual couples in 3 L plastic cages provided with water and food (sugar and protein at a 3:1 ratio) and an artificial egg-laying device [2.5 cm diameter agar sphere with green food coloring as described by Rull et al. 2010] from eclosion until death. Agar spheres were replaced and dissected under a microscope every 2–3 days. Eggs were lined over a piece of dark cloth placed over a piece of cotton moistened with a 2 % sodium benzoate solution on a Petri dish, incubated at 24 °C, and inspected after 6–9 days to record the number of hatched eggs. Experiments were carried out at the Instituto de Ecología (Inecol), Xalapa, Veracruz, Mexico.

### Behavioral Observations

On the day of emergence, adults were sorted by sex and placed in 30 × 30 × 30 cm transparent Plexiglass cages and fed ad libitum a diet consisting of sugar and hydrolyzed yeast (Yeast Hydrolyzed Enzymatic, MP Biomedicals ®), at a 3:1 ratio.

For behavioral and mating observations, 13–18 days after adult eclosion, two separate transparent 30 × 30 × 30 cm cages were set with four 5–10 cm *S. appendiculatum* twigs including 4–6 fruits hung on the cage ceiling with a paper clip. Five and four pairs of flies were released in each cage respectively. On the day before cage set up, flies were individually marked to ease identification and behavioral tracking, this was achieved by applying a dot of acrylic paint (Vinci – Dixon®, EdoMex, Mexico) on the noto-thorax, a procedure that does not affect behaviour or the sexual performance of flies. In cage 1, five colors were used (pink, yellow, white, green and blue), while in cage 2 four colors were used (the same mentioned above except yellow). Cages were maintained at 26 ± 2 °C, light was provided by a window and artificial lights switched on at 7:00 and off at 20:00 h. Pairs were examined and observed daily for 24 consecutive days. Observations

consisted in periodical scanning of cages (c.a. every 15 min) and recording of behavioral activities. Scanning encompassed a daily time period from 6:00 am in the morning until 22:00 pm in evening. Several random observations were made at irregular intervals from 22:00 to 6:00 a.m. to verify the general lack of nocturnal activity of flies.

### **Description of Pre-Copulatory, Copulatory, and General Behavioral Patterns**

Based on notes taken during observations, some typical pre-mating and mating sequences and some general behavioral patterns were described.

### **Daily Activity, Mating Patterns and Duration of Copulation**

General behavioral activities were categorized and recorded as follows: i) Rest (where all the flies in a cage were observed standing motionless); ii) Reduced activity (where flightless one or more flies were observed walking or grooming.); iii) Activity (when at least one fly was observed in flight, signaling, or egg laying). In the case of mating activity, mating events were recorded when there was at least one couple *in copula*. Two distinct copulation durations were observed and classified as follows: “short copulations”, which started and ended in the course of the same day, and “long copulations”, where the couple remained *in copula* overnight and separated the following day. Additionally, the place where the copulation started was recorded [over the host (on leaves or fruit) or over the cage (on walls, bottom, ceiling, or on diet trays or water containers)].

### **Morphological Features of Observed Adults**

At the end of observations, adults were placed in vials with 70 % alcohol and dissected under a stereoscopic microscope (Nikon SMZ 1500, Nikon Instruments®, Melville, NY). Relative adult size for all observed flies (both sexes) was estimated by measuring head width by means of NIS elements, AR 2.20® (Nikon instruments®, Melville NY). In the case of males, testes size, aedagus length, and clasper length was similarly measured. In the case of females, the size of the left and right ovarioles was measured, the number of mature eggs in each ovariole was counted. Additionally, both spermathecae were extracted and broken over a drop of saline solution on a microscope slide, covered and observed under a contrast phase microscope (Nikon Eclipse 50i, Nikon Instruments®, Melville NY). The relative sperm number according to three categories was established as none, few, and many. Transferred sperm categories were determined according to Segura et al. (2013), with none = no sperm found, few = few sperm easily individualized, and many = large bundles of sperm.

