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Short Communication

Differences in larval emergence chronotypes for sympatric *Rhagoletis brncici* Frías and *Rhagoletis conversa* (Bréthes) (Diptera, Tephritidae)

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ABSTRACT

In central Chile, *Rhagoletis brncici* and *R. conversa*, can be found in sympatry, associated with the fruit of their native host plants: *Solanum tomatillo* and *S. nigrum* (Solanaceae), respectively. Third-stage larvae must emerge from its host in search of pupation sites, and during this period larvae must find an appropriate pupation microhabitat while avoiding predation and adverse abiotic factors. In this study, we explored whether these sympatric species differ in terms of the timing of their larval exit from the host fruit in search of pupation sites. Field-collected fruits from host plants were checked daily for larval emergence, within 24 h, under laboratory conditions, in order to determine the time of the event. We found that these species differed significantly in their diel larval emergence. For *R. brncici*, most larvae left the host fruit between late evening and past midnight, meanwhile larvae from *R. conversa* concentrated their peak of emergence near midnight and early morning. We discuss these findings in terms of the ecological and evolutionary implications of the temporal separation of larval emergence regarding the use of pupation sites, abiotic stress and risk of predation for these sympatric species.

Niche partitioning through the use of different resources is a factor that promotes the coexistence of ecologically similar species in sympatry (Levine and HilleRisLambers, 2009). In insects, such divergence may be expressed as allochronic behaviors: differences in the timing of activity peaks that promote species spatial overlap (Schoener, 1974; Werner and Gilliam, 1984). Temporal segregation in foraging behavior facilitates the sympatric coexistence of insect species from an oak-defoliating assemblage (Kalapanida and Petrakis, 2012). Allochronic behavioral differences in populations of the same species could promote a sympatric speciation. Four sympatric species of ants from the Australian genus *Myrmecia*, for example, are capable of sharing foraging resources and space by being active at different times of the day (Narendra et al., 2016). In the same fashion, it has been found that in the dragonfly species *Anax imperator* Leach, 1815 and *A. parthenope* (Sélys, 1839) (Odonata: Aeshnidae), immatures select similar substrates as support to carry out ecdysis, but they emerge from the water asynchronously to do so, contributing this temporal differentiation to the sympatric coexistence of these species (Boucenna et al., 2018).

In the case of tephritid flies (Diptera), despite many species having been carefully studied due to their importance as pests on several fruit commodities (Uchôa, 2014), the information regarding larval behavior is scarce, and research concerning temporal patterns of larval activity is even less abundant (but see Aluja et al., 2005).

However, considering available studies in tephritid flies, it is proposed that among larvae behaviors, the last-stage larval exit from the host fruit to find pupation sites in the soil is one of the most critical events of this period. At the end of development, the larvae must find a suitable pupation microhabitat with specific soil, temperature and humidity characteristics (Thomas, 1995).

Moreover, wandering larvae must avoid adverse abiotic conditions, such as elevated temperatures that may kill them through dehydration (Aluja et al., 2005; Schwerdtfeger, 1976). On top of all this, while looking for pupation site, larvae must evade active predators and parasitoids (Fernandes et al., 2012; Thomas, 1993, 1995). It has been found that larvae searching for pupation sites may even try to prevent potential predation and/or parasitation during pupation (Bressan-Nascimento, 2001;Wang and Messing, 2004). This implies covering as much distance as possible from the host fruit's kairomones (used by parasitoids to find nearby pupae) and also from other already established pupation sites, as

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larval aggregations are also preferentially predated and parasitized (Guillén et al., 2002).

The genus *Rhagoletis* Loew (Diptera: Tephritidae), with 65 species distributed in the Nearctic, Pelearctic and Neotropical zones (Foote, 1981; Thompson, 1999; White and Elson-Harris, 1994), has been mostly studied regarding their reproductive behavior and oviposition mechanisms (Frías-Lasserre, 2015; Rull et al., 2016). Little information is available regarding other key life cycle events related to the larval stages, such as the timing of larvae rhythmic behaviors.

So far, it is known that after females from *Rhagoletis* species deposit their eggs inside host's fruits, larvae hatch and feed on the mesocarp and seeds, requiring an approximate minimum of two weeks inside the parasitized fruit to growth and develop (Duso and Lago, 2006; Frías, 1986). Following that, the third-instar larvae leave the fruit and enter the soil to pupate and overwinter for several months in diapause (Boller and Prokopy, 1976; Bush, 1992, 1966; Christenson and Foote, 1960; Frías, 1992). Despite the relevance of this temporal event, diel patterns of larval emergence are unknown for many *Rhagoletis* species.

