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Contrast in Post-Chill Eclosion Time Strategies Between Two Specialist Braconid Wasps (Hymenoptera: Braconidae) Attacking *Rhagoletis* Flies (Diptera: Tephritidae) in Western North America

Wee L. Yee,[1,4] Andrew A. Forbes,[2] and Jeffrey L. Feder[3]

[1]USDA-ARS, Temperate Tree Fruit and Vegetable Research Unit, 5230 Konnowac Pass Rd, Wapato, WA 98951, USA, [2]Department of Biology, The University of Iowa, Iowa City, IA 52242, USA, [3]Department Biological Sciences, University of Notre Dame, Notre Dame, IN 46556, USA, and [4]Corresponding author, e-mail: wee.yee@usda.gov

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Abstract

Parasitoids comprise a speciose insect group, displaying a wide array of life history strategies. In the Pacific Northwest of the United States, the tephritid fruit flies *Rhagoletis tabellaria* (Fitch) and *Rhagoletis indifferens* Curran infest red osier dogwood, *Cornus sericea* L. (Cornaceae), and bitter cherry, *Prunus emarginata* (Douglas ex Hooker) Eaton (Rosaceae), respectively. The flies are parasitized by different braconid wasps at different life stages; *Utetes tabellariae* (Fischer) oviposits into *R. tabellaria* eggs, whereas *Diachasma muliebre* (Muesebeck) oviposits into *R. indifferens* larvae feeding in cherries. Because *Rhagoletis* only have one major generation a year and the wasps attack temporally distinct fly life stages, we predicted that eclosion times of *U. tabellariae* should more closely follow that of its host than the larval-attacking *D. muliebre*. As predicted, *U. tabellariae* eclosed on average 6.0–12.5 d later than *R. tabellaria*, whereas *D. muliebre* eclosed on average 32.1 d after *R. indifferens*. Unexpectedly, however, longer chill duration differentially affected the systems; longer overwinters minimally influenced eclosion times of *R. tabellaria* and *U. tabellariae* but caused earlier eclosion of both *R. indifferens* and *D. muliebre*. Results imply that in temperate regions, diapause timing in braconid wasps evolves in response to both host life stage attacked and fly eclosion characteristics, possibly reflecting differential effects of winter on host plant fruiting phenology. Differences in phenological sensitivity of the lower host plant trophic level to variation in environmental conditions may have cascading effects, sequentially and differentially affecting eclosion times in higher frugivore (fly) and parasitoid (wasp) trophic levels.

Key words: *Rhagoletis tabellaria*, *Utetes tabellariae*, *Rhagoletis indifferens*, *Diachasma muliebre*

Parasitoids comprise one of the most speciose groups of insects (Strand and Obrycki 1996, Mayhew 2016, Forbes et al. 2018), displaying a wide array of life history strategies that take advantage of physiological, behavioral, and ecological traits of their hosts. Among these parasitoids are > 100 braconids, as well as diapriids and pteromalids, that attack tephritid fruit flies (Diptera: Tephritidae) in their egg, larval, larval-pupal, and pupal stages (e.g., Strand and Obrycki 1996, López et al. 1999, Ovruski et al. 2000, Costa et al. 2009, Forbes et al. 2009, 2018, Rull et al. 2009, Schliserman et al. 2016, Wharton and Yoder 2020). Because these fly life stages develop sequentially, specialist parasitoids attacking them must evolve to synchronize their adult eclosion and reproductive maturation times with when the appropriate host life stage is present. This is especially true of parasitoids of temperate fly species, as these flies only have one major generation and eclosion period per year (Frick et al. 1954) versus tropical fly species that have four or more overlapping generations per year (Bayoumy et al. 2020). For specialist parasitoids of temperate tephritids, timing of eclosion with flies is ultimately determined by host plant fruiting phenology that selects for fly eclosion time.

The tephritid genus *Rhagoletis* has about 70 species of mostly temperate flies (Norrbom 2006) that are attacked by specialist and

generalist parasitoids (e.g., Frick et al. 1954, Dean and Chapman 1973, Forbes et al. 2010). This genus includes the apple maggot fly (*Rhagoletis pomonella* [Walsh]), which has played a major role in elucidating mechanisms of ecological speciation via sympatric host plant shifting in insects, both for flies and their parasitoids (Forbes et al. 2009, Hood et al. 2015). The timing of the termination of the overwintering pupal diapause affecting when flies eclose as adults is a key life history adaptation involved in host shifts and population divergence. Because *Rhagoletis* flies have one major generation a year and adults survive only 30 d or less in nature (Frick et al. 1954, Dean and Chapman 1973), flies must break diapause to eclose as adults and reach sexual maturity at times matching host phenology to maximize ripe fruit availability to mate on and oviposit into. Fruit on the various host plants attacked by different *Rhagoletis* flies often ripen at different times of the year. Thus, shifting to a novel host commonly selects on flies to correspondingly shift their eclosion timing, generating a degree of allochronic premating isolation with the fly population attacking the ancestral host. Consequently, ecological life history adaptation accompanying host shifts can help initiate the origin of new biodiversity for *Rhagoletis* flies.

What is true for the flies is also the case for the wasps that attack the flies, at least for the braconids that parasitize sibling species belonging to the *R. pomonella* group (Forbes et al. 2009, Hood et al. 2015). However, how the eclosion timing of parasitoids at sympatric sites varies when they attack non-sibling fly species that are not members of the *R. pomonella* group remains unstudied. Such comparisons have the potential to contribute greatly to our understanding of how diverse insect parasite life history strategies may arise and evolve with the phenologies of their hosts.

In the Pacific Northwest of the United States, there are at least six native *Rhagoletis* species that co-occur in the region (Bush 1966, Yee 2008). One is *Rhagoletis tabellaria* (Fitch), a fly whose ancestral host is native red osier dogwood, *Cornus sericea* L. (Cornaceae) (Bush 1966, Yee et al. 2020). Another is the western cherry fruit fly, *Rhagoletis indifferens* Curran, whose ancestral host is native bitter cherry, *Prunus emarginata* (Douglas ex Hooker) Eaton (Rosaceae) but that also infests and has become a pest of introduced, cultivated cherries (Bush 1966, Yee et al. 2015). Red osier dogwood and bitter cherry are found in moist soil in sympatry at sites in ponderosa pine habitat throughout the Northwest (Esser 1995, Yee 2008, Gucker 2012), where ripe fruit of the two occur in July to September, depending on the site (Yee 2014, Bressette 2020).

Puparia of *R. tabellaria* and *R. indifferens* require chilling before adult eclosion occurs, with few or no flies emerging under no-chill conditions (Frick et al. 1954, Yee et al. 2020), an adaptation to cold winters that synchronize fly eclosion with when host fruit are present in the following summer. Eclosion timing of *R. tabellaria* and *R. indifferens* has been determined in the laboratory (Yee et al. 2015, 2020) but in disparate and not within simultaneously conducted experiments. Thus, direct species comparisons are lacking. Given that fruit ripening of bitter cherry and red osier dogwood overlap, it might be predicted that the eclosion timing of the two flies would be similar and also overlap greatly under controlled laboratory rearing conditions.

The two flies are attacked by specialist hymenopterous endoparasitoids (hereafter also referred to as wasps or parasitic wasps). *Rhagoletis tabellaria* is parasitized by the native, bisexual braconid *Utetes tabellariae* (Fischer) (Wharton and Yoder 2020, Yee et al. 2020), whereas *R. indifferens* is parasitized by the native, thelytokous braconid *Diachasma muliebre* (Muesebeck) (Muesebeck 1956, Yee et al. 2015). Based on the habits of its relative *U. canaliculatus* (Gahan) (= *U. lectoides* [Gahan]) (Forbes et al. 2010), *U. tabellariae* attacks eggs of *R. tabellaria*. Based on the habits of its relative *D. alloeum* (Muesebeck) (Forbes et al. 2008), *D. muliebre* attacks second and third instar larvae of *R. indifferens*. Because of the use of different, temporally separated fly life stages, it can be predicted that eclosion times of *U. tabellariae* and *D. muliebre* also differ. Indeed, depending on the chill duration of fly pupae, *U. tabellariae* ecloses on average 10–18 d after *R. tabellaria* ecloses (Yee et al. 2020), whereas *D. muliebre* ecloses on average 22–36 d after *R. indifferens* ecloses (Yee et al. 2015), conforming to this prediction. However, as with their fly hosts, eclosion timing of the two wasps has not been directly compared under controlled environmental conditions to verify this. Eclosion timing of wasps after winter diapause that is synchronized with timing of specific fly stage presence may be controlled by similar mechanisms regulating diapause of wasps attacking tropical tephritids, mechanisms that allow tropical wasps to survive periods when fruit fly hosts are scare or absent (Aluja et al. 1998).

