



## Research article

# Phenology model development for *Neodryinus typhlocybae*: Evaluation of phenological synchrony with its host, *Metcalfa pruinosa*

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## ABSTRACT

The invasive species *Metcalfa pruinosa* has inflicted significant economic losses in various European and Asian regions. To combat this pest, the parasitoid wasp *Neodryinus typhlocybae* has been effectively introduced in Europe. Despite its success, research on the field occurrence patterns of *N. typhlocybae*, particularly its phenology, remains scarce. This study aims to develop a degree-day model for predicting the adult emergence of *N. typhlocybae* from overwintering cocoons and to assess the phenological synchrony between *N. typhlocybae* adults and the nymphal stages of *M. pruinosa* in Korea. In this study, we estimated the thermal parameters of *N. typhlocybae* under field temperatures and six constant temperatures (13.92, 17.71, 18.53, 20.53, 22.78, and 24.03 °C) conditions. The lower developmental temperature was estimated using the values of the coefficient of variation for the cumulative degree days of emerged individual adults. The estimated lower developmental threshold temperature was 12.3 °C. With this developmental threshold, a degree-day model was developed, and this model well-predicted emergence in field conditions. By simulating this developed model with the actual occurrence of the nymphal stages of its host, *M. pruinosa*, adult wasp emergence was estimated to be 1.5 weeks later than the first instar nymph of the host but faster than other nymphal stages of *M. pruinosa*. Thus, the findings in this study would be helpful in determining the possibility of establishing *N. typhlocybae* and improving the management efficiency of *M. pruinosa*.

## 1. Introduction

The citrus flatid planthopper, *Metcalfa pruinosa* (Say) (Hemiptera: Flatidae), is native to North America [1] but has invaded non-native regions, including around 20 European and East Asia countries, including South Korea [2]. Its polyphagous characteristics

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combined with continual immigration into crop fields have been concerns in many countries where *M. pruinosa* has successfully established itself [3–7]. In addition to its agricultural pest impact, *M. pruinosa* has also been considered a nuisance to civilian life because it massively occurs around residential and urban areas, causing damage to ornamental trees and herbs [8,9]. Since it is expected that *M. pruinosa* will consistently cause damage in the future [10–12], proper actions to manage its abundance in non-native regions are necessary. Chemical application has been considered the major option in Korea to control the local abundance of *M. pruinosa*, mainly targeting agricultural fields [2]. Many studies supporting the effective chemical control of *M. pruinosa* have been performed as part of an integrated pest management (IPM) strategy [6,7,13]. These suggested strategies are very useful for the emergency control of *M. pruinosa* in agricultural fields. However, they are not long-term solutions for preventing consistent outbreaks [2] because *M. pruinosa* has already spread across Korea and occurs in a variety of landscapes, as well as around crop fields [9,14]. Even if *M. pruinosa* is properly managed for a year, it could cause damage in the following year due to its prominent dispersal when finding food or oviposition hosts [7]. This leads to repeated chemical spraying against the constantly immigrating *M. pruinosa* population, which can result in unintentional non-target effects on the environment.

Wilson and Lucchi [15] reported an enormous difference in the abundance of *M. pruinosa* between native and non-native regions and suggested that the difference was generated by the presence of a predator, as well as climatic factors. It is generally believed that the mass occurrence of an invasive species in novel habitats is associated with the absence of a natural enemy, as suggested by the enemy-release hypothesis (ERH) [16–18]. The ERH provides a theoretical basis for introducing the natural enemy of invaders in classical biocontrol [17]. Thus, introduction of *N. typhlocybae* would be a practical management options to manage invasive *M. pruinosa* as a long-term solution [19–23]. This parasitoid has been established in several European countries since its introduction from North America [14,22,24]. Introduction and release programs using mass-reared *N. typhlocybae* have also been considered in Korea at the government level, and the parasitoid populations were released limited area with allowance in farm scale where *M. pruinosa* had massively occurred [25]. However, environmental adaptability, such as the phenological synchrony of *N. typhlocybae*, which determines the successful management of *M. pruinosa*, has not been fully evaluated in Europe or Korea, where this biological control agent has already been released [2,23]. A study on the phenology of a biocontrol agent would aid in planning the optimal timing of a release program.

