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Research

Comparison of the Hatch of Newly Laid *Lycorma delicatula* (Hemiptera: Fulgoridae) Eggs From the United States After Exposure to Different Temperatures and Durations of Low Temperature

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Abstract

Comparisons were made of the effects of temperature and duration of low temperature on hatch of newly laid egg masses of the invasive spotted lanternfly, *Lycorma delicatula* (White). Egg masses were collected in mid-October 2019 and estimated to be less than 14 d old. There was a significant positive nonlinear relationship between temperature and developmental rate (1/d) for eggs held at constant temperatures. The lower threshold for egg development was estimated as 7.39°C. Eggs held at constant 10, 15, and 20°C were estimated to require 635, 715, and 849 $DD_{7.39}$, respectively, to develop. Egg hatch was variable, egg hatch rates were highest (58.4%) when held at a constant 15°C, though high rates (52.7%) were also obtained when eggs were held for 84 d at 10°C, then moved to 25°C. Almost all eggs enter diapause since very few eggs were able hatch when moved to 25°C after 7 d of chill at either 5 or 10°C. Chilling at 5 or 10°C increased percentage egg hatch as the duration in chill increased up to ~100 d and eggs held at 10°C were able to complete some or all the post-diapause development before being moved to 25°C. All egg masses were held at constant 16:8 (L:D) photoperiod and 65%RH. Our data suggest that temperature is the driving factor for diapause termination in spotted lanternfly, but other abiotic factors should be investigated. These identified developmental temperature threshold and degree day requirements for egg hatch will improve predictive distribution and phenological models.

Key words: overwintering, temperature, diapause, spotted lanternfly, egg

When developing phenology models, identification of abiotic factors contributing to the start of development is critical. For many temperate insect species development begins at the termination of diapause (Tauber and Tauber 1976) but the cues used to terminate diapause or quiescence and commence development vary depending on which life stage is overwintering. Diapause itself is a physiological adaption to synchronize occurrence of life stages with the environment and available food resources (Tauber and Tauber 1976). Insects often use temperature and/or photoperiod as cues to initiate and terminate diapause, and the stage at which the insect overwinters can determine which is the dominant cue. It is also possible for the diapause in eggs to be maternally determined (maternal inheritance, Mousseau and Dingle 1991).

We studied the effects of temperature on diapause termination and post-diapause development for the eggs of the spotted lanternfly, *Lycorma delicatula* (White) (Hemiptera: Fulgoridae), an invasive plant hopper in the United States of America (Dara et al. 2015) that overwinters in the egg stage. Winter diapause in eggs may provide protection against winter mortality and desiccation as it does in gypsy moth, *Lymantria dispar* L. (Lepidoptera: Lymantriidae) (Smitley et al. 1998). Hemiptera are well adapted to varying ecological processes and while most diapause occurs in the nymph or adult stage there are a few examples of egg diapause primarily in Miridae and Lygaeidae species (Musolin and Saulic 1999). Among the Hemiptera that have been studied, photoperiod during the nymphal stage

has been identified as the primary cue that initiates egg diapause, though temperature can also induce diapause (Musolin and Saulic 1999, Higuchi and Takahashi 2005). Cues to initiate and terminate diapause in Fulgoridae are relatively understudied as most species are not pestiferous. However, spotted lanternfly is invasive in the Republic of Korea and the eastern United States where it threatens specialty crops, particularly cultivated wine grapes (Dara et al. 2015, Leach and Leach 2020). Females oviposit 30–50 eggs on smooth vertical surfaces during the fall. These egg masses are coated with a waxy covering that may aid in preventing desiccation and buffering the eggs from temperature extremes. Studies carried out in the Republic of Korea (Park et al. 2009, Choi et al. 2012) documented hatch rates of 33–84%, and found decreased egg hatch with increasing incubation temperatures. These data were used to model lanternfly hatch in the spring (Lee et al. 2014) and to estimate a lower temperature threshold for egg hatch of 8.14°C (Choi et al. 2012). Other studies suggest that diapause termination in *L. delicatula* eggs requires a chilling period, and that a heat shock protein may play a role in the resumption of development (post-diapause development before hatch) once diapause is terminated (Shim and Lee 2015).

During an invasion event, the founding population carries with it only a small proportion of genetic diversity ('founder effect') which can cause the invading population to manifest biological characteristics that differ from the mean or median characteristics of the source population. For example, the loss of intercolony aggression in Argentine ants following a founder effect driven genetic bottleneck (resulting from a founder effect) is thought responsible for the loss of intercolony aggression, leading to the formation of super colonies in invasive populations (Suarez et al. 1999). These shifts in species biology and behavior highlight the importance of characterizing invasive populations found in novel regions.