### **Statistical Analysis**

Copulation frequency was compared among two hour time periods by means of a Chi-square test of total number of copulations observed during a two hour period vs a uniform distribution of copulations. Duration of copulations (from the time of the beginning of the copulation to its end) and mating latency (from time the lights were turned on until the beginning of copulation, in a given day) were compared between

short and long copulations with *t* tests for independent samples. A Pearson correlation was used to analyze the relationship between copulation duration and mating latency using all copulations (short and long copulations) and between copulation duration of short or long copulations and their respective mating latency. Only for correlation analysis, two outliers corresponding to “long copulations” were removed (of 2238 and 2452 min of copulation duration). To establish the relationship between mating success (total number of copulations achieved by an individual) and morphological features head width (relative size), size of ovarioles, egg load in the case of females and head width, testes size, and aedagus length in the case of males, correlation analyses were used.

## Results

### Life-History and Basic Biology

Monthly fruit collections revealed that infested *S. appendiculatum* fruit could be recovered almost all year round. A three month fruitless seasonal period was recorded from May to August, corresponding to the beginning of the rainy season in Xalapa (CNA 2005). Despite fruit availability during the rest of the season, *R. solanophaga* adults and larvae occurred at low densities, a single adult male, guarding fruit was sighted in September during fruit collections. An average of  $5.82 \pm 3.58$  % collected fruit was found to be infested, usually with a single larva. Infested *L. sideroxiloides* represents a new host plant record for *R. solanophaga*. Exposure of uninfested fruit in Plexiglass cages revealed that the egg-larvae period took an average of  $18.64 \pm 2.02$  days from fruit exposure to mated couples (egg laying) to pupation. Records from individual fruit and pupae revealed that adults took an average of  $34.41 \pm 5.28$  days to develop, implying that no individuals in this sample became dormant. Nevertheless, two individuals collected during December and January took 82 and 76 days from collection to adult eclosion respectively, implying that a small proportion of individuals in the population may enter diapause or become quiescent in response to unidentified environmental cues. For individual couples held in plastic 3 L cages, adult longevity was estimated at  $79.6 \pm 30.9$  (s.d.) days.

Three females observed in 3 L cages laid eggs in agar spheres, one female laid in four spheres 2 eggs in one occasion and one egg in three occasions, the second females laid on two spheres, 23 eggs and 5 eggs, and the third females laid in two spheres, 4 eggs and 1 egg. In all cases, all eggs were laid singly and eclosion ranged from 0 to 100 % with a mean of  $33.7 \pm 47.4$  %. All eggs that eclosed, did so between 4 and 6 days after sphere dissection (no daily records were made).

### Behavioral Descriptions

As for other members of the genus *Rhagoletis* no males were seen calling or emitting pheromone. When facing members of the opposite or same sex adults usually engaged in brief wing displays. No evident courtship behavioral sequence was observed. Mating approaches were brisk, with males usually abruptly jumping on females to mount them and attempt intromission. Females could resist penetration by shaking vigorously and

occasionally dropping to the cage floor and rolling. After egg laying, which could last for up to ten minutes females could engage in typical host marking behavior, dragging their extended aculeus over fruit, presumably to deposit host marking pheromone. No boxing or pawing behaviors between males were recorded on guarded fruit.