Different chill regimes consisting of varying temperatures and chill durations affect *R. tabellaria* and *R. indifferens* eclosion times (Yee et al. 2015, 2020) and therefore should affect eclosion timing of *U. tabellariae* and *D. muliebre* as well. Changes in fly physiology in response to chill temperatures or durations should also cause changes in wasp physiology that are reflected in eclosion responses. For example, if long chill duration results in shortened eclosion times in flies, it should select for shortened wasp eclosion to maintain synchrony with their hosts.

For *R. indifferens* attacking *P. emarginata*, site differences in eclosion times have been detected, appearing to affect eclosion times of *D. muliebre* from the sites as well (Yee et al. 2015). These differences appear genetic given site comparisons were determined under identical laboratory conditions. Temperatures affecting fruiting times and thus eclosion timing of *R. indifferens* and its wasp may similarly affect eclosion of *R. tabellariae* and its wasps. For example, temperatures at one site could cause earlier blooming of both dogwood and bitter cherry than at another site. However, if flies and wasps have adapted to local conditions and fruiting times, then relative eclosion times of flies in dogwood and in bitter cherry at the sites should be the same. If so, genetics of insects in both systems might change at similar rates in response to earlier or later fruiting of both host plants across sites.

In addition to eclosion timing, wasp lifespan is another life history trait that could impact fitness. Both eclosion timing and longevity could be selected to correspond with the periods when suitable fly stages are available. If the lifespan of wasps is short, then tight synchrony with host life stages is crucial for fitness. If wasps survive longer, then precise timing of eclosion would be less crucial and more variation tolerable, as wasp lifespans can overlap entirely with when susceptible fly stages are present. Although adult *U. tabellariae* can survive on average 29–38 d (Yee et al. 2020), suggesting a capacity to parasitize host flies over a major portion of the fly life cycle, the lifespan of *D. muliebre* has not been documented.

Here, we predicted that eclosion times of *R. tabellaria* and *R. indifferens* in the laboratory are similar as suggested by previous disparate experiments. However, as a result of attacking different temporally offset fly life stages (eggs vs. 2nd and 3rd instar larvae), the eclosion times of their major specialist wasp parasitoids should differ relative to their hosts. Specifically, *U. tabellariae* (egg parasite) should more closely follow that of its fly host *R. tabellaria* than the larval-attacking *D. muliebre* compared with *R. indifferens*. Effects of different chill regimes on eclosion timing were predicted to be similar for both fly-wasp systems, as were the lifespans of the parasitoids because the wasp experience and evolved under the same environmental stresses. We discuss the

implications of our findings for how the eclosion timing of phytophagous insects and their parasitoids may coordinately vary in different manners related to how host plant phenology is affected by environmental conditions.

Materials and Methods

Study Sites

Rhagoletis tabellaria and R. indifferens puparia originated as larvae infesting fruit collected in the towns of Roslyn and Nile in central Washington State, USA in 2019. Roslyn (see below for coordinates, elevations) is located in a ponderosa pine ecosystem in the foothills of the Cascade Mountain Range. The study site had a mix of native bitter cherry, dogwood, and snowberry (*Symphoricarpos albus* [L.] Sidney Fay Blake) and non-native trees such as sweet cherry (*Prunus avium* L.) and plum (*Prunus* spp.). Nile (coordinates below) is also located in the foothills of the Cascade Mountain Range in the ponderosa pine ecosystem, ca. 38 km due south of Roslyn. Native fruit plant diversity in Nile was similar to that in Roslyn, but non-native plant abundance was lower. In Roslyn, fruit from two dogwood patches (47°13′14.03″N, 120°59′18.80″W, 675 m; 47°13′10.79″N, 120°59′19.25″W, 670 m) and a patch of bitter cherry trees ca. 59–155 m away (47°13′15.75″N, 120°59′19.57″W, 676 m) were collected. In Nile, fruit were collected from three patches of dogwood and two patches of bitter cherry trees within 11.2 km, with the nearest dogwood and bitter cherry being 2.9 km apart (dogwood: 46°48′43.20″N, 120°56′48.09″W, 646 m; 46°50′38.12″N, 120°56′52.32″W, 621 m; 46°47′26.78″N, 120°57′22.65″W, 749 m; bitter cherry: 46°48′40.37″N, 120°56′50.72″W, 647 m; 46°53′20.82″N, 120°59′21.66″W, 668 m). Fruit from different collections were managed separately.

Fruit Collections

Within sites, fruit from red osier dogwood and bitter cherry were collected on the same dates whenever possible. In Roslyn on 9 August, a total of 4,491 bitter cherry fruit were collected; 12 August, 2,355 dogwood and 5,428 bitter cherry fruit; 14 August, 3,166 dogwood and 3,978 bitter cherry fruit; and on 30 August, 1,312 dogwood fruit (most bitter cherry had already been collected). In Nile on 12 August, a total of 1,773 dogwood fruit and 6,955 bitter cherry fruit were collected; 19 August, 12,091 dogwood and 6,036 bitter cherry fruit; and 30 August, 13,562 dogwood fruit (no bitter cherries).

Following collection, fruit were immediately transferred to a room held at 14:10 [L:D] h and 21–23°C. Fruit from different collections were held separately on hardware cloth in wooden frames in rubber tubs for larval emergence over a 4-wk period, after which emergence ended. Puparia that formed on the bottom of tubs were collected daily and placed in moist fine aquarium sand (CaribSea Inc., Ft. Pierce, FL) inside 473-ml clear plastic cups covered with a plastic lid (containers were not airtight). Pupae were held in the room for 14 d before chill treatments.

Chill Treatments

As stated above, *R. tabellaria* and *R. indifferens* pupae require chilling for high adult eclosion rates. Puparia of the two flies from each site were subjected to six temperature-chill duration treatments to determine if the treatments differentially affect fly and wasp eclosion. Puparia were held at 3°C or 5°C for 3, 4.5, or 6 mo inside low temperature incubators (0.57 m^3, Thermo Fisher Scientific, Asheville, NC) set at 9:15 [L:D] h to simulate short winter days. Cups containing fly puparia within the incubators were placed next to one another to ensure that insects experienced identical conditions within a given treatment.

Treatments were sequentially assigned to groups of puparia collected daily. Puparia formed 1 d were randomly assigned to the first treatment, puparia formed the next day were assigned to the second treatment, and so on until puparia were assigned to all six treatments, after which the sequence started anew. There were 50–347 *R. tabellaria* puparia allocated for each treatment, except from the Roslyn site, where relatively few larvae (n = 329) emerged and inadvertent uneven dividing of pupae across treatments resulted in 18 and 8 puparia in the 3°C, 6-month chill and 5°C, 3-mo treatments, respectively. There were 43–708 *R. indifferens* puparia per treatment. A total of 1,925 *R. tabellaria* puparia and a total of 4,428 *R. indifferens* puparia were used in the study.

After chilling, puparia were transferred to a room set at 16:8 [L:D] h and 21–23°C, with all treatments randomly arranged side by side on tables or shelves in the room. Flies and wasps eclosed from December 2019 to May 2020. Each cup was monitored for insect eclosion for 100 d after removal from chilling. The sand in cups was moistened ca. every 2 wk to maintain relative humidity at ca. 80–100% during the eclosion period. Flies were collected in vials and identified by W.L.Y. using morphological diagnostics in Bush (1966). Upon eclosion, wasps were either collected in vials and used for lifespan monitoring (see below) or were live killed in 90% ethanol. All wasps were identified by A.A. Forbes using keys in Wharton and Marsh (1978). Voucher fly and voucher wasp specimens are maintained at the USDA-ARS Temperate Tree Fruit and Vegetable Research Unit in Wapato, WA, and at the Department of Biology at the University of Iowa, respectively.