In the Neotropic, most *Rhagoletis* species are associated with Solanaceae (Foote, 1981; Frías et al., 2006; Frías, 2001). In central Chile, for example, *Rhagoletis brncici* Frías and *R. conversa* (Bréthes) (Diptera, Tephritidae) overlap their distributions associated to the phenological cycle of their respective host plants: *Solanum tomatillo* and *S. nigrum* (Solanaceae) (Frías et al., 1984). In this short paper we explore the circadian clocks for larval emergence in two sympatric and closely-related species: We evaluate if there is a diverging diel pattern for larvae leaving the host plant's fruits and discuss emergence trends in overlapping closely-related holometabolous insects.

Observations of larval emergence were carried out under laboratory conditions at 21 ± 1 °C, 65% relative humidity and 14 h: 10 h L(3600 lx):D photoperiod at Instituto de Entomología Universidad Metropolitana de Ciencias de la Educación, Santiago, Chile. The larvae of *R. brncici* and *R. conversa* were obtained with the fruits of their respective host plants, *S. nigrum* and *S. tomatillo* (Solanaceae), in the town of Pirque (33°38′00″S 70°33′00″W) in the Cordillera Province, located 21.3 km south east of Santiago, at an altitude of 697 m.a.s.l.

Between October and December 2017, six periodic fruit collections were made using plastic screw-top jars that had a fine mesh lid to ensure good ventilation. During this period, a total of approximately 6000 fruits of *S. nigrum* and 2000 fruits of *S. tomatillo* were collected. These fruits were transferred to the laboratory and placed on a thin grid on a plastic tray (40 cm × 35 cm × 8 cm). The grid allowed only the passage of the larvae that left the fruits, which fell onto the plastic tray and thus could be counted. During the 24-h larval emergence, records were taken at time intervals of 5 h (Table 1); considering dawn (06:00–11:59); midday (12:00–17:59), dusk (18:00–23:59) and night (00:00–05:59) time intervals (CLST, Chile Summer Time, UTC/GMT −3 h). This observation was repeated 22 times for *R. brncici* and 10 for *R. conversa*. For each species, at each interval, the larval emergence percentage was determined. With the information obtained, the percentage of larvae that emerged was estimated.

Circular statistical analysis was conducted in order to test the occurrence of a given diel pattern of emergence for these species (dos Santos et al., 2017; Fisher, 1993). To test the prevalence of emergence timing on a 24 hr clock for the *Rhagoletis* species, we used a Rayleigh (z) test in order to specify the significance of mean angle for the occurrence of this behavior. We considered as a null hypothesis time intervals for emergence that were evenly distributed around the diel clock. Alternatively, there could be a mean time interval where flies emergences are being concentrated. Furthermore, we compared diel emergence between *R. brncici* and *R. conversa* using Watson–William equal directions test (Morellato et al., 2010).

There was a clear tendency of the larvae of both the studied species to emerge in a time range from 18:00 PM to 5:59 AM. Eighty-three percent of *R. brncici* and 77% of *R. conversa* larvae emerged during this window. Despite the similarity of these emergence times, the larvae of these species, during the specified time window, displayed differences in the proportion of larvae exiting fruit at different times. In *R. conversa*, the highest percentage of larvae exiting fruit was between the hours of 00:00 to 5:59 (65% emergence), when only 39% of *R. brncici* larvae emerge. Between the hours of 18:00 to 23:59, 12% of *R. conversa* larvae emerged, while the emergence of *R. brncici* larvae in the same period was 44%. During daylight hours (6:00–5:59), the percentage emergence of the larvae of both species decreased (18% in *R. brncici* and 23% in *R. conversa*) (Table 1A).

The mean time of larval emergence showed a marked diel pattern for each *Rhagoletis* species with r corresponding to 0.5 and 0.7 for *R. brncici* and *R. conversa* respectively. The null hypothesis of uniform distribution for both species was rejected (Table 1B, Fig. 1). Furthermore, *R. brncici* and *R. conversa* also showed statistical differences, disproving the null hypothesis (Watson–Williams test, $F = 168.873$; $p < 0.0001$).

The sympatric *Rhagoletis* species *R. brncici* and *R. conversa* have a marked diel pattern of larval eclose from their hosts' fruits. Moreover the circadian rhythms for this activity were statistically different between these two closely-related taxa, perhaps as a product of selection produced by host plants that occupy different habitats.

Extrapolating our experimental data to what could happen in nature, the scarce emergence of larvae of both species during daytime may allow them to avoid the higher temperatures recorded for these times in the field (reaching around 30 °C), during the spring–summer season in the Matorral of central Chile (Armesto et al., 2007; Rundel, 1981), thus avoiding dehydration.

Table 1

A: Larval emergence (percentage and sample size) in relation to diel time intervals for *Rhagoletis brncici* and *R. conversa*. B: Circular statistics uniform distribution showing sample size, Raleight test value and statistical significance for both sympatric species.