*Metacalfa pruinosa* is a univoltine insect, while *N. typhlocybae* has both univoltine and bivoltine life cycles [1,26]. *Neodryinus typhlocybae* adults effectively parasitize and lay eggs on either the third, fourth, or fifth instars of *M. pruinosa* [23,27]. The ectophagous larva of *N. typhlocybae* preys on the immature stages of *M. pruinosa*. The bivoltine individuals of the parasitoid firstly emerge as adults in the late spring season and the next summer population parasitize the nymphal stage of *M. pruinosa* again [28]. Both the univoltine and summer populations of bivoltine parasitoids overwinter as mature larvae by forming silky cocoons on leaves after leaving from their hosts. The adults of wasp emerge the following year when the nymphal stages of *M. pruinosa* are present. Therefore, phenological synchrony between *N. typhlocybae* and *M. pruinosa* would be a significant component for successful biological control of *M. pruinosa* [28]. The longevity of *N. typhlocybae* adult females is shorter than the nymphal period of *M. pruinosa* [26], so adult wasp emergence must not be too early but occur early enough to synchronize with the occurrence of *M. pruinosa* nymphs. Moreover, the co-occurrence of adult wasps and older instars (fourth and fifth instars) of the host could determine the persistency of this biological agent. This is because male-biased offspring is generated from the younger nymphs of *M. pruinosa* (before the fourth instar) [29]. Therefore, it is important to evaluate whether an introduced parasitoid could persist in the novel environment by completing its lifecycle in proper synchrony with the host species or not.

In this study, we aimed to develop a predictive model for the adult emergence of *N. typhlocybae*, which would be helpful in adjusting the timing of the release of the parasitoid. To achieve this, we investigated variations in the emergence time of *N. typhlocybae* with respect to experienced temperature regimes and developed a degree-day model. This model was subsequently validated under field conditions in Korea. Additionally, the phenology model was compared to independent data on the occurrence of *M. pruinosa* nymphs to evaluate phenological synchrony between *N. typhlocybae* adults and the nymphal stages of *M. pruinosa* in Korea. Based on the results, we discussed the potential effectiveness and persistence of *N. typhlocybae* when this parasitoid was released in non-native environments.

## 2. Material & methods

### 2.1. Study insects

In April 2017, approximately 80 two-year-old mulberry (*Morus alba*) seedlings were planted in a greenhouse (8 m × 50 m) at the Rural Development Administration (RDA; 35.8293434 N, 127.0408896E, Jeonju, Korea). The greenhouse, constructed using meshed cloth (60 mesh per square inch, Greenponex Corp.; Hwaseong, Korea) rather than plastic vinyl, was designed to synchronize interior and exterior environmental conditions while preventing the intrusion of unwanted insects. This setting facilitated the mass rearing of *Neodryinus typhlocybae*. In late May 2017, *M. pruinosa* nymphs, hatched from overwintering eggs, were collected from fields using insect aspirators (SL 13015, Shinill Science Inc.; Paju, Korea) and then transferred to new mulberry shoots within the greenhouse. If the target nymph density of over 100 nymphs per tree was not achieved by June 2018, additional *M. pruinosa* nymphs were introduced onto the mulberry leaves. Cocoons and newly emerged adults of *N. typhlocybae*, sourced from the University of Padova, Italy, in collaboration with the Animal & Plant Quarantine Agency, Korea, were released in the greenhouse. To enhance the survival rate of overwintering cocoons, newly formed *N. typhlocybae* cocoons were artificially collected from September 2018. These cocoons were stored in an open-air warehouse at the RDA, where the average temperature and relative humidity (RH) during the preservation period were 7.2 °C (±6.7 °C, SD) and 70% (±12%, SD), respectively. Starting in 2019, *M. pruinosa* nymphs were introduced annually to

maintain the desired population density. Overwintered *N. typhlocybae* cocoons were then released using customized cages (6 cm diameter × 7.5 cm height) onto new mulberry shoots to sustain the experimental population (Fig. 1).

### 2.1.1. Experimental design

Overwintered *N. typhlocybae* cocoons were individually put in an insect breeding dish (5 cm diameter × 1.5 cm height) (#310050, SPL Life Sciences). Forty prepared dishes were put in a plastic basket (Mirae Scientific; Daejeon, Korea). Each basket was then randomly assigned to one of three temperature conditions on two separate occasions: April 23, 2020 (18.53, 20.53, and 24.03 °C) and March 30, 2021 (13.92, 17.71, and 22.78 °C). This experimental design encompassed temperature regimes ranging from 14 to 24 °C, with 2 °C intervals, simulating the natural conditions that *N. typhlocybae* cocoons may encounter during spring temperature range of Korea. Although the constant temperature rooms (walk-in environmental room) were maintained within  $\pm 1$  °C, some discrepancies were observed between the set and actual temperatures. This experiment was conducted under a photoperiod of 14:10 (L:D) h with a RH of 55–65 % in constant temperature rooms in the RDA. The temperature and relative humidity of these rooms was monitored with HOBO data loggers (U12-012, OnSet Computer Corp.; Pocasset, MA, USA). Due to the limitation in the number of constant-temperature rooms in the RDA, the experiment was conducted over two years. Every two days, the emergence of adult *N. typhlocybae* was checked. After observing the first early adult in the experimental populations (approximately the middle of May) among 40 dishes, the dishes were checked daily for adult emergence.

