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The Effect of Winter Length on Duration of Dormancy and Survival of *Rhagoletis completa* (Diptera: Tephritidae) and Associated Parasitoids From Northeastern Mexico

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Abstract

The walnut husk fly *Rhagoletis completa* (Cresson), native to the Midwestern United States and Mexico, is invasive in California and Europe. It is one of the most important pests of walnuts in areas gathering 30% of the world production. Knowledge of life-history regulation is important for the design of management strategies. Research on dormancy has been performed on invasive populations, and not on populations at the southern extreme of its native range. Here, we examined the effect of winter length on fly and parasitoid emergence, survival, and duration of dormancy. Percent emergence was higher for chill periods at 5°C ranging from 8 to 20 wk. No or insufficient chill resulted in low emergence and a significant proportion of individuals in prolonged dormancy (>1 yr). Duration of dormancy was longer for pupae at constant temperatures and a 4-wk chill period than longer winter durations. Dormancy was longer for Mexican than that reported for U.S. populations, suggesting the existence of a latitudinal cline where populations at southern latitudes have evolved slower metabolic rates. Three parasitoid species were found associated with *R. completa* (*Aganaspis alujai* (Wharton and Ovruski) (Hymenoptera: Figitidae), *Diachasmimorpha juglandis* Muesebeck, and *Diachasmimorpha mellea* Gahan) (Hymenoptera: Braconidae). Results suggest that rearing of *R. completa* is possible by subjecting pupae to chill periods between 8 and 20 wk. Overwintering mortality of flies and *A. alujai* could be further reduced above 5°C. Our findings can contribute for the accurate development of predictive models on invasion potential, development, fly and parasitoid rearing, and biological control.

Key words: Walnut husk fly, dormancy, prolonged dormancy, pest management, biological control

Tephritid fruit flies in the genus *Rhagoletis* are mainly univoltine, specialized stenophagous frugivores that exploit groups of plants with discrete yearly phenology (Bush 1966). Adults have to emerge in synchrony with ripe fruit on which they meet, mate, and deposit eggs to give rise to a new generation (Prokopy and Papaj 2000). Among other specialized herbivorous insects exploiting seasonal resources, *Rhagoletis* fruit flies synchronize emergence with suitable host fruit through dormancy (Bush 1966, Boller and Prokopy 1976, Prokopy and Papaj 2000). This life-history strategy enables flies to exploit relatively predictable host plants (Bush 1966). While most *Rhagoletis* adults emerge during the following season, a proportion of individuals emerges without becoming dormant or after a multi-year or prolonged diapause (Boyce 1931, Dean 1973, Moraiti et al. 2012, Rull et al. 2016, Neven and Yee 2017). A complex interaction between genes and environment is responsible for dormancy regulation (Feder and Filchak 1999, Dambroski and Feder 2007). For the genus *Rhagoletis*, the length of dormancy varies among populations

and species differentially adapting them to variation in host fruiting phenology (Berlocher 2000). Duration of dormancy has been found to result in partial allochronic reproductive isolation among host races of the apple maggot fly *Rhagoletis pomonella* Walsh (Feder and Filchak 1999), and among sister species of walnut infesting *Rhagoletis* (Tadeo et al. 2018).

The walnut husk fly *Rhagoletis completa* Cresson is native to an area encompassing the mid-western and south-central United States to North Eastern Mexico (Bush 1966, Rull et al. 2013, Yee et al. 2014). The species expanded its range to California around 1926 (Boyce 1933, Berlocher 1984, Chen et al. 2006), was introduced to Switzerland in the late 1980s (Merz 1991) and has since invaded most of western Europe (Verheggen et al. 2017, Augustinos et al. 2019). The walnut husk fly is currently one of the most important pests of walnuts in California and Western Europe (Yee and Goughnour 2008, Solar et al. 2019), which together produce 30% of the commercial walnuts worldwide (Shah et al. 2018). Infestation can reach up

to 95% of fruit in unsprayed orchards (Kasana and AliNiazee 1996). Economic losses are the result of nut staining, increased percentage of adhering hull, and % shrivel, and reductions in edible yield and relative value (Coates 2005, Solar et al. 2019). Synthetic pesticide use restrictions have fostered the development of environmentally sound management tactics (Sarles et al. 2015, 2017, 2018) which could include biological control. The only major walnut producing area that has not been invaded by the walnut husk fly is China.