Wasp Lifespan

Subsets of wasps eclosed from dogwood and bitter cherry fly puparia in 3°C and 5°C for the 3- and 4.5-mo chill treatments were monitored daily for survival until all wasps were dead. Upon eclosion, wasps were held inside 355-ml white cups (6.5 cm high, 9.7 cm diameter; Prime Source, Hazelwood, MO) with an 11-ml glass vial containing a 10% sucrose solution. The vial was plugged with a cotton wick that partially drew up the solution, providing wasps both water and food. Cups were covered with fine clear mesh (ca. 0.4 × 0.4 mm openings). Depending on how many insects eclosed per day, one to five wasps were placed in a cup at 16:8 [L:D] h and a mean of 22°C (71% and 69% of cups from dogwood and bitter cherry treatments, respectively, had one wasp) and ca. 20–40% relative humidity. There were 32 and 186 cups of Roslyn and Nile wasps, respectively, from dogwood fly puparia and 123 and 20 cups of Roslyn and Nile wasps, respectively, from bitter cherry fly puparia, with 1–50 cups per site-temperature-chill duration treatment. After death, wasps were preserved in 90% ethanol and later identified as stated above.

Statistical Analyses

Residual plots (generated from the 'Proc univariate DATA=outweight NORMAL plot; VAR resid' command in SAS software; SAS Institute, Inc. 2016) indicated that eclosion day data were normally distributed. Analysis of eclosion times was conducted by estimating the regression of eclosion on four predictors of site (categorical factor), insect type (categorical), temperature (continuous), and chill duration (continuous) and their 2-, 3-, and 4-way interactions (IDRE 2020). Site was Roslyn or Nile; insect type was *R. tabellaria* (sexes combined), male *U. tabellariae*, female *U. tabellariae*, *R. indifferens* (sexes combined), or *D. muliebre* (all females); temperature was 3°C

or 5°C; chill duration was 3, 4.5, or 6 mo. Proc glm in SAS software (SAS Institute, Inc. 2016) was used to estimate the model. Each fly and wasp was treated as a replicate for analysis. However, as information on the tree or bush that fruit were sampled from was not recorded at sites, these units were not analyzed as replicates.

In addition to the four-way interaction model, differences between mean eclosion days of flies and their wasps were analyzed using randomized complete block analysis of variance (ANOVA) followed by the Tukey HSD test. Each site-temperature-chill duration treatment was a blocking factor (10 blocks to make a complete design, excluding three treatments where differences could not be calculated). Temperature effects on eclosion times among the different insect types at the Roslyn and Nile sites were determined by regression analysis (3°C and 5°C as predictors of eclosion time) using Proc reg in the SAS software package (analysis of covariance was not conducted, as the parallel slope assumption was not met, in addition to a significant temperature × insect type interaction being detected in the first model). To determine eclosion time differences among species, one-way ANOVA was conducted on eclosion day within each temperature-chill duration treatment, followed by the Tukey HSD test. To determine the effects of chill duration (3, 4.5, and 6 mo) within a given temperature treatment on eclosion time, a linear regression was run. To further determine the relationship between eclosion day of wasps and their host flies, Pearson correlations between mean day of fly and wasp eclosion for site-temperature-chill duration treatments were conducted (12 data points, unless there was no eclosion in a treatment, which occurred for *R. tabellaria* versus male *U. tabellariae*, to give $n = 11$, and for *R. tabellaria* versus female *U. tabellariae*, to give $n = 9$). Roslyn versus Nile site effects within each insect were determined using paired *t*-tests (pairing each temperature-chill duration treatment between sites).

Lifespans of male and female *U. tabellariae* and *D. muliebre* were compared using one-way ANOVA followed by the Tukey HSD test. Within sites, no significant differences were detected among temperature-chill duration treatments ($P > 0.05$), so data from wasps across treatments within sites were combined. Each wasp was considered a replicate. Throughout the results, means ± SEM are presented where appropriate.

Results

Overall Analysis of Treatment Effects on Fly and Wasp Eclosion Times

The estimated linear model (Table 1) generated an R^2 of 0.8212, indicating that the eclosion times of flies and wasps were significantly and highly explained by the main effects of site, insect type, temperature, and chill duration. Significant two-way interactions were also observed for site x insect, temperature x insect, and chill duration x insect, indicating that eclosion times for insects were differentially affected by site, temperature, and chill duration. Significant three- and four-way interactions were also detected and indicated complex relationships among factors affecting eclosion, necessitating analyses of single factors (i.e., insect type, temperature, chill duration, and site) one at a time. The lack of a significant temperature x site interaction (Table 1) indicated that temperature effects on eclosion of insects from Roslyn and Nile did not differ.

As predicted, the eclosion time of *U. tabellariae* was similar to or only slightly later than that of *R. tabellaria*, with female *U. tabellariae* eclosing later than males. In contrast, the eclosion time of *D. muliebre* occurred much later than that of *R. indifferens* (Figs. 1–4; Table 2). Averaged across all treatments, the difference in mean eclosion time (10 treatments) between male *U. tabellariae* and *R. tabellaria* was 6.0 ± 0.5 d, which was less than half the difference between female *U. tabellariae* and *R. tabellaria* (12.5 ± 1.0 d). In comparison, the mean differences in eclosion time between *D. muliebre* and *R. indifferens* was 32.1 ± 1.5 d ($F = 131.37$; df = 2, 17; $P < 0.0001$).

Effects of Specific Factors on Fly and Wasp Eclosion Times

Temperature effects (3°C vs. 5°C) on eclosion times were weaker for *R. tabellaria* and *U. tabellariae* compared with those for *R.*

Table 1. Estimated regression of eclosion days on the four-way interaction of site (categorical factor), insect type (categorical), temperature (continuous), and chill duration (continuous)

Source	df	*F*-value	*P*-value	R^2
Model	39	527.42	< 0.0001	0.8212
Error	4480			
Corrected Total	4519			
Source	**df**	***F*-value**	***P*-value**	
Site (2 levels)	1	1847.94	< 0.0001	
Insect (5 levels)	4	3777.01	< 0.0001	
Site x insect	4	50.09	< 0.0001	
Temperature (2 levels)	1	152.10	< 0.0001	
Temperature x site	1	0.38	0.5374	
Temperature x insect	4	36.78	< 0.0001	
Temperature x site x insect	4	1.82	0.1228	
Duration (3 levels)	1	2356.94	< 0.0001	
Duration x site	1	336.16	< 0.0001	
Duration x insect	4	44.04	< 0.0001	
Duration x site x insect	4	9.85	< 0.0001	
Temperature x duration	1	114.39	< 0.0001	
Temperature x duration x site	1	27.00	< 0.0001	
Temperature x duration x insect	4	7.33	< 0.0001	
Temperature x duration x site x insect	4	6.74	< 0.0001	

Site: Roslyn and Nile; insect: *Rhagoletis tabellaria*, male *Utetes tabellariae*, female *U. tabellariae*, *Rhagoletis indifferens*, and *Diachasma muliebre*; temperature: 3°C and 5°C; chill duration: 3, 4.5, and 6 mo.