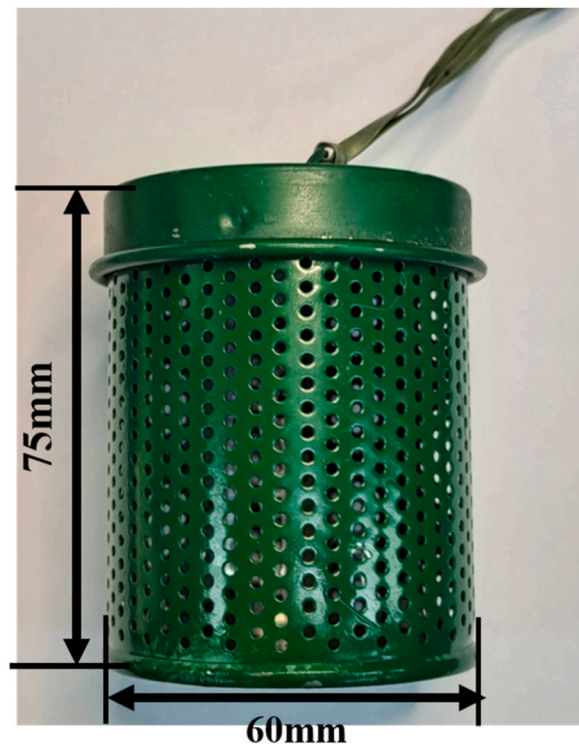
### 2.2. Statistical analysis

After the experiment, normality and variance homogeneity of the developmental data of *N. typhlocybae* cocoon were checked with Shapiro-Wilk and Bartlett tests in SAS [30]. ANOVA was applied to find out the effects of temperature on development of *N. typhlocybae* using PROC GLM in SAS [30]. Tukey HSD test ( $P < 0.05$ ) and Bonferroni correction were applied for mean separation in SAS [30].

The overwintered cocoon survival was calculated by dividing the number of emerged adults by the initial number of individuals (i. e., 40) at each temperature. The effect of temperature on the survival of overwintered cocoons was tested by the Chi-squared test [30].

### 2.3. Development of the phenology model

The experiments for the phenology model development were conducted under mixed conditions, incorporating both field overwintering and development of overwintered cocoons at constant temperatures, to encompass the entire period required for



**Fig. 1.** *N. typhlocybae* release cage. The lid (height 15 mm) had no hole but was attached to aluminum straps for tree hanging. There were 33 and 800 holes with diameters of 2.2 and 1.78 mm on the underside and sides of the cage, respectively. *N. typhlocybae* adults from overwintered cocoons emerged through these holes to escape from the cage.

*N. typhlocybae* adult emergence. The lower developmental temperature (LDT) for overwintered *N. typhlocybae* cocoons has not been previously established. Generally, the LDT of an insect species is estimated using a linear relationship between development time and temperature. However, in this study, the linear model was not applicable for estimating the LDT of overwintered *N. typhlocybae* cocoons due to the inability to determine the precise start time of development for each individual. Therefore, the LDT was estimated using the coefficient of variation (CV) for the cumulative degree days (CDDs) associated with the emergence of 172 adults from a total of 240 cocoons. Among all potential temperatures, the one with the lowest CV was considered the most accurate estimate for the LDT.

To estimate the LDT of overwintered *N. typhlocybae* cocoons, the CDDs for each individual were calculated by summing the CDDs at the overwintering site (open-air warehouse at the RDA) and in the constant temperature room. For field conditions, CDDs from January 1, and for laboratory conditions, from the start date of the experiment to the adult emergence date, were computed. The field CDDs were calculated using the Degree-Day Utility (UC IPM Online) with the single-sine method, based on temperature data (i.e., daily maximum and minimum air temperatures) from the nearest KMA weather station, about 10 km from the overwintering site. In the constant temperature room, CDDs were computed by multiplying the number of days to complete development by the difference between the experimental temperature and candidate LDTs. These candidate LDTs, ranging from 7 to 15 °C in 1 °C increments, were applied to calculate daily degree days for both field and laboratory conditions. For more precise LDT estimation, CV values were compared at 0.1 °C intervals between two temperatures with the lowest CVs.