Although dormancy is exceptionally well known at the pupal stage for several *Rhagoletis* species inhabiting temperate areas (Prokopy 1968; Baker and Miller 1978; Van Kirk and AliNiazee 1982; Feder et al. 1997, 2010; Filchak et al. 2000), few studies have been done to understand its regulation for *R. completa*. With some exceptions, the majority of such studies have been performed on Californian or European introduced populations. In California, Boyce (1931) revealed a multi-year dormancy, experimented with the depth in soil from which adults are capable of emerging, and tested the effect of various chemicals attempting to break dormancy of pupae. Gibson and Kearby (1978) described adult phenology of black walnut *Juglans nigra* L. infesting populations in Missouri and reported a proportion of individuals undergoing 2-yr dormancy periods. Kasana and AliNiazee (1994) examined the effect of various constant temperatures on pupal development. Duso and Lago (2006) described the phenology and life cycle of Italian populations. Chen et al. (2006) compared genetic composition and diapause length between Californian introduced and native *R. completa* populations, and Guillén et al. (2011) compared diapause length of Swiss populations stemming from different *Juglans regia* cultivars. More recently, Emery and Mills (2019a) examined the effect of temperature and other environmental factors on post-diapause duration of Californian *R. completa*.

From an applied perspective, it is important to predict seasonal adult emergence from overwintering pupae (Emery and Mills 2019b), in order to optimize the timing and frequency of control measures before fruit damage. Specialized natural enemies can exhibit durations of dormancy synchronized with availability of suitable host larvae in infested fruit (Ovruski et al. 2007). In consequence, dormant parasitoid species are likely to be highly specific and therefore safer candidates for biological control than generalist species. The effect of the winter length on duration of dormancy and percentage and time to emergence for flies and associated parasitoids has not been examined for *R. completa* populations at the southern limits of its native range. This latter information can be of use to accurately delimit areas that this invasive fruit fly can colonize (Kumar et al. 2014, 2016; Qin et al. 2015) especially because they inhabit the warmest area of the species native range. Results of this study can also shed some light on inherent variability and/or plasticity for the species. Additionally, biological control agents absent from currently invaded areas can be identified and some of their biological traits characterized. This work therefore evaluates the effect of winter length on percent emergence, survival, and time to emergence of *R. completa* and associated parasitoids infesting walnut fruits in northeastern Mexico.

Material and Methods

Biological Material

Infested fruits of *Juglans hirsuta* Manning were collected at a single site under the canopy of several trees in the locality of San Juan Bautista, Nuevo León (25°23′36.6″ N, 100°18′05.1″ W; 1,385 masl) on the first week of September 2017. The area is characterized by temperate humid climate with a mean temperature of 14°C, an average rainfall between 600 and 900 mm per year and with cold

winds from the north during the winter. The coldest months of the year are January and February with mean monthly minimal temperatures of 3.5 and 4.5°C, respectively. Collected fruits were taken to the laboratory at the Instituto de Ecología AC and handled as described by Rull et al. (2006) to recover pupae. Pupae were removed from trays every 3–4 d and placed in groups of 100 in transparent plastic 200-ml cups containing 5 g of vermiculite previously moistened with a sodium benzoate solution (3 g/liter) using a hand sprayer. Cups were covered with an aerated mesh lid and taken to a laboratory under controlled environmental conditions at a 24 ± 1°C temperature, a 65 ± 5% HR and a 12:12 (L:D) h photoperiod for 7 d. A total of 30 cups, with 100 pupae each, were prepared and distributed according to 6 different temperature regimes (described below) with five replicates per regime. Following the first week, all cups remained under a 24 ± 1°C pre-winter period during four additional weeks at in the lab. Thereafter, six temperature regimes were evaluated: a first group was maintained under laboratory conditions 24 ± 1°C without undergoing a chilling (winter) period (0 wk). The other cups were transferred to a conventional refrigerator (5 ± 1°C), (roughly corresponding to the average mean minimal winter temperatures of the collection site) in the dark for a winter period of different duration; 4, 8, 12, 16, and 20 wk (hereafter referred to as 4 wk, 8 wk, 12 wk, 16 wk, and 20 wk). Twenty weeks has been used as the optimal chill duration for dormancy studies on *R. completa* Northern populations (Emery and Mills 2019a). After each winter period, cups were returned to the lab, under similar conditions of the pre-winter period, for emergence. Emergence date and sex of flies and parasitoids were recorded twice a week (Monday and Friday) until the end of the experiment; 375 d after pupal recovery from field infested fruit. The duration of the pre-winter period (c.a. 40 d from fruit collection) was established to record proportions of nondormant flies (Rull et al. 2016). Vermiculite of all cups was moistened twice a week during the entire experimental period with a sodium benzoate solution (3 g/liter). At the end of the adult emergence (29 September 2018) uneclosed pupae for all treatments and replicates were inspected under a dissecting microscope to establish the proportion of the pupae having entered a long diapause (live pupae) and the proportion of uneclosed pupae having suffered mortality or puparia failing to form pupae. Time to eclosion for flies and parasitoids was corrected for winter length (substracted). Parasitoids were identified using morphological traits by Larissa Guillen (INECOL). The rate of parasitism (percent) was calculated by dividing the number of emerged parasitoids over the sum of emerged parasitoids and emerged flies.