(A)			(B)					
Species	Time Intervals	Larval Emergence	Sample Size	Mean Direction	Circular Deviation	Length of mean vector (r)	Rayleigh's Stat.	Rayleigh's Prob.
R. brncici	00:00–05:59	39% (335)	867	23.189	4.191	0.548	558.957	<0.001
	06:00–11:59	8% (69)						
	12:00–17:59	10% (84)						
	18:00–23:59	44% (379)						
R. conversa	00:00–05:59	65% (194)	299	2.593	3.391	0.674	302.355	<0.001
	06:00–11:59	22% (66)						
	12:00–17:59	1% (4)						
	18:00–23:59	12% (35)						

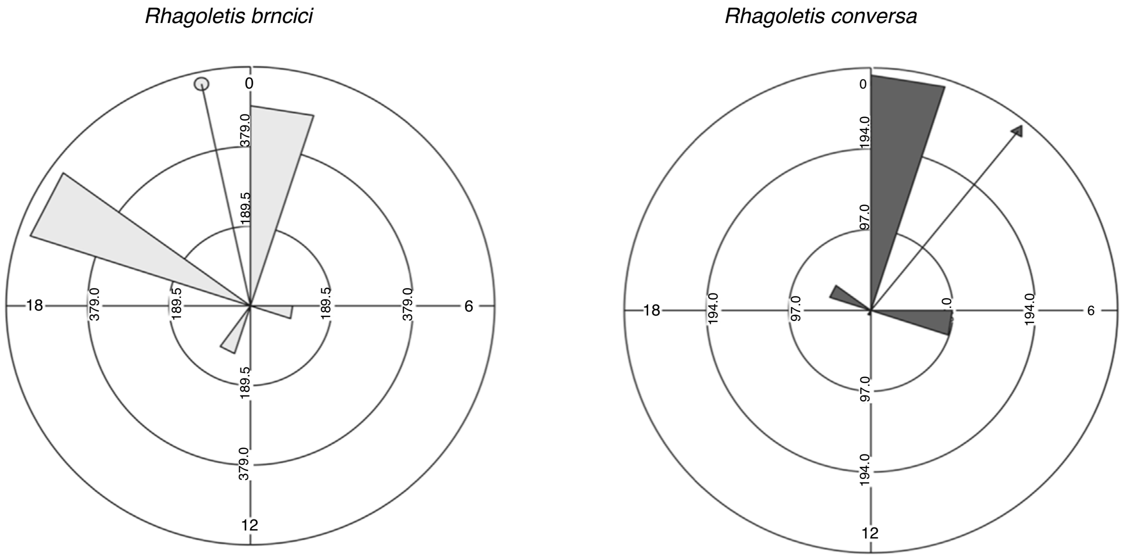

Fig. 1. Circular histogram rose plot showing *Rhagoletis brncici* and *R. conversa* larval emergence from fruit in a 24-h clock. For both graphs, the arrow shows the mean time of emergence. Main numbers in sections correspond to time in hours. Numbers accompanying circumferences refer to number of individual larvae replicates.

It has been documented that predation at the time of third-instar emergence from fruits is one of the factors that affects larval survival (Aluja et al., 2005). The other factor that may influence larval emergence behavior in the species studied is the evasion of potential diurnal predators that have been described for Tephritidae larvae, such as mice (Thomas, 1993, 1995), ants and wasps (Aluja et al., 2005), and also several parasitic microhymenopteran species (Maier, 1981; Ovruski et al., 2000; Bomfim et al., 2007; Hernández-Ortiz et al., 1994; Sivinski et al., 2001, 1997; Taira et al., 2013).

Moreover, the interespecific allochrony detected in larvae emergence, for both *Rhagoletis* species, may be also be related to the differences in geographical distribution found in these species; *R. brncici* tends to inhabit cooler places in the sub-Andean region of central and southern Chile, whereas *R. conversa* is distributed in warmer localities in central and northern Chile (Frías et al., 1984; Frías, 2001). This kind of pattern has been found in species of other genera of Thephritidae such as *Bactrocera* (Danjuma et al., 2014) and *Ceratitis* (Duyck and Quilici, 2002).

As a final remark, we can comment that it is necessary to further study larval exit timing differences in these two *Rhagoletis* species in allopatric populations, considering climatic variables and their respective *Solanum* host plants, in order to test if temporal difference between *R. conversa* and *R. brncici* larvae may fit to potential physiological adaptations of these flies within their distribution. Moreover, it would also be of great interest to compare this with sympatric populations in order to test for differences in emergence regarding allopatric populations. These comparisons may allow us to disentangle whether changes in larval exit evolved as an isolating mechanism when these two species overlap.

Conflicts of interest

The authors declare no conflicts of interest.

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