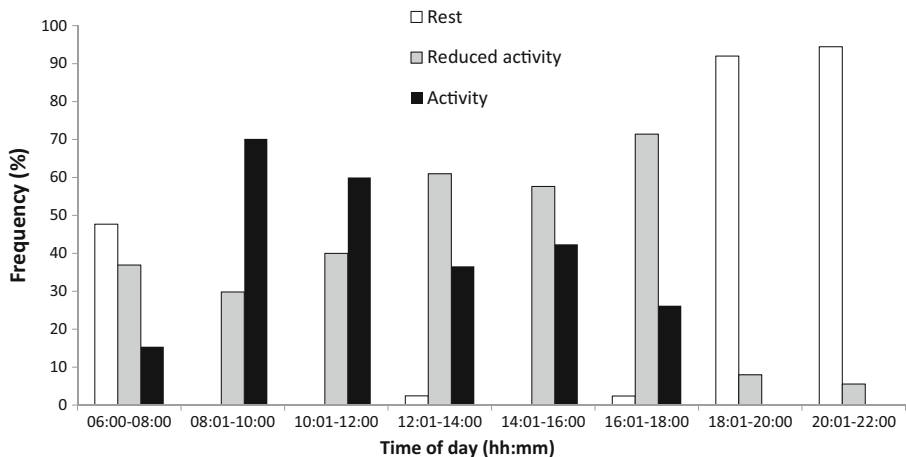
### Diel Activity and Mating Patterns

Flies became active soon after sunrise and artificial lights were turned on with natural and artificial light. There was a mid-morning activity peak between 08:00 am and 12:00 pm with activity declining gradually towards dusk (Fig. 2). Flies were generally observed at rest at night, with couples engaged in long copulations also remaining motionless. The peak of mating activity occurred between 08:00 pm and 10:00 pm. There were more copulations than expected from a uniform distribution of mating activity during the 8:00–10:00, 10:00–12:00 and 14:00–16:00 observational periods (Chi-square = 38:48;  $p < 0.01$ ). Mating however could initiate at any time during periods of daylight (Fig. 3).

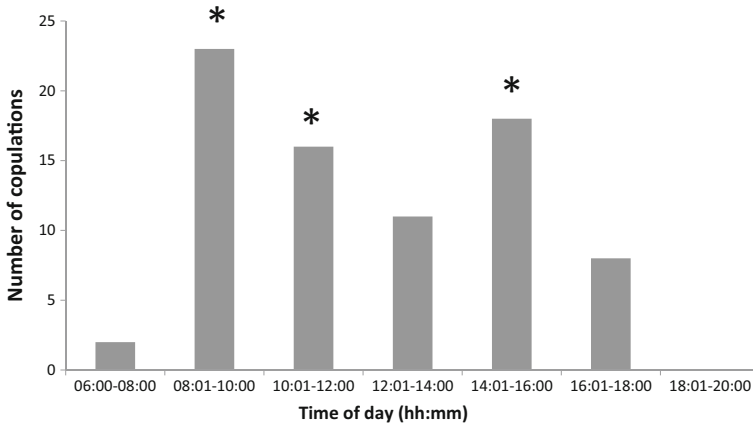
### Duration of Copulations

In total, 79 copulations were recorded, 54 in cage 1 and 25 in cage 2. Of these, 31 were “short copulations” ( $475 \pm 46$  (S.E.) min) and 48 were “long copulations” ( $1077 \pm 44$  (S.E.) min). Copulation duration and mating latency differed between short and long copulations (Fig. 4). Short copulations were significantly shorter than long copulations ( $t$ -test,  $t = -8.67$ ;  $df = 77$ ;  $P < 0.0001$ ). Short copulations began earlier ( $196 \pm 22$  min) in the day than long copulations ( $373 \pm 24$  min) copulations (mating latency (mean  $\pm$  S.E.) from lights on (7:00 A.M.) to intromission) ( $t$ -test,  $t = -4.94$ ;  $df = 77$ ;  $P < 0.0001$ ).