Statistical Analyses

Cumulative percent post-winter adult emergence for *R. completa* was compared among temperature regimes and sex by means of a GLM with normal distribution (Kolmogorov–Smirnov d = 0.089, P = 0.729), followed by Tukey's HDS mean comparisons.

Cumulative percent post-winter adult emergence for *D. juglandis* was compared among temperature regimes by means of a GLM with normal distribution (Kolmogorov–Smirnov, d = 0.136, P = 0.639), followed by Tukey's HDS mean comparisons. The length of time from the end of artificial winter to adult emergence in dormant individuals, including flies and parasitoids held at constant temperatures, was compared among treatments and sex by means of GLMs with Quasi-Poisson distribution for over dispersion, followed by Bonferroni mean comparisons.

Mean pupal mortality was subjected to a one-way ANOVA, followed by Tukey's HDS mean comparisons. Means percentage of both parasitoid emergence and length of dormancy at constant

temperature were compared with Student *t*-test, considering Welch's test for unequal variances. All analyses were performed using the R-based program Jamovi v.0.9.1.12 (Jamovi 2018).

Results

Percent Emergence of Flies and Parasitoid According to Temperature Regimes

No flies or parasitoids emerged from cups during the pre-winter period (40 d) without becoming dormant. After the pre-winter period, a total of 1,005 *R. completa* adult flies emerged from all pupae reared in the experiment (30 cups, 100 pupae each one), with 557 males (55%) and 448 females (45%). A significantly higher percent *R. completa* emergence was observed for pupae exposed to winter lengths of 8, 12, 16, and 20 wk (38–43%), than for pupae maintained under laboratory constant environmental conditions or for a shorter winter length period of 4 wk (18–25%) ($F = 15.35$; df = 5,48; $P < 0.001$) (Fig. 1a). There was also a statistically significant effect of sex on adult emergence ($F = 12.65$; df = 1,48; $P < 0.001$), and a significant interaction between sex and temperature regime ($F = 5.49$; df = 1,48; $P < 0.001$) with higher proportions of males emerging under the no chill and 4-wk winter period.

A total of 147 parasitoids emerged from cups. Total percent parasitism reached 12.8%. Nineteen of those were identified as *Anagaspis alujai* Wharton & Ovruski and 128 were *Diachasmimorpha* spp. All specimens of *A. alujai* parasitoids were females whereas *Diachasmimorpha* parasitoids were either *Diachasmimorpha*

juglandis (Muesebeck, 1961) (75 wasps, 21 females, and 54 males) or *Diachasmimorpha mellea* (Gahan, 1915) (53 wasps, 21 females, and 32 males). *Diachasmimorpha* species were identified at the end of the experiment due to their morphological similarity. In consequence, emergence and mean duration of dormancy was estimated without distinction between these two species. *Aganaspis alujai* parasitoids only emerged from pupae after dormancy when held at constant temperature, whereas no emergence of this species was observed from any of the winter length treatments. In consequence, all comparisons between *A. alujai* and *Diachasmimorpha* spp. were only made for this temperature regime. There were no significant differences in mean percent emergence between both parasitoids under constant temperature ($t = 0.962$; df = 8; $P = 0.364$), with 3.8 ± 0.9% (mean ± SE) for *A. alujai* and 4.8 ± 0.4% (mean ± SE) for *Diachasmimorpha*. *Diachasmimorpha* emerged from pupae under all temperature regimes, with no significant differences in percent emergence (3 to 5.6%) among different temperature regimes ($F = 0.750$; df = 5,24; $P = 0.594$) (Fig. 1b).