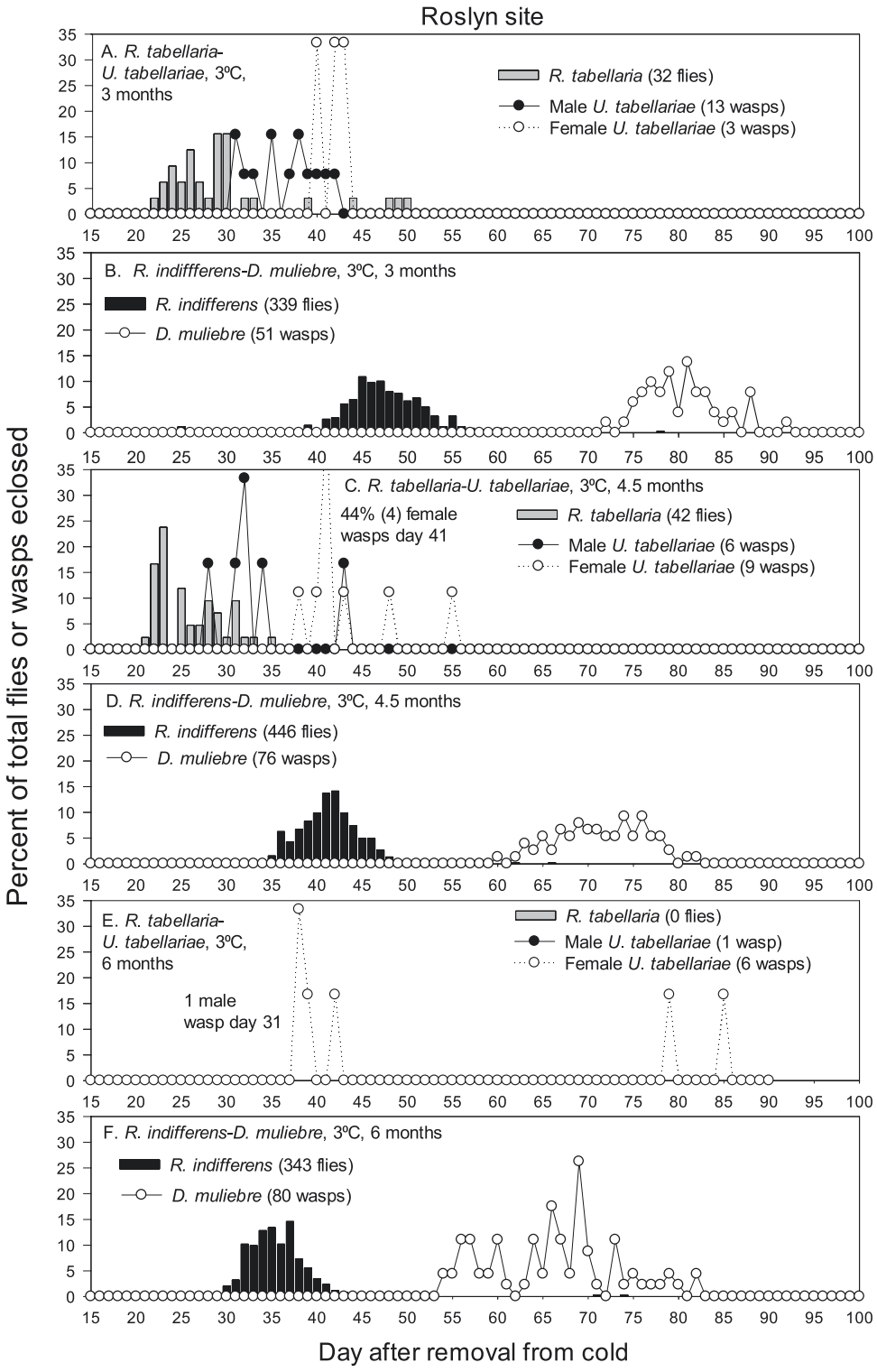

Fig. 1. Percent daily eclosion of *Rhagoletis tabellaria, Rhagoletis indifferens,* and their respective wasp parasites *Utetes tabellariae* and *Diachasma muliebre* from the Roslyn site (Washington State, USA) in the laboratory after fly pupae were chilled at 3°C for (A and B) 3 mo, (C and D) 4.5 mo, and (E and F) 6 mo.

indifferens and *D. muliebre* (Table 2). For *R. indifferens* from Roslyn and Nile, flies eclosed significantly earlier when they experienced 3°C than 5°C as pupae for 3 and 4.5 mo, but not 6 mo. The same was also true for *D. muliebre*, except for wasps in the 3-mo treatment at the Nile site. There was a consistent pattern in eclosion times within 3°C and 5°C treatments across all chill durations: ranking of earliest to latest mean eclosion time was always *R. tabellaria* < male *U. tabellariae* < female *U. tabellariae* < *R. indifferens* < *D. muliebre* (Table 2).

With respect to chill duration (Table 3), there were significant negative regressions between the 3-, 4.5-, and 6-mo chill treatments on eclosion times for Roslyn and Nile *R. tabellaria*, *R. indifferens*, and

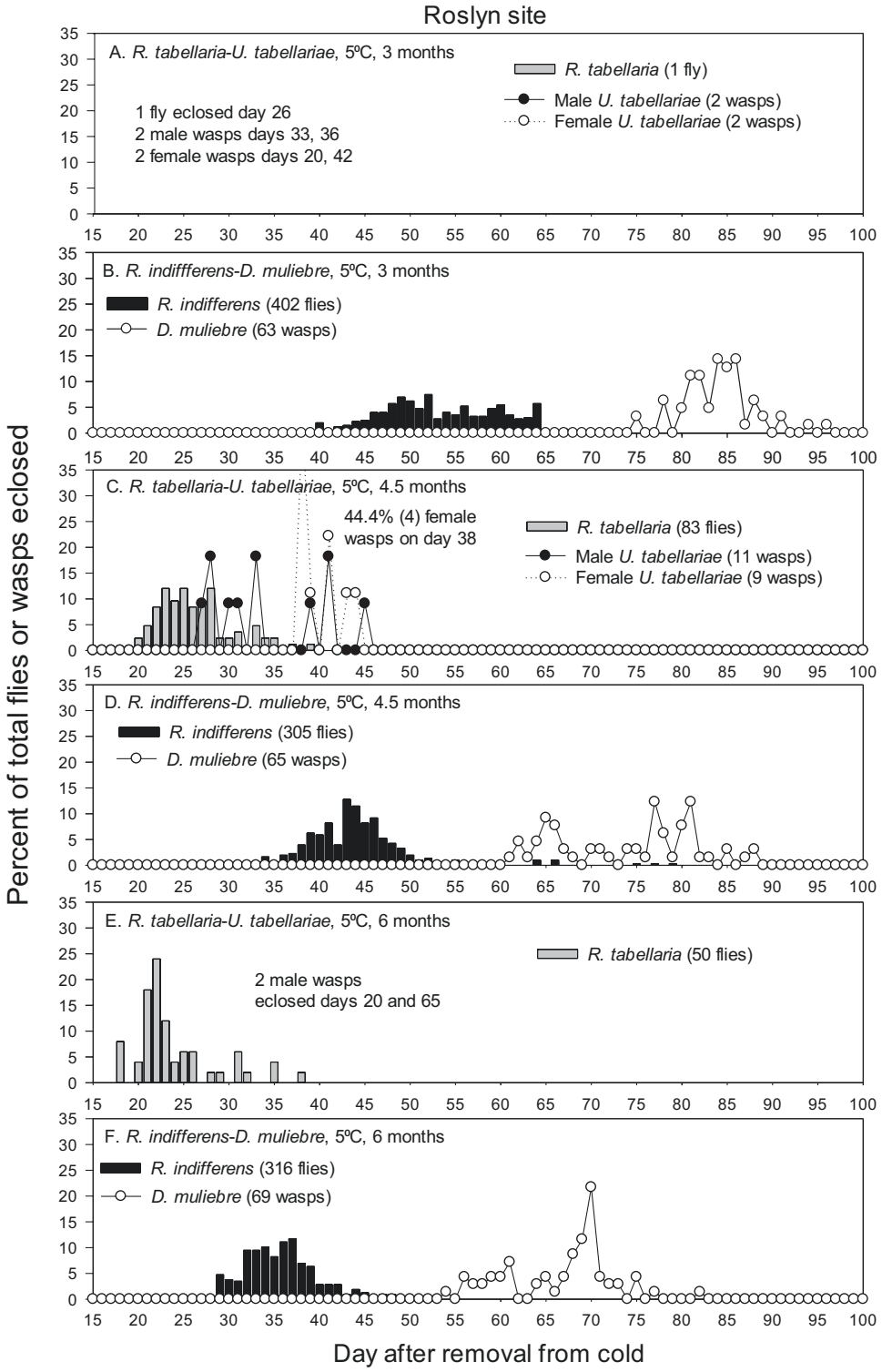

Fig. 2. Percent daily eclosion of *Rhagoletis tabellaria, Rhagoletis indifferens,* and their respective wasp parasites *Utetes tabellariae* and *Diachasma muliebre* from the Roslyn site (Washington State, USA) in the laboratory after fly pupae were chilled at 5°C for (A and B) 3 mo, (C and D) 4.5 mo, and (E and F) 6 mo.

D. muliebre, indicating that longer chill duration resulted in earlier eclosion times for these insects. However, regressions for *U. tabellariae* were not significant except for Nile males in the 3°C treatment. The R^2 values for *R. tabellaria* (0.0580–0.2608) and for *U. tabellariae* (0.0107–0.3167) were also generally lower than those for *R. indifferens* and *D. muliebre*, which ranged from 0.2657 to 0.5862 (Table 3).