There was no correlation between copulation duration and mating latency when overall copulations were included (short plus long copulations) (Fig. 5). When both



**Fig. 2** Diel activity patterns for two cohorts (4 and 5 couples) of *Rhagoletis solanophaga* sexually mature adults (13–18 day old) over 24 observational days

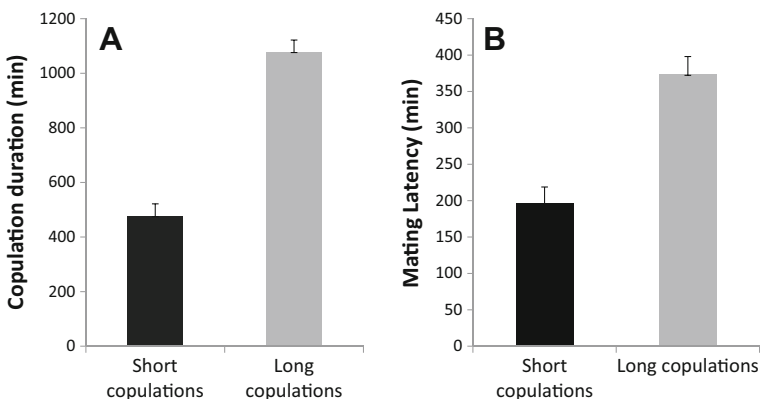


**Fig. 3** Temporal distribution of mating activity (total observed copulations) for two cohorts (4 and 5 couples) of *Rhagoletis solanophaga* sexually mature adults (13–18 day old) over 24 observational days

types of copulation were analyzed separately, there was a negative correlation between copulation duration and mating latency (Fig. 6). That is, both in short and long copulations, copulation duration were shorter when they began later.

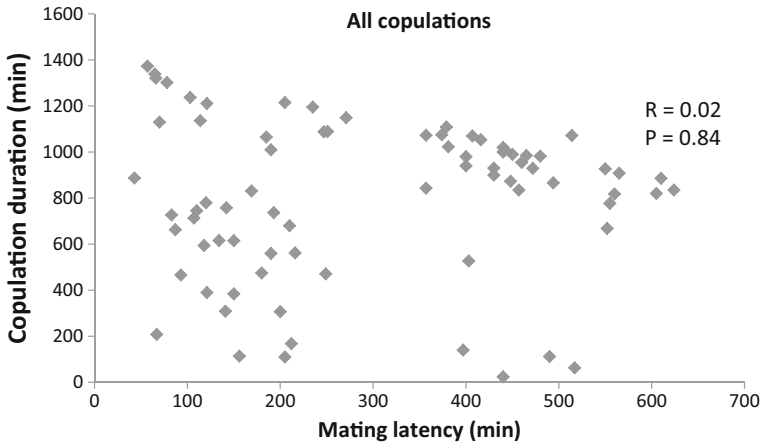
### Mating Success

Of the total number of observed copulations ( $N = 79$ ), 13 started over the host, 23 on the mesh, 15 on the floor, 14 on the roof, 4 on the food, 5 on the water bottle and for 5 copulations the place of copulation initiation was not recorded. Mating success (total number of individual copulations achieved throughout the entire observational period) differed markedly among females and males (Table 1) ranging between 0 and 17, and 0 and 26 respectively. There was no correlation between mating success and relative female size (head width) ( $P = 0.59$ ), size of ovarioles ( $P = 0.1$ ), or egg load ( $P = 0.49$ ), nor between mating success and relative male size ( $P = 0.98$ ), testes size ( $P = 0.81$ ), or aedeagus length ( $P = 0.62$ ). All dissected females except an unmated one in cage 2



**Fig. 4** Average  $\pm$  s.e. duration (a) in minutes and latency (b) (time elapsed in minutes from 6:00 a.m. [lights on] to the beginning of copulation) to mate for short (started and ended in the course of the same day; black bars) and long (couple remaining *in copula* overnight; grey bars) copulations in *Rhagoletis solanophaga*