Duration of Dormancy According to Temperature Regimes

The mean emergence time for *R. completa* adults stemming from pupae subjected to constant temperature or different winter lengths was also significantly different ($\chi^2 = 1334.9$; df = 5; $P < 0.001$), although no statistical difference was observed between sexes ($\chi^2 = 0.055$; df = 5; $P = 815$) or the interaction between sex and mean emergence time ($\chi^2 = 10.8$; df = 5; $P = 0.056$). The mean emergence time under constant temperature, ~278 d, was reduced to ~133 d after a winter length of 20 wk, although this reduction was not linear along the winter length duration (Fig. 2a). A large reduction in the meantime to adult fly emergence was observed from constant temperature to a winter length of 4 wk, followed by 8 wk, with a significant reduction in the time to emergence for pupae under winter lengths of 8, 10, 16, and 20 wk.

Parasitoids entered a pronounced dormancy. The mean time to adult emergence for *A. alujai*, was 318 ± 84 d (mean ± SD), and for *Diachasmimorpha* parasitoids, 321 ± 33 d (mean ± SD). The duration of such periods was not statistically different (Welch's $t = 0.182$; df = 22.3; $P = 0.857$). As expected, under constant temperature parasitoid species emerged significantly later than their fly host ($\chi^2 = 228.0$; df = 2,130; $P < 0.001$). Significant differences were observed in the mean emergence time of *Diachasmimorpha* adults when pupae were subjected to constant temperature or different winter lengths ($\chi^2 = 1338$; df = 5; $P < 0.001$) (Fig. 2b). A significant reduction in the meantime to emergence of *Diachasmimorpha* adults was observed from constant temperature to a winter length of 4 and 8 wk, with a slight reduction in the meantime to emergence from 8 wk up to a winter length of 20 wk. As expected, for all different temperature regimes, *Diachasmimorpha* parasitoids emerged as adults later than their fly host ($\chi^2 = 125.59$; df = 5; $P < 0.001$) with a lag emergence time of ~30–40 d.

Survival at the End of the Experiment

A mean (±SE) of 26.6 ± 2.3% of pupae subjected to constant temperature were alive at the end of the experiment (375 d) exhibiting a prolonged dormancy. Pupal survival under constant temperature was not significantly different from pupae subjected to a winter length of 4 wk (21.6 ± 2.4% of pupae alive, mean ±SE), but both treatments had a higher percentage of survival than those held under a winter length of 8 wk (4.6 ± 1.4%, mean ±SE). Longer winter lengths (12, 16, and 20 wk) did not result in any pupal survival (Fig. 1a, indicated above bars).
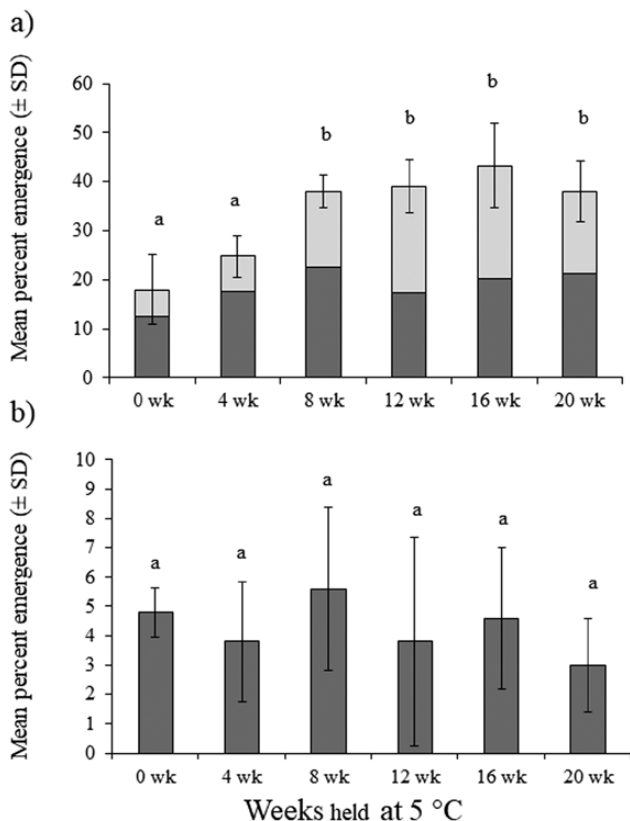