As of 2020, spotted lanternfly egg eclosion in Virginia, New Jersey, and Pennsylvania typically begins in mid-May, although first-instar nymphs can be observed through early July suggesting intrapopulation plasticity in duration of diapause (Tauber and Tauber 1976). Feeding occurs on over 70 host plants (Dara et al. 2015, Lee et al. 2019) with early nymphs preferring to feed on phloem from younger and softer vegetative tissues, while later instars and adults tend to feed on the phloem through the bark. First instars appear to have a broad host range and the lack of selectivity by females for oviposition sites (oviposition is commonly observed on non-host surfaces including concrete and stone) suggests the absence of a preference–performance link (Price et al. 1980, Thompson 1988), so female choice of oviposition sites may have little effect on the nymphs finding suitable food. Further, a diverse nymphal diet increases survivorship to adulthood and egg production (Uyi et al. 2020). Key host plants of spotted lanternfly include *Ailanthus altissima* (Miller) Swingle (Sapindales: Simaroubaceae), *Vitis* sp. L. (Vitales: Vitaceae), *Betula nigra* L. (Fagales: Betulaceae), *Acer rubrum* L. (Sapindales: Sapindaceae), and *Juglans nigra* L. (Fagales: Juglandaceae). Of primary economic concern is the feeding on cultivated grape vines and the build-up of the population when egg masses are laid on vines or on posts within vineyards (Leach and Leach 2020).

To support management of this high impact pest and to understand the potential ecological roles, we investigated the effect of chill duration and rearing temperature on spotted lanternfly egg hatch rates and timing. These data are necessary to accurately predict the potential success and timing of egg hatch across the landscape. The lower developmental threshold and required number of degree days (DD) for egg hatch were also determined; these data permit identification of an in-field biofix and estimated timing of first-instar occurrence.

Materials and Methods

Egg Mass Collection

Five-hundred and forty-six spotted lanternfly egg masses were collected in Pennsylvania, USA on 15–16 October 2019 and brought to the USDA Forest Service Quarantine Laboratory, Ansonia, CT under valid state and federal permits. An additional fourteen egg masses were laid by females collected at the same time. The timing of egg mass collection was chosen to ensure that the eggs had been laid within the last 2 wk (most in the last few days based on observations made by PA Department of Agriculture staff) and had minimal exposure to temperatures ≤ 5°C. Sixty-five egg masses were collected in Hellertown, PA in the Water Street Park along the Saucon Rail Trail (40.85°N 75.34°W). Four of these egg masses were collected from *Acer negundo* L. (Sapindales: Sapindaceae), 7 from *J. nigra*, 7 from *Malus pumila* Miller (Rosaceae: Rosales) and the remaining 47 from *A. altissima*. Sixty-four egg masses were collected off *A. altisima* in Whitehall township on the Ironton Rail Trail (40.67°N 75.51°W) and the remaining 417 were from *A. altisima* in Pennsburg, PA (40.41°N 75.48°W). An additional 14 egg masses were laid in the laboratory by adults collected at the Pennsburg, PA location. Voucher specimens of adults reared from these egg masses have been deposited at the Entomology Division, Yale Peabody Museum of Natural History, New Haven, CT. Each egg mass was carefully removed from the host tree by cutting through the bark around the egg mass and then peeling the section of bark off with the egg mass attached. The bark sections were dried for 30–60 min sitting on a raised screen under a laminar flow hood at ambient room temperature to reduce the moisture in the bark to inhibit fungal growth. Egg masses were then placed individually in 60 mm diameter × 15 mm deep petri dishes (Falcon 1007, Corning, Corning, NY).

Treatments and Data Collection

There were 15 total temperature treatments to which egg masses were exposed, each of these treatments used the same photoperiod of 16:8 (L:D) and ≥ 65% RH. Five different environmental chambers (20°C Percival, Perry, Iowa; 5 and 10°C Puffer Hubbard, Minnesota company that is no longer in business; and 15 and 25°C Environmental Growth Chambers, Chagrin Falls, Ohio) were used for the experiments and chart recorders were placed in each chamber to ensure the set points were resulting in the desired temperatures (± 1°C). Three of the temperature treatments involved holding the egg masses at a constant temperature (10, 15, or 20°C). After being air dried as stated above, egg masses were immediately placed at those temperatures. Egg masses that were to be chilled at 5 or 10°C for varying lengths of time followed by incubation at 25°C (11 treatments) were held for 7 d at 20°C before the treatments began to provide the eggs with additional development time (pre-diapause) before entering diapause. At both 5 and 10°C, there were 7, 28, 56, or 84-d chill treatments. Treatments in which eggs were held for 112 and 140 d at 5°C, and 216 and 250 d at 10°C were added after results from the first treatments indicated the full range of durations over which eggs could hatch was not fully captured. Egg masses from each collection location were divided evenly across the constant temperature and 7–84 d chill treatments: each treatment received 5–6 egg masses each from Hellertown and Whitehall and 28–29 from Pennsburg for a total of 40 egg masses per treatment. Forty Pennsburg, PA egg masses (either collected or lab laid) were used for the 112, 140, and 216 d treatments. At 250 d, half of the egg masses held at a constant 10°C were moved to 25°C to create the 250 d at 10°C treatment.