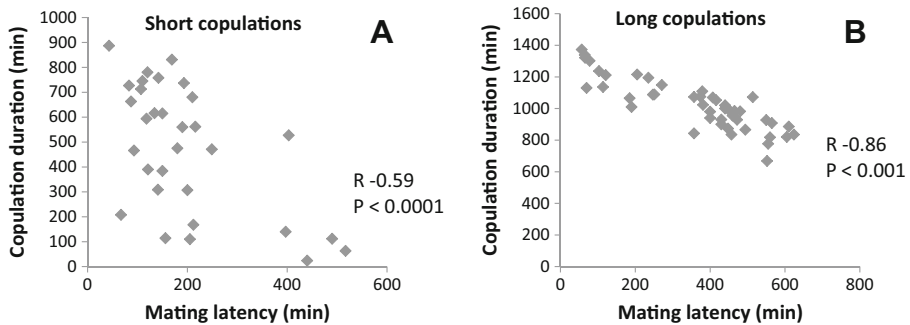


**Fig. 5** Correlation between latency (time elapsed in minutes from 6:00 a.m. [lights on] to the beginning of copulation) to mate and duration of copulation in minutes for all observed copulations among two cohorts (5 couples and 4 couples) of sexually mature *Rhagoletis solanophaga* (13–18 days old) over 24 observational days

(Fig. 7a) and a virgin control had many sperm (large bundles of sperm in which no spermatozoid could be individualized) in their spermathecae (Fig. 7b), suggesting that similar sperm transfer occurs even when few copulations (2) are achieved by individual females.

**Discussion**

Host plant surveys revealed that except for a three month period from May to August corresponding to the end of the dry season, *S. appendiculatum* fruit can be found in Xalapa most of the year. Over the 9 month fruiting period, a small proportion of fruit (< 10 %) was found to be infested usually with a single larva of *R. solanophaga*. The average duration of *R. solanophaga* lifecycle (c.a. 140 days from egg laying to death of adults) exceeded the three month fruitless period of the dry season. Additionally we



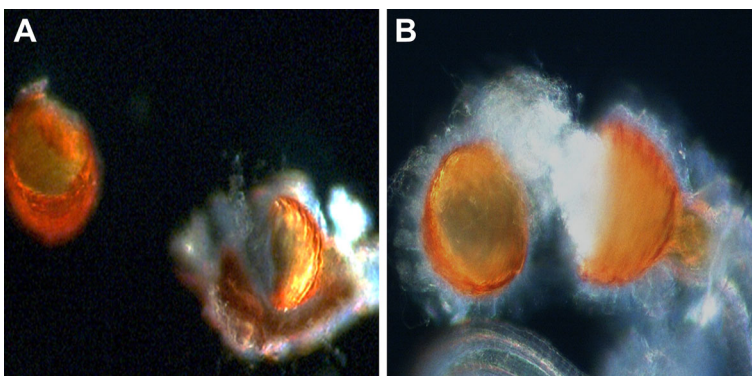
**Fig. 6** Correlation between latency (time elapsed in minutes from 6:00 a.m. [lights on] to the beginning of copulation) to mate and duration of **a** short (started and ended in the course of the same day) and **b** long (couple remaining *in copula* overnight) copulations in minutes among two cohorts (5 couples and 4 couples) of sexually mature *Rhagoletis solanophaga* (13–18 days old) over 24 observational days

**Table 1** Individual total number of copulations for male and female sexually mature *Rhagoletis solanophaga* (13–18 days old) over 24 observation days

Color		Pink	White	Blue	Green	Yellow
Cohort 1	Female	9	12	17	16	0
	Male	0	9	4	15	26
Cohort 2	Female	6	17	2	0	-
	Male	7	15	1	2	-

discovered that *R. solanophaga*, as other members of *Rhagoletis*, is oligophagous, as it was capable of exploiting Solanaceous plants in at least two genera. These features and particular environmental conditions could have selected for a non-diausing species of *Rhagoletis*, a genus where most species are univoltine (Boller and Prokopy 1976). Notwithstanding the above, some individuals in the population became dormant. As other members of the genus, *R. solanophaga* exhibited a resource defense mating system with forced copulations and multiple matings. Long copulations were observed, perhaps functioning as a form of mate guarding.