At each treatment temperature, the cumulative proportion (%) of adult emergence was transformed from the frequency data of the developmental time of 40 individuals. The cumulative proportion was fitted with a two-parameter Weibull cumulative function against cumulative degree days to describe the variation in adult emergence of overwintered *N. typhlocybae* cocoons (Eq. (1)).

$$P(DD_x) = 100 \left( 1 - e^{-\left(\frac{DD_x}{\alpha}\right)^\beta} \right) \quad (\text{Eq. 1})$$

where  $P(DD_x)$  is the cumulative proportion of individuals that completed development at degree days ( $DD_x$ ), and  $\alpha$  and  $\beta$  are parameters to be estimated. The Weibull function parameters were estimated with PROC NLIN function in SAS [30].

#### 2.4. Model validation

To validate the adult emergence model of *N. typhlocybae* developed in this study, adult occurrence was monitored in 2021 under open-air conditions at three locations in Korea: the open-air warehouse of RDA in Jeonju-Si, the roof-top of RDA in Jeonju-Si, and the balcony of the Forest Medicinal Resources Research Center (FMRRC) in Yeongju-Si. Overwintered *N. typhlocybae* cocoons, stored at the RDA's open-air warehouse, were placed on March 9 at the RDA roof-top and on April 7 at the FMRRC balcony. Each cocoon was housed in an individual insect breeding dish (5 cm diameter × 1.5 cm height), and these dishes were placed in shaded, rainfall-protected areas at all sites. Adult emergence was checked daily from the first occurrence until June 30, 2021. The total numbers of emerged adults were 187 at the RDA open-air warehouse, 34 at the RDA roof-top, and 53 at the FMRRC.

CDDs were calculated individually for each site by adding the CDDs from the overwintering location and each validation site. These calculations were performed using the Degree-Day Utility (UC IPM Online) with the single-sine method. Temperature data, including daily maximum and minimum air temperatures, were sourced from KMA weather stations located within 20 km of each experimental site. The LDT estimated in this study was used for the degree-day calculations. The validity of the developed model was assessed by comparing the deviations in Julian dates between the predictive model and actual field observations at 10, 30, 50, 70, and 90% cumulative frequencies of emergence. The degree days corresponding to each cumulative frequency in the field were estimated through linear interpolation of the nearest two samples.

#### 2.5. Phenological synchrony with host nymphs

The developed phenology model for *N. typhlocybae* adult emergence was compared to the nymphal periods of *M. pruinosa* estimated by the first instar and adult emergence models suggested by Kim et al. [2] and Kim and Lee [7]. The nymphal period was estimated as the duration between the 50% occurrence time of the first instars and the adults of *M. pruinosa*. Average temperature data, including daily maximum and minimum values for 30 years (1991–2020) across 219 weather stations (KMA) of the whole territory of Korea, were used for phenological simulations. The CDDs for the development of *M. pruinosa* eggs and nymphs were computed using an LDT of 10.1 °C with the single-sine method. Each date corresponding to 361 and 1173° days was regarded as the 50% occurrence time of the first instar and adult of *M. pruinosa*, respectively, based on predictive models from previous studies. Among 219 simulated locations, one (Daegwallyeong) was excluded because *M. pruinosa* adults were predicted not to occur due to low temperatures. The 50% emergence time of *N. typhlocybae* estimated by our model was checked to determine if it fell within the estimated *M. pruinosa* nymphal occurrence period.

In addition, the predicted emergence time of *N. typhlocybae* was also compared to the actual occurrence data of each developmental stage of *M. pruinosa* provided by Kim and Lee [7]. These data of *M. pruinosa* were constructed by field sampling (one-week intervals) using clear sticky traps at the border of a soybean field in Yeoncheon-Gun in Korea in 2017. For a clear comparison between adult *N. typhlocybae* emergence and the nymphal stages of its prey, the phenology model was transformed to a probability density function form based on the estimated parameters in the model developed in this study. The transformed model and nymphal occurrence data for each developmental stage were then standardized by dividing each maximum value and visualizing it on the Julian

date scale. Deviations between the peak times of parasitoid adult emergence and host nymphal occurrence at each development stage were calculated to evaluate phenological synchrony.

### 3. Results

#### 3.1. Development of the phenology model and its validation

Overwintered *N. typhlocybae* cocoons, which were moved to controlled temperature conditions, could complete their development into adults from 17.71 to 24.03 °C (Table 1). However, no individuals occurred as adults at 13.92 °C over six months. The developmental rate of the cocoon was affected by experimental temperatures in this study ( $F = 1027.22$ ;  $df = 4, 167$ ;  $P < 0.001$ ) (Table 1). However, its survivorship was not affected by experimental temperatures ( $X^2 = 3.508$ ;  $df = 4$ ;  $P = 0.5481$ ) (Table 1).