Fig. 1. Mean (±SD) percent emerged (a) *Rhagoletis completa* adult flies (males in dark grey bars followed by females in clear grey bars) and (b) *Diachasmimorpha* spp. adult parasitoids according to different winter lengths. Zero (0 wk) represents the constant temperature regime. Different letters above bars indicate significant differences ($P < 0.05$), Tukey HSD.
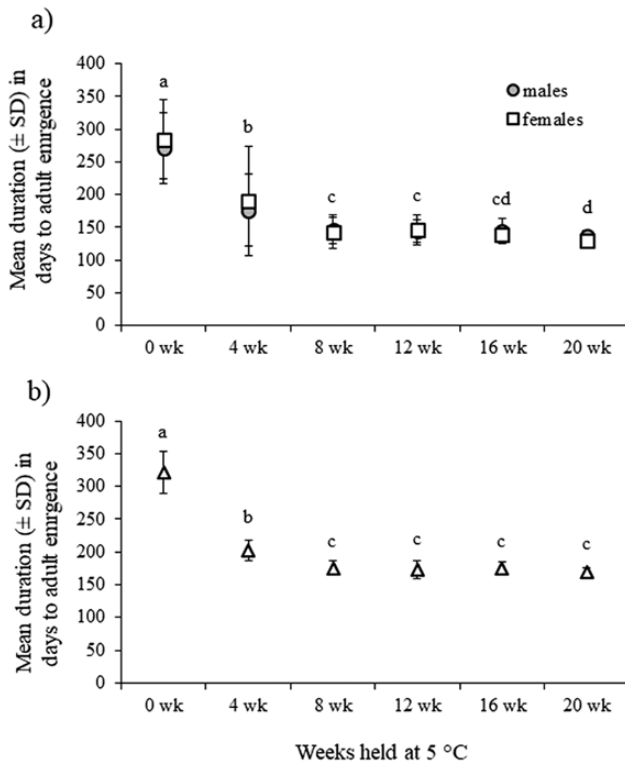
Fig. 2. Mean days (±SD) to adult emergence of (a) *Rhagoletis completa* flies and (b) *Diachasmimorpha* spp. parasitoids according to different winter lengths. Zero (0 wk) represents the constant temperature regime. Different letters above bars indicate significant differences ($P < 0.05$), Bonferoni.

Considering the sum of fly emergence and survival, all treatments produced similarly large proportions (54–62%) of pupae that died without emerging as adults and that were not statistically different in mortality among temperature regimes ($F = 1.17$; df = 5,24; $P = 0.269$).

Discussion

The effect of winter length on fly emergence, survival, and duration of dormancy was explored for *R. completa* exploiting *J. hirsuta* at one collection site in northeastern Mexico. Out of 3,000 pupae handled during this study, no flies or parasitoids emerged without becoming dormant. Boyce (1931) observed that only 0.6% of a 700 pupae *R. completa* lot recovered from Persian walnut *J. regia* L., in California, emerged without becoming dormant when placed under heated laboratory conditions. In the case of other closely related Mexican walnut infesting species, no nondormant flies were recorded for *Rhagoletis zoqui* Bush infesting *Juglans pyriformis* Liebm (Rull et al. 2016). A facultative second generation under particularly favorable environmental conditions has been recorded for several species of *Rhagoletis* at northern latitudes (Boller and Prokopy 1976, Feder and Filchak 1999, Teixeira and Polavarapu 2002). What factors drive the low frequency and persistence of this trait at southern latitudes are currently unknown. Perhaps, because lethal thermal minima are seldom reached in the south, long-lived flies are equally likely to survive after the end of the host availability period than nondormant flies and eventually reproduce under exceptionally long or delayed fruiting seasons and therefore the frequency of the trait may be diluted in comparison with flies at northern latitudes that are killed by cold temperatures.