Overall, there was no significant correlation in eclosion times of male or female *U. tabellariae* and *R. tabellaria* across all the different temperature-chill duration treatments performed in the study (Fig. 5A). Thus, combinations of treatment conditions associated with earlier or later eclosion time for *R. tabellaria* were not strongly associated with having the same effect for *U. tabellariae*. However,

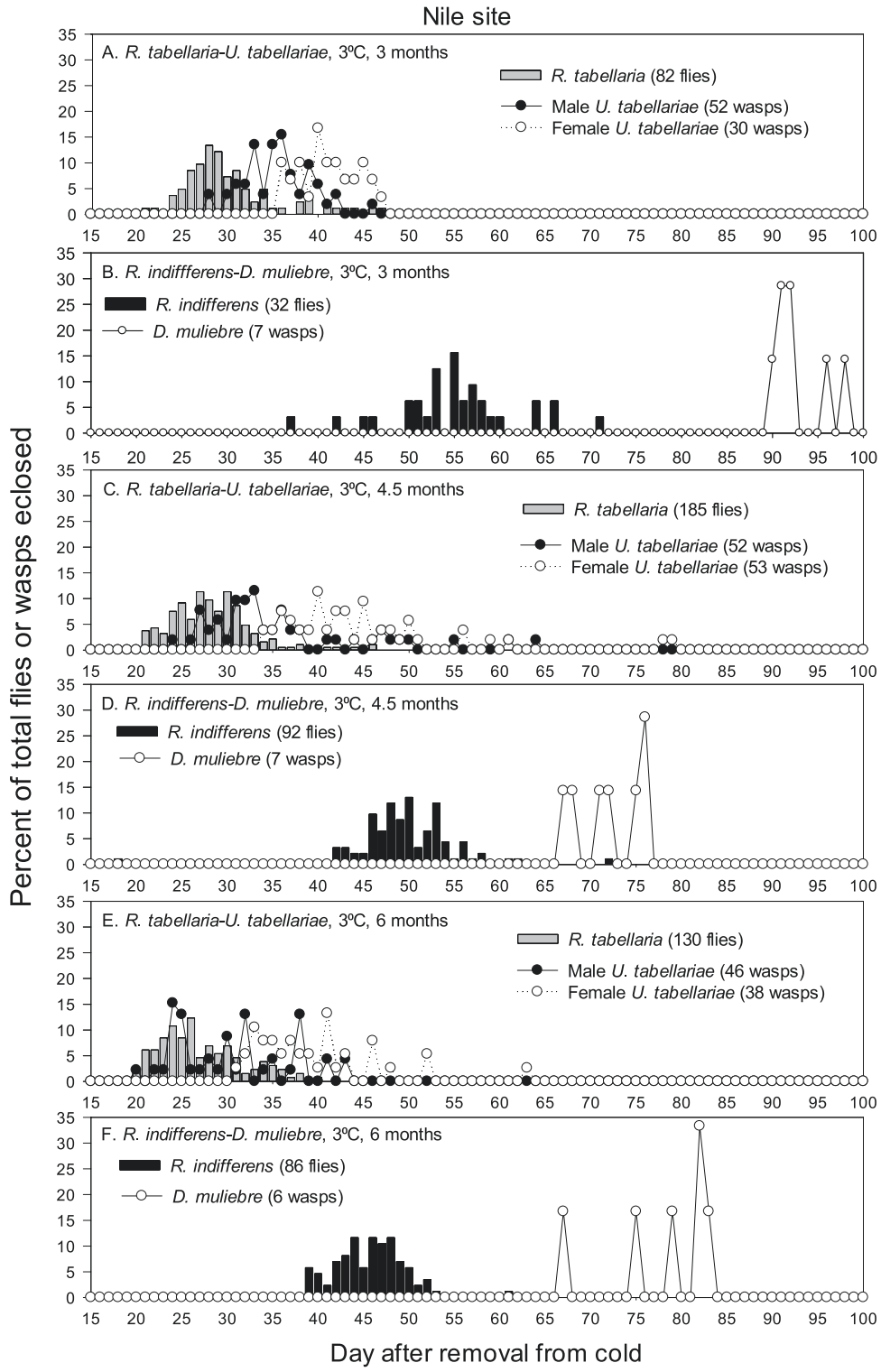

Fig. 3. Percent daily eclosion of *Rhagoletis tabellaria*, *Rhagoletis indifferens*, and their respective wasp parasites *Utetes tabellariae* and *Diachasma muliebre* from the Nile site (Washington State, USA) in the laboratory after fly pupae were chilled at 3°C for (A and B) 3 mo, (C and D) 4.5 mo, and (E and F) 6 mo.

eclosion times of *R. indifferens* and *D. muliebre* were strongly and significantly correlated across the different combinations of treatment conditions (Fig. 5B).

Site effects were seen for *R. tabellaria*, *R. indifferens*, and *D. muliebre* (Figs. 1–4; Table 2). *Rhagoletis tabellaria* from Roslyn eclosed significantly earlier than those from Nile (26.5 ± 1.0 d vs. 29.6 ± 1.1 d) (t = –3.28, df = 4, P = 0.0306). However, there was no statistical difference between male *U. tabellariae* from Roslyn (36.2 ± 2.4 d) and Nile (34.2 ± 1.1 d) (t = 0.68, df = 5, P = 0.5246) or between female *U. tabellariae* from Roslyn (41.9 ± 3.6 d) and Nile (42.4 ± 1.0 d) (t = –0.13; df = 4; P = 0.9040). However, as was the case for *R. tabellaria* flies, *R. indifferens* from Roslyn eclosed

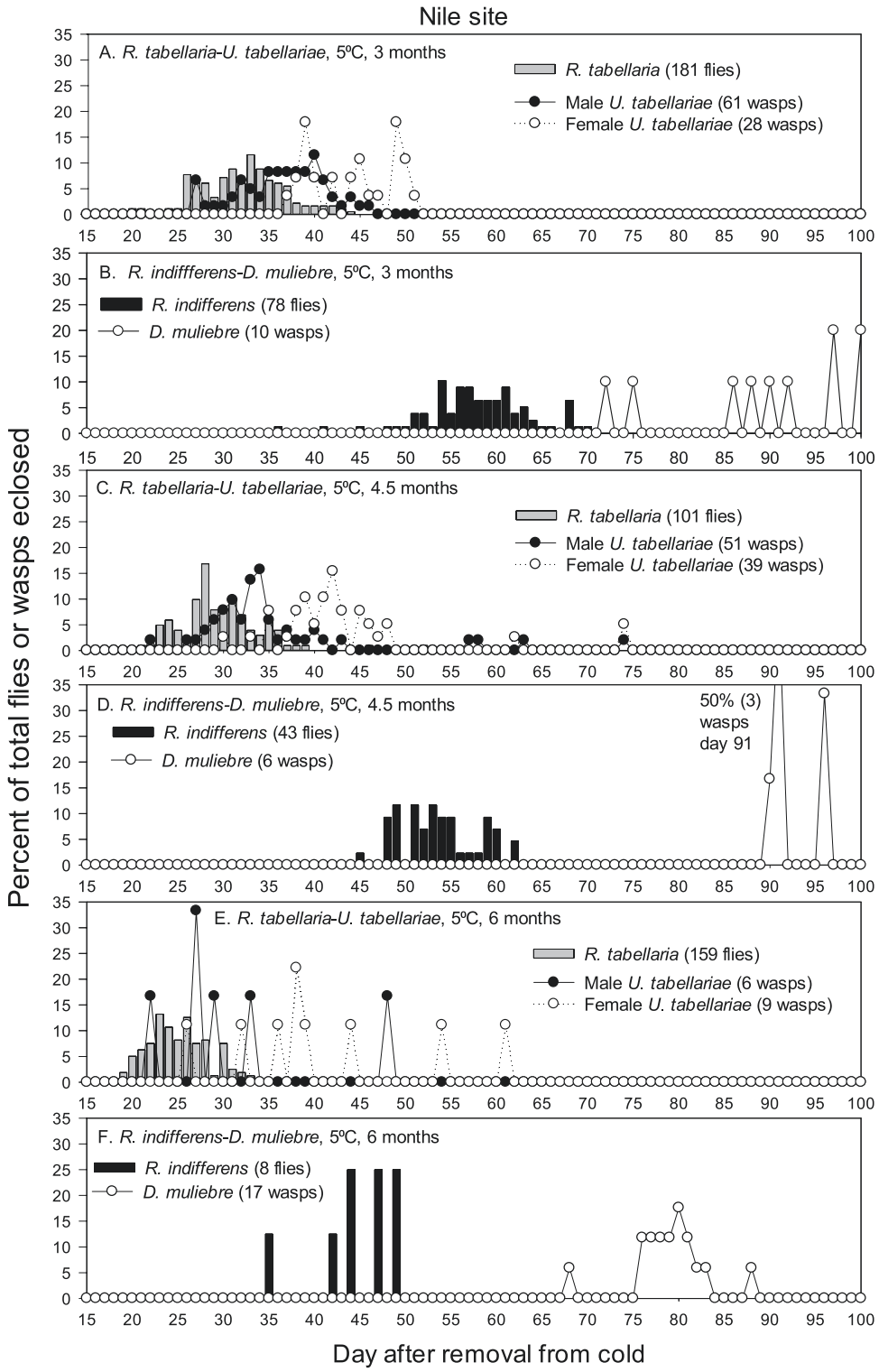

Fig. 4. Percent daily eclosion of *Rhagoletis tabellaria*, *Rhagoletis indifferens*, and their respective wasp parasites *Utetes tabellariae* and *Diachasma muliebre* from the Nile site (Washington State, USA) in the laboratory after fly pupae were chilled at 5°C for (A and B) 3 mo, (C and D) 4.5 mo, and (E and F) 6 mo.

significantly earlier than those from Nile (43.0 ± 2.8 d vs. 51.1 ± 2.2 d) (t = −10.68; df = 5; P < 0.0001). Correspondingly, *D. muliebre* from Roslyn also eclosed earlier than *D. muliebre* from Nile (71.8 ± 4.3 d vs. 84.0 ± 3.6 d) (t =3.54; df = 5; P = 0.0166).