The CV values for the CDDs required for adult emergence of each *N. typhlocybae* individual showed a decrease up to 12.3 °C, and then an increase with higher potential lower LDTs (Fig. 2). Consequently, 12.3 °C was determined to be the optimal LDT for the development of overwintered *N. typhlocybae* cocoons. The variability in adult emergence from these overwintered cocoons was significantly explained by two parameters of the Weibull function ( $F = 232.1$ ;  $df = 1, 44$ ;  $P < 0.0001$ ;  $r^2 = 0.92$ ) (Fig. 3). The parameters of this model were calculated to be  $\alpha = 449.700 \pm 3.8059$  and  $\beta = 13.045 \pm 2.3260$ .

There was an observed average difference of 2.3 days in the emergence of *N. typhlocybae* adults from overwintered cocoons between the developed predictive model and the actual field observations (Table 2). This discrepancy generally decreased from the early occurrence up to the 70% occurrence mark and then increased again during the later stages of occurrence.

Adult *N. typhlocybae* were predicted to emerge during the nymphal occurrence period of *M. pruinosa* in all 218 locations examined in Korea, regardless of the timing of the phenological events at each site (Fig. 4). The predicted time of 50% *N. typhlocybae* emergence was later  $20.6 \pm 2.13$  days than first instar of host, but faster  $29.2 \pm 1.83$  days than that of the adult across Korea. The predicted emergence time of *N. typhlocybae* overlapped with the actual occurrence of the nymphal stages of *M. pruinosa*, indicating that this wasp would have a chance to exploit its host under field conditions (Fig. 5). The adult wasp emergence was estimated to be 1.5 weeks later than the first instar of the prey but faster than other nymphal stages based on the peak timing of phenological events. The predicted emergence time distribution of *N. typhlocybae* adults was estimated to be faster than the occurrence of *M. pruinosa* adults (Figs. 4 and 5).

### 4. Discussion

As the phenomenon is well known, temperature plays a pivotal role in determining insect phenology [31]. An insect species requires a certain amount of heating units to complete its development from one point in its lifecycle to another, and thus this can be exploited to predict occurrence time of the insect [33]. In this study, we developed degree-days-based model to forecast the phenology of *N. typhlocybae* under field conditions. This model has advantages, such as ease calculating heat units and relatively good performance [34]. The phenology model of *N. typhlocybae* adults developed in this study also showed high accuracy in predicting their occurrence in fluctuating temperature conditions. This phenomenon indicated that this model could be applicable to predict the occurrence of *N. typhlocybae* adults in field conditions.

To develop a degree-day model of an insect species, the LDT is required to calculate thermal requirement of target species. The LDT of a target species is generally estimated by the linear regression of its developmental rate in constant-temperature experiments [32]. However, it was not feasible in this study because the physiological age of each overwintering cocoon and experimental years were different. Further each individual cocoon was exposed to both fluctuating (preserving temperature for overwintering) and constant (experimental temperature for developmental experiment) temperatures. Even though it was suspected that overwintering *N. typhlocybae* cocoons would require a certain low-temperature period to complete their overwintering, the critical temperature and duration have not yet been determined. Thus, we needed to provide enough chilling periods to activate their development after overwintering. Moreover, we wanted to minimize the experimental errors caused by observing experimental units in laboratory conditions. Thus, strict temperature-controlled rooms were used instead of experimental chambers required observation in laboratory

**Table 1**

Developmental period (days, mean  $\pm$  SD) and survival (%) of the cocoon of *N. typhlocybae* at different temperatures.

Transferred date <sup>a</sup>	Temperature (°C)	N <sup>b</sup>	Development time	Survival <sup>e</sup>
April 23, 2020	13.92	- <sup>c</sup>	-	-
	17.71	35	78.2 $\pm$ 4.82 a <sup>d</sup>	87.5 a (35/40)
	18.53	26	62.0 $\pm$ 2.87 b	65.0 a (26/40)
March 30, 2021	20.53	39	44.5 $\pm$ 1.79 c	97.5 a (39/40)
	22.78	38	37.2 $\pm$ 3.84 d	95.0 a (38/40)
	24.03	34	35.4 $\pm$ 2.52 d	85.0 a (34/40)