The mean percent adult emergence of *R. completa* adults was significantly lower when held at constant temperature or under a winter length treatment of 4 wk. This result is consistent with findings by Emery and Mills (2019b) who found lower percent adult emergence for unchilled pupae. Insufficient chilling may be an environmental cue indicating potential problems in synchronizing adult emergence with fruit availability during the following season. It has been concluded, in the case of *R. cerasi*, that plastic responses to interannual climatic variability result in long life cycles of prolonged dormancy due to insufficient or extended cold (Moraiti et al. 2014). Because individuals engaging in prolonged dormancy incur fitness costs (Moraiti et al. 2012), it has been proposed that this life-history trait may be a bet-hedging strategy to cope with unpredictable acyclic environmental variability (Moraiti et al. 2012, Rull et al. 2017). Similar conclusions were reached for weevils exploiting chestnuts in Europe, which are characterized by frequent masting events (Menu and Desouhant 2002). Like chestnuts, walnuts are known for periodic masting followed by sharp drops in fruit production that may select for variability in life cycle duration in the case of *R. completa*.

Although the effect of winter length has been examined for several species in the genus *Rhagoletis* (Feder et al. 1997; Rull et al. 2016, 2017), until very recently it had not been specifically examined for *R. completa*. Emery and Mills (2019a) did not find significant differences in percent emergence among pupae exposed to various chill duration regimes ranging from 0 to 21 wk (0 to 3,600 chill hours). Here, *R. completa* pupae emerged as adults in similar proportions when exposed to chill periods of either 8, 12, 16, or 20 wk. It has been conventionally considered that a chill period of at least 3 mo is required to obtain large proportions (50–75%) of adult emergence of *R. completa*. Such duration may have been arbitrarily established and adopted as a convention. Additionally, Emery and Mills (2019a) report that chill periods starting from 45 d (1,080 chill hours) produce good synchronization of adult emergence. In consequence, for artificial rearing or research purposes, *R. completa* can be handled in the laboratory by exposing pupae to artificial winters for periods starting at 45 d in duration.

Males emerged in higher proportions under constant temperatures or a short winter length (4wk), than under longer winter lengths. This result does not appear to be explained by sex related size differences in *R.completa*, since both sexes are roughly the same size (Emery and Mils 2019a). In general, female arthropods have greater lipid content than males (Lease and Wolf 2011). Among *Rhagoletis* flies, lipid stores play an important role on survival during the final stages of dormancy when metabolic rates increase (Ragland et al. 2009). The observed pattern could be explained by differential energetic budgets between sexes. Insufficient chilling or extended pre-chill periods could have resulted in a depletion of energy stores more acute for one sex than the other. Survival of pupae engaging in a prolonged dormancy, was higher under constant temperature and a winter length of 4 wk, followed by an 8 wk, a winter length over which there was no survival at the end of the experiment. Winter lengths above 8 wk did not improve emergence but rather reduced *R. completa* survival. Large proportions of live non-emerging individuals were observed for pupae exposed to a 20 wk long artificial winter (5°C) in the case of *R. cerasi* (Moraiti et al. 2014) or *Rhagoletis indifferens* Curran (Neven and Yee 2017). However, our results are not in accordance with those observations because no survival was recorded for pupae subjected to extended winter lengths (10-20wk). Winter temperature could be detrimental for pupal survival in our case, as was observed by Moraiti et al. (2014) for *R. cerasi* in Greece, where survival was reduced under long winters at 1 and 5°C, in comparison to warmer 8, 10 or 12°C temperatures.

Perhaps, for subtropical highland populations of *R. completa* in Mexico, winter temperatures of 5°C are too cold and long exposure produces mortality. This could explain the overall lower adult emergence proportions obtained in our study (around 50%) in comparison to findings by Emery and Mills (2018a) for Californian populations (around 75%).

Winter length had a significant effect on the duration of dormancy of *R. completa*. The number of days elapsed from pupation to adult emergence (corrected for winter length) was significantly reduced as winter length increased from 0 to 8 wk, the reduction in duration almost disappeared as winter lengths increased from 8 to 20 wk. A similar pattern, in which longer periods under low temperatures (winter) resulted in faster and synchronous adult eclosion after re-exposure to warm temperatures, was observed for *Rhagoletis cingulata* Loew (Rull et al. 2017), *R. indifferens* (Brown and AliNiazee 1977, Yee et al. 2015) and Californian populations of *R. completa* (Emery and Mils 2019a).