Wasp Lifespan

All wasps from Roslyn and Nile sites survived on average >28 d (Table 4). However, while female *U. tabellariae* and *D. muliebre* had similar lifespans, both survived significantly longer than male *U. tabellariae*.

Discussion

Five main findings emerged from the current study bearing on our understanding of the evolutionary relationship of diapause life history timing between parasitic wasps and their fly hosts, as affected

Table 2. Mean eclosion time in days ± SEM of *Rhagoletis tabellaria* and its parasite *Utetes tabellariae* (males and females), and *Rhagoletis indifferens* and its parasite *Diachasma muliebre* after fly pupae were chilled at 3°C and 5°C in the laboratory

Roslyn site			
Insect type	3°C	5°C	R^2; *P*-value from linear regression
3-month chill			
R. tabellaria	30.2 ± 1.3d (32)	26 (1)[a]	—
Male *U. tabellariae*	36.3 ± 1.1c (13)	34.5 ± 1.5c (2)	0.0313; 0.5282
Female *U. tabellariae*	41.7 ± 0.9bc (3)	31.0 ± 11.0c (2)	0.3563; 0.2879
R. indifferens	47.2 ± 0.3b (339)	53.2 ± 0.4b (402)	0.1622; < 0.0001
D. muliebre	80.4 ± 0.6a (51)	84.0 ± 0.5a (63)	0.1619; < 0.0001
	F = 469.01; df = 4, 433; *P* < 0.0001	*F* = 369.18; df = 3, 465; *P* < 0.0001	
4.5-month chill			
R. tabellaria	25.9 ± 0.6d (42)	26.3 ± 0.5d (83)	0.0023; 0.5964
Male *U. tabellariae*	33.3 ± 2.2c (6)	34.3 ± 1.9c (11)	0.0053; 0.7809
Female *U. tabellariae*	43.1 ± 1.7b (9)	40.0 ± 0.8bc (9)	0.1421; 0.1230
R. indifferens	41.4 ± 0.2b (446)	44.3 ± 0.4b (305)	0.0730; < 0.0001
D. muliebre	71.4 ± 0.6a (76)	73.9 ± 0.9a (65)	0.0381; 0.0203
	F = 1078.63; df = 4, 574; *P* < 0.0001	*F* = 576.81; df = 4, 468; *P* < 0.0001	
6-month chill			
R. tabellaria	None (0)	23.9 ± 0.6c (50)	—
Male *U. tabellariae*	31 (1)[a]	47.5 ± 17.5ab (2)	—
Female *U. tabellariae*	53.5 ± 9.1b (6)	None (0)	—
R. indifferens	35.9 ± 0.3c (343)	35.7 ± 0.2b (316)	0.0004; 0.6044
D. muliebre	66.1 ± 0.8a (80)	54.7 ± 2.6a (69)	0.1173; < 0.0001
	F = 874.67; df = 2, 426; *P* < 0.0001	*F* = 114.64; df = 3, 433; *P* < 0.0001	
Nile site			
3-month chill			
R. tabellaria	30.6 ± 0.6e (82)	32.0 ± 0.4e (181)	0.0185; 0.0274
Male *U. tabellariae*	35.5 ± 0.5d (52)	36.5 ± 0.6d (61)	0.0131; 0.2269
Female *U. tabellariae*	41.1 ± 0.6c (30)	44.1 ± 0.9c (28)	0.1281; 0.0058
R. indifferens	55.0 ± 1.2b (32)	57.8 ± 0.7b (78)	0.0388; 0.0392
D. muliebre	92.9 ± 1.1a (7)	89.7 ± 3.1a (10)	0.0426; 0.4269
	F = 349.80; df = 4, 198; *P* < 0.0001	*F* = 568.18; df = 4, 353; *P* < 0.0001	
4.5-month chill			
R. tabellaria	29.9 ± 0.4e (185)	29.8 ± 0.5e (101)	0.0142; 0.0439
Male *U. tabellariae*	36.0 ± 1.2d (52)	35.3 ± 1.3d (51)	0.0014; 0.7022
Female *U. tabellariae*	44.4 ± 1.1c (53)	43.1 ± 1.4c (39)	0.0052; 0.4948
R. indifferens	49.8 ± 0.6b (92)	53.6 ± 0.7b (43)	0.1025; 0.0002
D. muliebre	72.1 ± 1.4a (7)	92.5 ± 1.1a (6)	0.9175; < 0.0001
	F = 232.83; df = 4, 384; *P* < 0.0001	*F* = 205.61; df = 4, 235; *P* < 0.0001	
6-month chill			
R. tabellaria	27.0 ± 0.4e (130)	25.7 ± 0.4c (159)	0.0222; 0.0112
Male *U. tabellariae*	30.5 ± 0.9d (46)	31.0 ± 3.7c (6)	0.0007; 0.8495
Female *U. tabellariae*	39.4 ± 1.1c (38)	40.9 ± 3.6b (9)	0.0062; 0.5978
R. indifferens	45.7 ± 0.4b (86)	44.6 ± 1.6b (8)	0.0065; 0.4400
D. muliebre	78.0 ± 2.5a (6)	79.0 ± 1.0a (17)	0.0097; 0.6553
	F = 310.34; df = 4, 301; *P* < 0.0001	*F* = 439.77; df = 4, 194; *P* < 0.0001	

Results for linear regression analyses for 3°C vs. 5°C temperature conditions on eclosion time are given in rows, while analysis of variance results are presented in columns.

—, not conducted.

[a]Not included in analysis of variance. Sample sizes inside parentheses. Mean eclosion days within month and within temperature columns followed by the same letter are not significantly different (Tukey HSD test, *P* > 0.05).

by host plant fruiting phenology. First, *R. tabellaria* eclosed earlier than *indifferens*, selecting for distinct eclosion time traits of *U. tabellariae* and *D. muliebre*. Second, eclosion of *U. tabellariae* occurred earlier after eclosion of *R. tabellaria* than eclosion of *D. muliebre* after eclosion of *R. indifferens*, to synchronize the reproductive activity of the two wasps with the different immature fly life stages they attack. Third, the strength of eclosion time responses to temperature and chill duration between flies differed significantly, with variable effects on wasp responses. Fourth, geographic differences in eclosion timing of flies and wasps were detected, but inter-species differences remained similar, suggesting consistent adaptation by wasps to flies under different environmental conditions. Finally, the lifespans of *U. tabellariae* and *D. muliebre* were longer than have been previously reported for some wasps attacking *Rhagoletis* flies (Hood et al. 2015), which has implications for eclosion timing, fly presence, and wasp fitness.