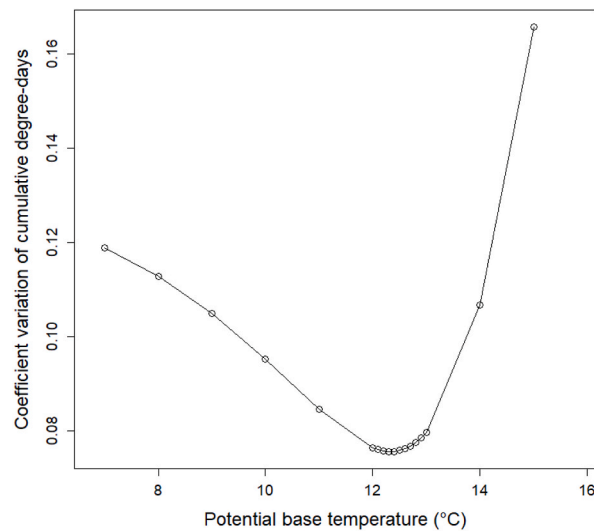
<sup>a</sup> Date transferred to constant-temperature room from overwintering site in RDA.

<sup>b</sup> Number of emerged adults at each temperature regime.

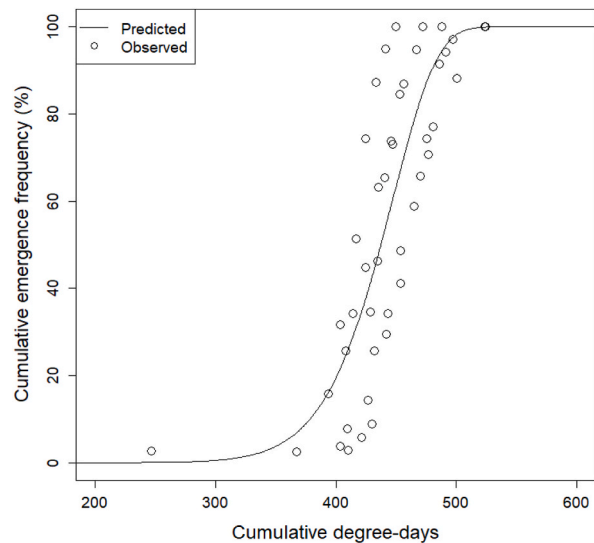
<sup>c</sup> No individual emerged.

<sup>d</sup> Means within a column followed by the same letter are not significantly different ( $P > 0.05$ ).

<sup>e</sup> Numbers in parentheses indicate the numbers of individuals that survived/total number of individuals tested.



**Fig. 2.** Coefficient of variation values of the degree days required for adult emergence from overwintered *N. typhlocybae* cocoons according to potential lower developmental threshold temperatures.



**Fig. 3.** Cumulative distribution (%) of adult emergence from *N. typhlocybae* cocoons vs. cumulated degree days.

conditions. Since we had only three temperature-controlled rooms, this experiment required two years to complete.

The LDT of overwintered *N. typhlocybae* cocoons was estimated at 12.3 °C in this study. This LDT theoretically indicates that temperatures over 12.3 °C would activate the development of overwintered cocoons. However, no individuals successfully developed into adults at 13.92 °C during the experimental period of six months. In this study, the thermal requirement of overwintered *N. typhlocybae* cocoons to adults was estimated to be 437.3°-days. Even though considering the accumulated degree days of overwintered *N. typhlocybae* cocoons during preserving periods, populations at 13.92 °C would require 248 days after transferring to a temperature-controlled room, explaining why no individuals completed their development to adults at 13.92 °C. To successfully become adults within six months, a temperature of at least 14.6 °C was required in the conditions in this experiment.

When we simulated the phenology of *M. pruinosa* nymphs and *N. typhlocybae* adults (Fig. 4), the emergence time of *N. typhlocybae* adults was within the nymphal period of the host. Because the wasp was predicted to emerge closer to the timing of the first instar occurrence than that of the adults of the host, it is expected that *N. typhlocybae* will have enough time for the predation and parasitism of *M. pruinosa*. Compared to actual host nymph occurrence, the estimated emergence time of *N. typhlocybae* overlapped with the first instars of its prey but was slightly later relative to the peak timing. The peak emergence time of adults was faster than the second, third, fourth, or fifth developmental stages of *M. pruinosa* nymphs but was estimated to partially overlap with the prey occurrence time.