Although the general pattern found for Mexican highland populations was similar to those of other species and populations, the overall mean duration of dormancy is longer than that reported for other *R. completa* populations (Kasana and AliNiazee 1994). Such a feature is consistent with latitudinal clines recorded for *R. pomonella* (Dambroski and Feder 2007) and also for Californian populations of *R. completa* (Emery and Mils 2019a). As proposed by Feder et al. (1993; 1997; 1999) flies exposed to longer periods of warm weather, either because they exploit plants with earlier fruiting phenology, or because they inhabit lower latitudes, tend to evolve slower metabolic rates than those occurring at higher latitudes and/or exploiting late fruiting hosts.

Overall percent parasitism (12.8%) by *A. alujai* and *Diachasmimorpha*, was almost identical to previously reported results for walnut fruits collected in the same locality in 2009 (Rull et al. 2013). However, parasitism rate could have been underestimated due the large numbers of dead (54–62%) pupae, of which a disproportionate number of deaths could have been be caused by successful parasitism leading to unsuccessful life cycle completion of both the host and the parasitoid. Additionally, because *R. completa* was collected as larvae within infested fruit, pupal parasitoids and a proportion of parasitoids infesting late instar larvae having left fruit before collection were not represented in our study. For an accurate estimation of the impact of parasitoids on *R. completa* populations, studies including collections at the pupal stage and including inspection and dissection of field-collected pupae aimed at quantifying the proportion of pupal mortality caused by unsuccessful parasitism, are necessary.

Rull et al. (2013) reported the emergence of only *D. juglandis* parasitoids for pupae collected in the same locality. Although it is well known that *D. mellea* is distributed in central-northern Mexico and have been reared from different *Rhagoletis* species such as *R. pomonella*, *R. mendax*, *R. zephyna*, and *R. cingulata* (Rull et al. 2009, Forbes et al. 2010, Rull 2011), to our knowledge, this is the first time that *D. mellea* parasitoids have been isolated from *R. completa*. In northeastern Mexico, *D. mellea* was found to parasitize 20% of *R. cingulata* pupae (Rull 2011).

Unlike *Diachasmimorpha* parasitoids, *A. alujai* only emerged from pupae subjected to constant temperature and were not recorded from any of the winter length regimes. While the geographical range of *D. juglandis* extends to the native area of distribution of *Rhagoletis junglandis* and *Rhagoletis boycei* in Arizona (Wharton and Marsh 1978), *D. mellea* has been recorded as far north as Canada (Monteith 1971), and *A. alujai* has only been recorded in Mexico at lower latitudes (Ovruski et al. 2007, Rull et al. 2013). Perhaps the thermal minima for these parasitoid species are different

and *A. alujai* is more susceptible to low temperatures. Consequently, overwintering parasitoids within pupae subjected to chill periods at 5°C, could have suffered mortality.

As expected, the mean number of days to emergence for both parasitoids (reared under constant temperature) were similar (318–321 d), but slightly shorter than values previously observed for parasitoids of *R. completa* from the same locality held under uncontrolled ambient temperature in the laboratory at Xalapa, Veracruz (356–358 d) (Rull et al. 2013). The mean time to emergence of *Diachsmimorpha* parasitoids showed similar patterns than fly emergence but with a temporal delay resulting in adult parasitoids emerging later than their fly host. The time lag of host/parasitoid emergence, held true for all temperature regimes, showing that there is strong selection for specialized parasitoids that adjust their life cycle to that of their host, as found for other parasitoid species specialized in flies in the genus *Rhagoletis* (Ovruski et al. 2007, Forbes et al. 2009, Rull et al. 2009). Such feature suggests that *D. juglandis*, *D. mellea*, and *A. alujai* are good candidates for classical or augmentative biological control in areas were *R. completa* is currently invasive. In the case of *A. alujai*, this may be true only for areas experiencing mild winters, such as for example, low elevation areas in Italy (Poggetti et al. 2019) or for Spain, where another species in the genus *Aganaspis* has been found to be associated with the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann (de Pedro et al. 2018).

Our results contribute to a better understanding of the effect of winter length on overwintering survival, percentage emergence and time to emergence of *R. completa* flies, and their parasitoids and are useful for development of rearing techniques. The need to examine overwintering temperatures (above 5°C) for *R. completa* and *A. alujai* was unveiled. Biological control agents were identified, and useful information to model the potential distribution of invasive pestiferous *Rhagoletis*, which may include areas with milder winters than previously though was obtained.

Acknowledgments

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