Eclosion Time of Flies in Relation to Eclosion Time of Wasps

In the laboratory, *R. tabellaria* eclosed on average from 11.8 to 27.2 d earlier than *R. indifferens*, depending upon the particular

Table 3. Results of linear regression of the effects of chill durations (x) of 3, 4.5, and 6 mo on eclosion time in days (y) of *Rhagoletis tabellaria*, male and female *Utetes tabellariae*, *Rhagoletis indifferens*, and *Diachasma muliebre* from Roslyn and Nile sites for 3°C and 5°C temperature treatments

	ANOVA			Linear regression[a]	
Insect type	*F*	df	*P*-value	Parameter estimates	R^2
Roslyn site, 3°C					
R. tabellaria[b]	10.12	1, 72	0.0022	y = 38.71 – 2.84x	0.1232
Male *U. tabellariae*	3.23	1, 18	0.0892	y = 41.93 – 1.88x	0.1520
Female *U. tabellariae*[b]	4.17	1, 9	0.0715	y = 13.00 + 6.00x	0.3167
R. indifferens	893.46	1, 1126	< 0.0001	y = 58.48 – 3.77x	0.4424
D. muliebre	185.26	1, 205	< 0.0001	y = 93.55 – 4.66x	0.4747
Roslyn site, 5°C					
R. tabellaria[c]	9.97	1, 132	0.0020	y = 33.26 – 1.55x	0.0703
Male *U. tabellariae*	1.92	1, 13	0.1893	y = 16.50 + 4.33x	0.1286
Female *U. tabellariae*	2.20	1, 16	0.1570	y = 25.10 + 4.47x	0.1211
R. indifferens	1446.23	1, 1021	< 0.0001	y = 70.59 – 5.82x	0.5862
D. muliebre	146.52	1, 195	< 0.0001	y = 114.96 – 9.80x	0.4290
Nile site, 3°C					
R. tabellaria	24.30	1, 395	< 0.0001	y = 33.85 – 1.16x	0.0580
Male *U. tabellariae*	13.28	1, 148	0.0004	y = 41.43 – 1.64x	0.0823
Female *U. tabellariae*	1.29	1, 119	0.2591	y = 45.22 – 0.70x	0.0107
R. indifferens	75.26	1, 208	< 0.0001	y = 63.54 – 2.99x	0.2657
D. muliebre	12.56	1, 18	0.0023	y = 104.12 – 5.19x	0.4111
Nile site, 5°C					
R. tabellaria	154.92	1, 439	< 0.0001	y = 38.57 – 2.12x	0.2608
Male *U. tabellariae*	2.75	1, 116	0.1000	y = 40.40 – 1.24x	0.0232
Female *U. tabellariae*	1.04	1, 74	0.3115	y = 47.06 – 0.94x	0.0138
R. indifferens	46.23	1, 127	< 0.0001	y = 68.87 – 3.61x	0.2669
D. muliebre	17.53	1, 31	0.0002	y = 103.19 – 3.84x	0.3612

[a]*P*-value for linear regression same as for ANOVA.
[b]3 and 4.5 mo; none eclosed from 6-mo chill treatment.
[c]One fly from 3-mo chill treatment.

treatment regime. Thus, part of the difference in eclosion time between the braconid parasitoids attacking these two flies appears due to a difference in when their respective hosts eclose, in addition to the difference in the life stage attacked by the wasps. The earlier eclosion time of *R. tabellaria* also implies that fruit on red osier dogwood is ready for fly attack earlier in the season than bitter cherry, as synchronization between fly eclosion time and host fruiting phenology is a hallmark in *Rhagoletis* flies (Feder et al. 1993, Feder and Filchak 1999). In the current study, however, there was a considerable seasonal overlap in when dogwood and bitter cherry fruits were collected for rearing larvae. The overlap was due, in large part, to dogwood having a protracted period of fruit ripening, with berries turning from green (unripe) to white (ripe) from July through August and into early September. Thus, despite the overlap in collecting time, the majority of dogwood fruit ripened earlier than bitter cherry in 2019, consistent with previous extensive observations at the Roslyn site in 2018 (W. L. Y., unpublished data) indicating that most red osier dogwood fruit had ripened by 16 July. In contrast, at the Roslyn site in 2011, most bitter cherry fruit did not ripen or turn red until 26 August at the earliest (Yee 2014). It therefore appears that the difference in adult eclosion time between the morphologically distinct *R. tabellaria* and *R. indifferens* reflects differential adaptation in diapause life history timing to variation in the fruiting times of their respective hosts, as has been determined for sibling species of *Rhagoletis* flies in the *R. pomonella* group (Dambroski and Feder 2007, Lyons-Sobaski and Berlocher 2009).

Relative Eclosion Times of Wasps

The differences in eclosion timing between *U. tabellariae* and *D. muliebre* relative to their hosts represent different life history strategies, likely an adaptation to the use of different fly life stages by the wasps, which in the past may have benefitted them from parasitizing either eggs or larvae but not both (Strand and Obrycki 1996). In this regard, if *R. tabellaria* reaches sexual maturity and begins ovipositing ca. 7–10 d post-eclosion (as for *R. indifferens*; Frick et al. 1954), then the eclosion time of female *U. tabellariae*, about 2 wk after flies eclose, is well-timed with the presence of dogwood fly eggs. Although not studied in bitter cherry, later instars of *R. indifferens* in sweet cherry appear about 3–4 wk after first fly eclosion (Frick et al. 1954), coinciding with eclosion times of *D. muliebre*.

Other aspects of the biology of the parasitoids also appear to be attuned to those of their fly hosts. Wasp ovipositor length is related to where a host fly lives, whether exposed or inside a substrate (Sivinski and Aluja 2003). *Utetes tabellariae* females in the current study had a mean ± sem ovipositor length of 0.77 ± 0.06 mm (n = 15), sufficient to access eggs laid <1 mm below the surface of dogwood fruit (*R. tabellaria* aculeus length is ca. 0.70–0.75 mm [Bush 1966]). In comparison, *D. muliebre* evolved to eclose later than *R. indifferens* to track larval presence. Correspondingly, *D. muliebre* in the current study had a mean ovipositor length 2.2 times greater than that of *U. tabellariae*, at 1.67 ± 0.16 mm (n = 15), long enough to reach larvae in 7–10 mm diameter bitter cherries. Thus, both the life history and morphology of these two parasitoids appear to have evolved to adapt

them to variation in the temporal and spatial apparency of their host flies.

Strength of Eclosion Time Responses to Temperature and Chill Duration Between Flies and Effects on Wasps

Not all of our *a priori* predictions were met, however, as contrary to expectations, different chill regimes had disparate and not the same effect on eclosion timing in the two fly-wasp systems. Previous studies have shown that longer chill duration reduces days to eclosion of tephritids, as well as their wasp parasitoids (e.g., Yee et al. 2015, Rull et al. 2019). The same trend was also generally apparent in the current study for all insects except *U. tabellariae*, where the R^2 for chill time for *R. tabellaria* was weak. In addition, temperature effects of 3°C versus 5°C on eclosion times were weaker for *R. tabellaria* and *U. tabellariae* compared with those for *R. indifferens* and *D. muliebre*. As a result, the eclosion time responses of *U. tabellariae* did not closely track those of *R. tabellaria* across the different temperature-chill duration treatments in the study but were strongly associated for *R. indifferens* with *D. muliebre*.

We speculate that the difference in chill regime effects between the two systems may reflect blooming and fruiting times of red osier dogwood being less responsive to variation in climatic conditions than bitter cherry. This, coupled with the wider temporal window of dogwood fruit availability, may select for diapause termination in *R. tabellaria*, and subsequently *U. tabellariae*, to be less sensitive to winter temperature and duration. Rather, following the onset of warm conditions, it may be more important for these flies and their parasitoids to quickly reinitiate development to consistently eclose early to take full advantage of the entire and more predictable temporal spectrum of host availability. In contrast, bitter cherry may represent a more seasonally variable moving target across years. Hence, diapause termination in *R. indifferens*, and subsequently *D. muliebre*, is affected more by overwintering conditions and thus was strongly correlated with one another in the current study. Further field and laboratory work are needed, however, to support this hypothesis.