*Neodryinus typhlocybae* was reported to prefer older than third instar stage of *M. pruinosa*, and the females can more successfully

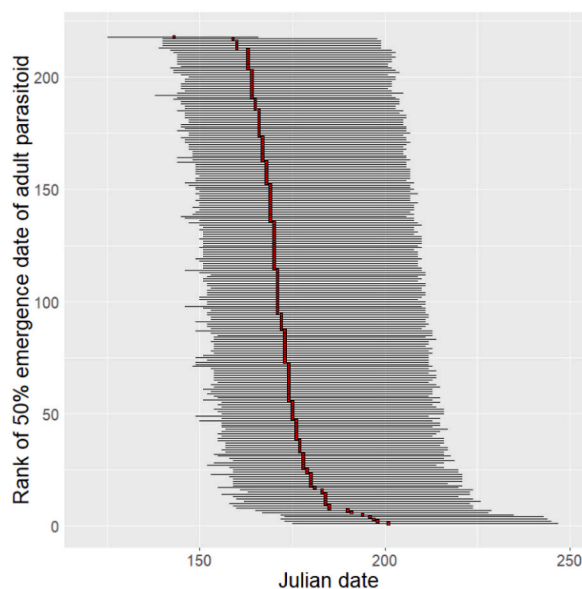
**Table 2**Comparison between observed and predicted *N. typhlocybae* adult occurrence from overwintered cocoons.

Sites	Occurrence (%) <sup>a</sup>	Observed DD <sup>b</sup>	Predicted DD <sup>c</sup>	Difference (days)
RDA open-air warehouse (Jeonju-Si)	10	332.5	355.7	2
	30	372.4	390.0	2
	50	397.2	410.0	1
	70	433.5	427.5	1
	90	467.1	448.9	2
RDA roof-top (Jeonju-Si)	10	326.8	355.7	3
	30	367.8	390.0	4
	50	387.0	410.0	2
	70	443.6	427.5	1
	90	512.2	448.9	5
FMRRC balcony (Youngju-Si)	10	366.2	355.7	1
	30	376.7	390.0	2
	50	393.7	410.0	2
	70	414.8	427.5	1
	90	537.5	448.9	7
Average				2.3

<sup>a</sup> Each occurrence was estimated from the nearest two points in linear regression.

<sup>b</sup> Observed DD indicates the observed cumulative degree days at each cumulative occurrence point (%) of *N. typhlocybae* adults in each site.

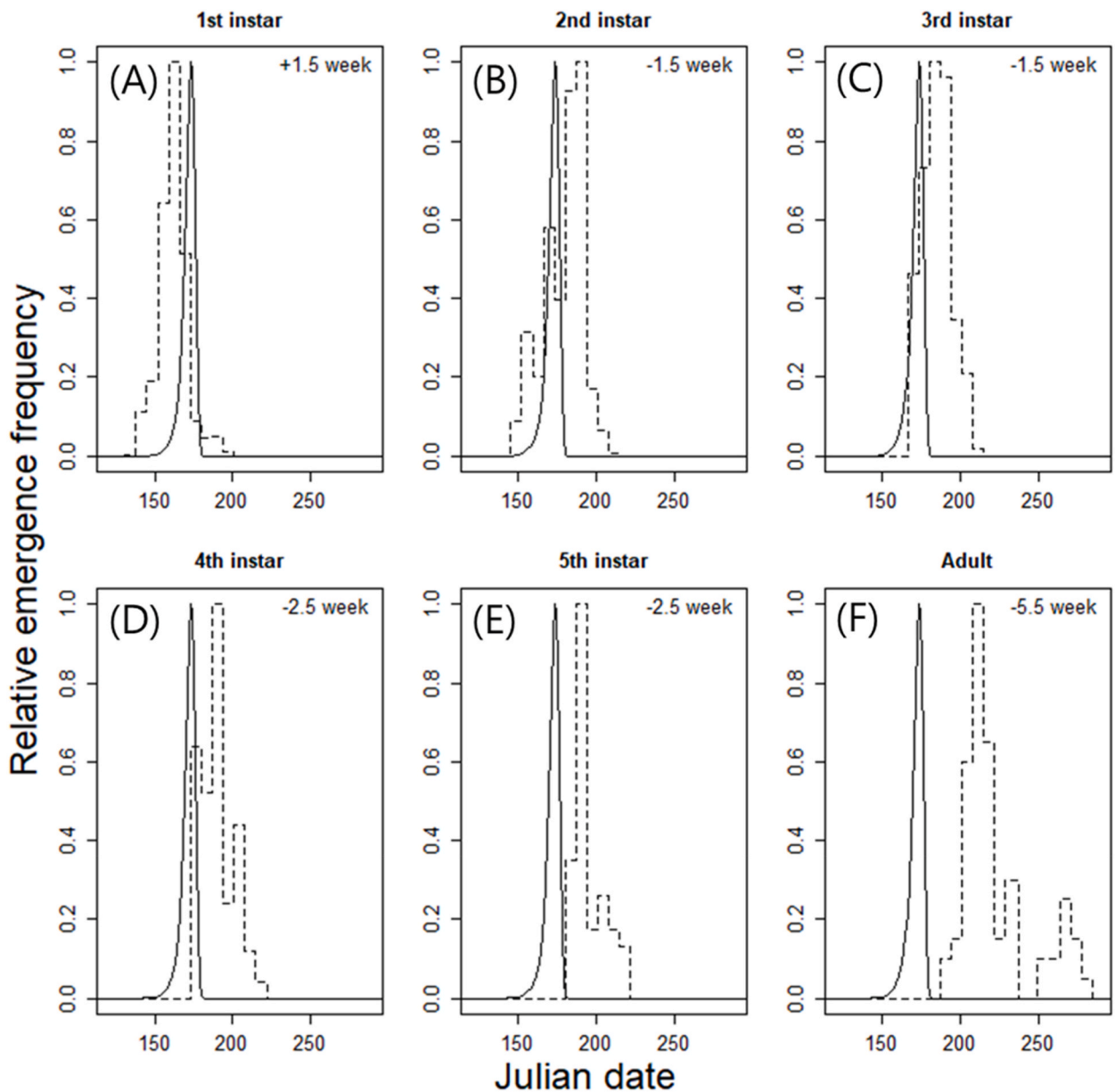
<sup>c</sup> Predicted DD indicates the cumulative degree days at each cumulative occurrence point (%) of *N. typhlocybae* adults predicted by the model in this study.