Most species in the genus *Rhagoletis* are oligophagous, univoltine and undergo pupal diapause (Boller and Prokopy 1976, Prokopy and Papaj 2000). Dormancy can be conceived as a state of suppressed development that evolved among animals to cope with long periods of adverse conditions (Kostal 2006). In temperate areas, such periods are characterized by the prevalence of extreme low temperatures where insects typically undergo an endogenously centrally mediated diapause process (Danks 1991). The relative importance of biotic and abiotic conditions on diapause regulation in the genus *Rhagoletis* in particular, and sub-tropical species of insects in general, is poorly understood (Denlinger 1986, Huestis and Lehmann 2014). At mid elevations in central Veracruz, within the native range of *R. solanophaga*, winters are milder than those in areas where overwintering strategies of most species of *Rhagoletis* have been studied in detail. Factors affecting diapause such as day length, pre-winter temperatures, and winter duration (Prokopy 1968, Filchak and Feder 1999, Feder et al. 2010) are less variable in tropical and sub-tropical highlands than at more northern latitudes. In

**Fig. 7** Spherical spermatheca of mated *Rhagoletis solanophaga* female containing **a** no sperm and **b** many sperm (large bundles of sperm in which no spermatozoid can be individualized) after mating

Xalapa, *R. solanophaga* is sympatric with *R. zoqui* and *R. turpiniae*, two univoltine species that do enter diapause (Rull et al. 2016a, b). While the three species experience identical physical environmental conditions (temperature, humidity, day length), *R. turpiniae* and *R. zoqui* exploit host plants with a single discrete fruiting period (*Turpinia insignis* (Kunth) and *Juglans Pyriformis* Liebm.) (Rull et al. 2016a, b) and *R. solanophaga* does not. Sampling during our study revealed that egg laying sites are available almost all year and demographic studies demonstrated that a single cohort can outlive periods of host scarcity at the end of the dry season. *R. solanophaga* belongs in the *striatella* species group (Hernández-Ortiz and Frías 1999). While the range of *Rhagoletis striatella* (Wulp) reaches as far north as Wisconsin (Bush 1966), phylogenetic studies have dated a putative split between North American and South American Solanaceous infesting *Rhagoletis* at about 4.33 Mya (Ramirez et al. 2008). For all studied species of South American *Rhagoletis* a large proportion of the population is multivoltine (Frías 1986, Frías et al. 1991, Hernández-Ortiz and Frías 1999) with some species bridging fruitless periods of main Solanaceous hosts by exploiting alternate plants in the same family with different fruiting phenology (Frías et al. 1984, Frías 1986). It is therefore currently unknown if non-diapusing *R. solanophaga* originated from non-diapusing South American ancestors or were directly selected for by host plant availability and benign environmental conditions.

All studied species in the genus *Rhagoletis* are highly promiscuous, and the last male to copulate fertilizes most of the eggs laid by the female (Prokopy and Papaj 2000, Opp and Prokopy 2000). Such a condition may favor the evolution of mate guarding among competing males (Alonso-Pimentel and Papaj 1996, Opp et al. 1996). Males may engage in long copulations with high quality females to preclude competing males from mating, transfer sufficient sperm to prevent remating due to sperm depletion, and/or flood out previously deposited sperm from rivals (Carsten and Papaj 2005). Long copulations in contrast may be disadvantageous for females if they reduce time available for egg laying, increase the risk of inbreeding, reduce fertility assurance, and/or the opportunity to mate with better males (Keller and Waller 2002; Chapman et al. 2003; Carsten and Papaj 2005). *Rhagoletis solanophaga* could be a good model system to study sexual conflict over mating rate.