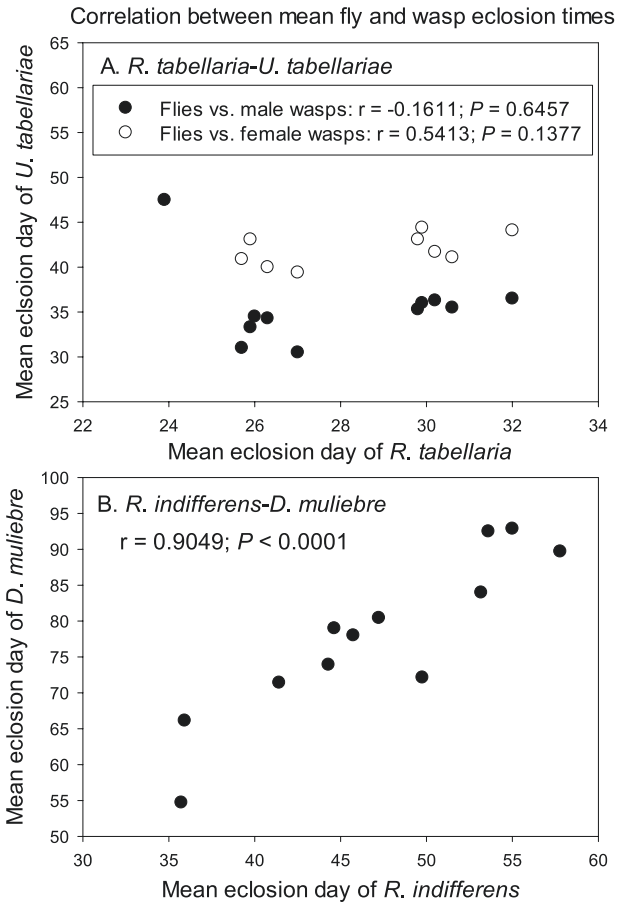

Fig. 5. Correlations between mean eclosion time in days of host flies and their associated wasp parasites experiencing different combinations of chill temperatures and durations in the study: (A) *Rhagoletis tabellaria* vs. male and female *Utetes tabellariae*; (B) *Rhagoletis indifferens* vs. *Diachasma muliebre*.

Geographic Differences in Eclosion Timing of Flies and Wasps

Eclosion times were generally earlier in *R. tabellaria*, *R. indifferens*, and *D. muliebre* from Roslyn than Nile. This suggests that initial fruiting of both red osier dogwood and bitter cherry is earlier at the Roslyn than the Nile site, based on well-established relationships within *R. pomonella* showing that when a host fruit species develops early, flies adapted to that fruit eclose earlier than flies adapted to later-developing fruit species (Hood et al. 2015, Mattsson et al. 2015). This may be due in part to flies having large genetic variation for development-related traits, as in *R. pomonella* (Feder and Filchak 1999, Doellman et al. 2018, 2019). Results here suggest that genetics controlling eclosion timing in both *R. tabellaria* and *R. indifferens*, and their respective wasps, changed similarly in response to different fruiting times of their host plants at Roslyn and Nile sites, located only 38 km apart.

Table 4. Mean lifespans in days ± SEM of adult *Utetes tabellariae* and *Diachasma muliebre* from two sites in Washington State, USA maintained under laboratory conditions of 16:8 [L:D] h, mean temperature of 22°C, and ca. 20–40% relative humidity

Wasp type[a]	Roslyn site		Nile site	
	N	Lifespan	*N*	Lifespan
Male *U. tabellariae*	19	34.5 ± 3.7[b]	167	28.5 ± 1.0[b]
Female *U. tabellariae*	15	50.5 ± 3.5[a]	115	43.0 ± 1.4[a]
D. muliebre[b]	151	48.2 ± 1.5[a]	20	50.9 ± 3.8[a]
ANOVA	$F = 5.21$; df = 2, 182; $P = 0.0063$		$F = 50.63$; df = 2, 299; $P < 0.0001$	

[a]Wasps from different temperature-duration treatments (3°C and 5°C for 3 or 4.5 mo) combined because there were no significant treatment differences (ANOVA, $P > 0.05$).

[b]*Diachasma muliebre* is an asexual species. Means within columns followed by the same letter are not significantly different (Tukey HSD test, $P > 0.05$).

The ability of a wasp to modulate its eclosion time to track regional variation in fruiting phenology and host fly stage availability can contribute to its success in colonizing broad geographic areas (Rull et al. 2019). Our results suggest that at least *D. muliebre* has adapted to different fly eclosion times and therefore fruiting phenologies in Roslyn and Nile, even if fruit timing differences between sites may only be ca. 1 wk (W.L.Y., unpublished data). Differences in insect responses between sites are likely genetic, which is the case for other *Rhagoletis* flies (Ragland et al. 2017, Doellman et al. 2018, 2019; Dowle et al. 2020, Meyers et al. 2020), as eclosion times were determined under identical laboratory conditions. Site differences were not detected for *U. tabellariae* either because of a statistical artifact due to relatively low numbers from Roslyn or because the small difference in eclosion timing of Roslyn versus Nile *R. tabellaria* was not sufficient to select for different *U. tabellariae* eclosion phenotypes between sites.

Lifespans of Wasps and Implications for Eclosion Timing and Wasp Fitness

Lifespans for both *U. tabellariae* and *D. muliebre* in the laboratory of about a month for males and up to 50 d for females were longer than predicted based on previous longevity data of braconid parasitoid species (10–14 d) attacking *Rhagoletis* flies (Hood et al. 2015). Indeed, lifespans for female parasitoids in the current study were comparable to those of female *R. tabellaria* (Yee et al. 2020) and female *R. indifferens* (Yee 2003) in the laboratory; in addition, *R. indifferens* can oviposit for > 50% of their lifetimes (Yee 2003). It would seem logical that increased wasp longevity to overlap more extensively with host fly resources should evolve, when possible, to increase parasitoid fitness. *Rhagoletis indifferens* larvae are present in sweet cherries for ca. 2–3 wk (Frick et al. 1954) and probably as long or longer in bitter cherry (here, larval emergence from bitter cherry occurred for ca. 1 mo). Also, prolonged wasp lifespan could mean that mismatches of wasp and fly eclosion (e.g., if wasps eclose one week before fly egg or larval presence) would minimally affect wasp fitness.

Whether lifespans of wasps fed sucrose in the laboratory are remotely approached by wasps in nature remains unknown. Sucrose or honeydew can prolong the survival of certain braconid wasps (e.g., Hagley and Barber 1992, Costamagna and Landis 2004, Biondi et al. 2013). However, other braconids fed honey have relatively short lifespans, e.g., 8 to < 20 d for females (Costamagna and Landis 2004, Liu and Kainoh 2020), more similar to those recorded previously for parasitic wasps of *Rhagoletis* (Hood et al. 2015). Thus, while it would appear increased adult longevity has also evolved in *U. tabellariae* and *D. muliebre*, further study is needed to confirm that our results obtained under ideal laboratory conditions translate to the field.

Implication of Global Warming on Fruit Timing and Insect Eclosion

Global warming has increased winter temperatures (Lindsey and Dahlman 2021) but how this has or will affect chill units needed by fly pupae and wasps to terminate diapause requires study. However, given that *R. indifferens* and its wasp are more sensitive to long chill durations than *R. tabellaria* and its wasp, it can be predicted that eclosion times of *R. tabellaria* and its wasp would be changed less by warmer winters. Effects of warmer post-chill temperatures on insect eclosion were not studied here but may be more predictable than effects of chill temperatures. As the earth warms due to climate change, fruiting times and thus fly and wasp eclosion times may be accelerated. Data for effects of global warming on red osier dogwood and bitter cherry apparently are absent, but spring plant events have advanced an average of 1.9 d per decade for 650 temperate plant species (Khanduri et al. 2008). Assuming this figure for bitter cherry, that *R. indifferens* shifted in under 100 years from later developing bitter cherry to earlier developing cultivated cherry (Frick et al. 1954, Bush 1966), which ripen 2–4 wk earlier, and that genetically determined fly eclosion times are variable (data in current study), flies from bitter cherry probably could adapt to earlier fruiting times due to climate change over the short term. Phenology models also predict that *R. indifferens* eclosion time in the spring is flexible, occurring earlier when it is warmer than cooler (AliNiazee 1979, Jones et al. 1991). *Diachasma muliebre* might also have the genetic variation to eclose earlier in response to global warming, as do *R. tabellaria* and *U. tabellariae*, given variable eclosion times seen here. In plant-insect associations where insect emergence is not tightly associated with that of the host plant, climate change can be detrimental (Singer and Parmesan 2010). This is not the case for *Rhagoletis* flies and their wasps, whose eclosion is tightly synchronized with that of their hosts. If warming proceeds at a faster rate than 1.9 d per decade, outcomes for host plant, fly, and wasp eclosion might be different.

Conclusions

Our current study suggests that braconid wasps have evolved different diapause strategies in response to not just differences in the eclosion times of their *Rhagoletis* hosts but also to the specific fly life stage they attack. Moreover, differences in the phenological sensitivity of the lower host plant trophic level to variation in environmental conditions through time and space may also have cascading effects that sequentially and differentially affect eclosion time in the higher frugivore (fly) and parasitoid (wasp) trophic levels. Whether our findings are generally applicable to other phytophagous specialist insect and parasite systems in temperate environments will require additional research.

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