**Fig. 4.** Estimated nymphal period for *M. pruinosa* in 218 regions throughout Korea, represented by black horizontal lines. The estimates were calculated based on the time interval between 50% occurrence of both first instar and adult stages. The red vertical line represents the projected 50% emergence time for *N. typhlocybae* adults across the 218 regions in Korea. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

develop from older instar stages (i.e., fourth and fifth instar stage) than the third instar stage of *M. pruinosa* nymphs due to the size of the prey [29]. Because female *N. typhlocybae* adults could be alive for about 2–4 weeks despite the estimation in our laboratory conditions [26], our simulation results potentially suggested that the emerged female wasps would encounter older instar stages of their prey. Moreover, female wasps experienced with younger instar prey could have greater rates of producing female offspring on fifth nymphs compared to females that had never encountered younger instars [29]. Therefore, our simulation results indicated that *N. typhlocybae* could establish themselves in novel conditions in Korea with proper synchrony with their prey, along with the European countries where their successful establishment and spread have already been well-reported.

The synchrony observed between *N. typhlocybae* adults and *N. pruinosa* nymphs in field conditions showed a high likelihood of successful establishment for released *N. typhlocybae* populations in Korea. Nevertheless, releasing *N. typhlocybae* adults during the occurrence of *M. pruinosa* fourth or fifth instars in field conditions could enhance efficiency, given their preference for older nymphal stages (i.e., fourth and fifth instars) [29]. The findings from this study could contribute to better management of *N. typhlocybae* cocoon



**Fig. 5.** Phenological distribution of *N. typhlocybae* adults and developmental stages of *M. pruinosa*. The occurrence of *N. typhlocybae* adults is represented by a solid line and was estimated using the phenology model, while the developmental stages of *M. pruinosa*, observed in the field, are indicated by dashed lines. The different developmental stages of *M. pruinosa* are depicted in each panel: (A) first instar, (B) second instar, (C) third instar, (D) fourth instar, (E) fifth instar, and (F) adult.

development in laboratory (controlled) conditions and inform optimal release timing under field conditions.

## 5. Conclusions

In summary, this study provided fundamental information related to the temperature-dependent development of overwintered *N. typhlocybae* cocoons. The finding would be applicable in adjusting development time in controlled conditions for purpose of release program. We also found proper synchrony with the occurrence of *N. typhlocybae* adults and their host in Korea. This information should be helpful in evaluating the possibility of establishing *N. typhlocybae* in novel country conditions when considering the intentional introduction of this biocontrol agent.



## Additional information

No additional information is available for this paper.

## Data availability statement

The data presented in this study are available upon request from the corresponding author.

## CRediT authorship contribution statement

**Sunghoon Baek:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Min-Jung Kim:** Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Formal analysis, Data curation, Conceptualization. **Bo Yoon Seo:** Writing – review & editing, Writing – original draft, Methodology, Investigation, Conceptualization. **Kwang-Ho Kim:** Methodology, Investigation. **Chang-Gyu Park:** Methodology, Investigation. **Jumrae Cho:** Writing – original draft, Methodology, Investigation. **Hong-Hyun Park:** Writing – review & editing, Writing – original draft, Supervision, Software, Resources, Methodology, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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