We found that *R. solanophaga* is highly promiscuous. Although weather or not there is last male sperm precedence for this species is currently unknown, *R. solanophaga* has large spherical spermathecae (Hernández-Ortiz and Frías 1999), a condition supposed to favor sperm mixing. In the case of South American species of *Rhagoletis* (as far as we know all associated to plants in the Solanaceae), it is interesting to note that species on the Pacific slopes of the Andes have elongated spermathecae, while for all species on the Eastern side they are spherical in shape (Ramirez et al. 2008). Comparing the mating system of species on both sides of the Andes may shed some light on the effect of these morphological features on mating behavior.

Oviposition site guarding is common among several species of Tephritidae and in particular among all walnut infesting species of *Rhagoletis* (Papaj 1994). Such behavior has been hypothesized to increase access to females, but could also function as a form of male paternity assurance strategy. High variability in copula duration has been reported for other Solanaceous infesting Tephritids (Rull et al. 2016a, b), and has also been hypothesized to constitute a male paternity assurance strategy. We observed high variability in mating duration during our study, a feature that has been found among

Diptera possessing spherical spermathecae when there is sperm competition (Wilkinson and Johns 2005). The sperm loading hypothesis states that males engage in long copulations to transfer more sperm (Dickinson 1986). Alternatively, male dung flies have been found to engage in longer copulations when mating with previously mated than with virgin females, presumably to displace rival sperm (Simmons and Parker 1992). We found that regardless of the number of lifetime copulations female *R. solanophaga* stored large quantities of sperm, suggesting that a single copulation is sufficient to supply enough sperm for egg fertilization. Comparing mating duration between couples of previously mated and virgin females could provide support for the sperm displacement hypothesis. However, as it has been documented for *Haywardina cuculi* Hendel, we observed that copula duration was longer when copulations began late in the day (Rull et al. 2016a, b), a phenomenon also reported for *Phyrocoris* fireflies (Schöfl and Taborsky 2002). Because flies are not active at night, this finding suggests that males could remain *in copula* overnight to ensure that females engage in egg laying the following day before copulating with a competing male. Males of several species of Tephritidae have been found to guard egg laying sites (Papaj 1994, Dodson 1997, Prokopy and Papaj 2000). In the case of walnut infesting *R. zoqui*, males guard fruit after mating and re-copulate with females when a second male interferes with egg laying of recently copulated females or if such females leave guarded fruit and return for other reasons (Guillen unpublished results). Support for this hypothesis could come from field observations of egg laying behavior, although such study may prove to be difficult considering that *R. solanophaga* seems to occur at low densities.

Copulation duration could also be the result of copulatory courtship where females have control of sperm transfer or storage (Eberhard 1991). Copulatory courtship has been documented among several species of Diptera (Eberhard 1994) and in some cases has been demonstrated to influence mating duration, sperm storage, and fertilization success (Otronen and Siva-Jothy 1991). For example, some male features such as relative body size or male clasper shape have been shown to influence copulatory courtship, mating duration, and fertilization success of *Dryomyza anilis* (Fall) flies (Otronen 1997, 1998). Alternatively, it has been shown that large male dung flies displace sperm faster than small males (Simmons and Parker 1992) and engage in longer copulation with large females having greater egg loads than small ones (Parker et al. 1999). Although none of the male or female features that we measured after our observations were related to mating success, our sample size was too small to reach any robust conclusion, and we did not evaluate fertilization success. Comparing copulation duration among male and female *R. solanophaga* of different conditions (size, age, mating status, nutritional state), coupled with close observation of copulatory behavior could be an initial test of the copulatory courtship and sperm displacement hypotheses.

Our study unveiled interesting aspects of the life history and mating behavior of *R. solanophaga* that can lead to finer scrutiny through experimental studies whose results could contribute to the understanding the evolution of dormancy and mating behavior. Several hypothesis regarding variability in mating duration surfaced during our observations and highlight the value of examining behavior of little known species of no economic importance to broaden our understanding of factors that favor the evolution of mating systems and behavioral and life history strategies.

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