Some dishes contained more than a single egg mass because they were laid too close to separate and were treated as a single egg mass for the purposes of the study. The petri dishes containing the egg masses were stacked in clear 355 ml plastic cups (Solo TP12 cold drink cup, Solo Cup Operating Corp., Dallas, TX) which were held in large clear plastic boxes. Egg hatch was evaluated by counting and removing nymphs daily following transfer from chill to 25°C, and after first hatch was observed in the constant temperature treatments. The constant 10°C treatment was checked three times per week for first hatch (Monday, Wednesday, and Friday) and the 15 and 20°C treatments were checked daily. Daily hatch checks continued for each egg mass for 30 d after the last non-zero observation.

When daily checks for hatch ended, the gray waxy coating covering the eggs was carefully removed using a paintbrush. Egg hatch was confirmed by the presence of an egg lid (often still attached) or the hole that is left when it is pushed up (Liu 2019a). All hatched and unhatched eggs were counted, and percentage hatch of eggs within each egg mass was calculated. The number of days from study initiation at 10, 15, 20, or incubation at 25°C to first hatch and the duration of hatch also was determined for each egg mass.

Statistical Analysis

Statistical analyses were performed using SAS 9.4 (SAS Institute 2015). The Shapiro–Wilk and Anderson–Darling tests were used to evaluate the normality of the data. When the data were not normally distributed PROC UNIVARIATE was used to assess the fit of the data to a gamma distribution which can handle data with long right tails. PROC GLIMMIX (a generalized linear mixed model) was used to evaluate the effects of temperature treatments on days to hatch and DD requirements for individual eggs and percent hatch, days to first hatch, and hatch duration for egg masses. The site that the egg mass was collected, or the egg mass the egg was from were treated as a random effect in the models for egg mass data and individual egg data, respectively. The percentage hatch was fitted to a beta distribution with a logit link function while days to hatch, days to first hatch, and duration of hatch were fitted to a gamma distribution with a log link function. Proportion values zero and 1, which are cannot be fit in a Beta distribution, were replaced with 0.0001 and 0.9999 before analyses. The Tukey–Kramer post-hoc analysis at $\alpha = 0.05$ was used to determine if means were significantly different in pairwise comparisons. The residuals for each model were evaluated for normality and the homogeneity of variance in each temperature treatment was checked using Levene's test.

To determine the best nonlinear model to use for each of the following relationships, we selected the one with both the best visual fit and adjusted r^2 among commonly used models. The relationship between percentage of egg masses hatching and days held at 5 or 10°C were both fitted to a quadratic equation ($Y = a + bx + cx^2$) using PROC NLIN and the Marquardt convergence method (SAS Institute 2015). The relationship between egg developmental rate (1/d) and temperature (10–20°C) was also fitted to a quadratic equation. The relationship between average percentage hatch and days at temperature was also fitted to a quadratic equation for time at 5°C or a third level polynomial equation ($Y = a + bx + cx^2 + dx^3$) for time at 10°C. In each case the points at which the fitted model crossed the *x*-axis were determined by solving for y+ 0.

The number of DD required for each egg to hatch (all temperature treatments) was calculated using the lower threshold of 7.39°C, which was estimated based on the relationship between developmental rate and temperature. Based on the DD calculations there appeared to be two different processes occurring (see Results), presumably diapause (occurring above the lower threshold) and post-diapause development.

For the diapause development model, the post-diapause DD estimated for each individual egg were subtracted from the total DD for the treatments involving constant temperatures and 7–28 d exposure at 5 and 10°C. The number of DD subtracted was kept proportional to the position of the individual egg in the distribution of total DD for these treatments. Accumulated DD required by 10, 50, and 90% of the eggs to complete a phase were calculated.

The post-diapause development relationship between cumulative proportion of eggs hatching and accumulated DD was modeled using the Gompertz function, $P = \exp[-\exp(-b * DD + a]$ with PROC NLIN and the Marquardt convergence method. The post-diapause model used the treatments that involved 56–112 d exposures at 5 and 10°C.

Results

Time to first hatch within an egg mass and mean days to hatch for individual eggs decreased with increasing constant temperature, while duration of hatch (time between first and last egg hatch) for individual egg masses did not significantly change (Fig. 1A, Table 1). Percentage hatch within egg masses was significantly higher for 15°C than for either 10 or 20°C. Only 20% of the egg masses held at constant 10°C had any hatch, while 97.5% and 90% of the egg masses at 15 and 20°C had at least some hatch, respectively. The average percentage hatch of egg masses that had hatch was 13.1, 65.9, and 13.9% for 10,

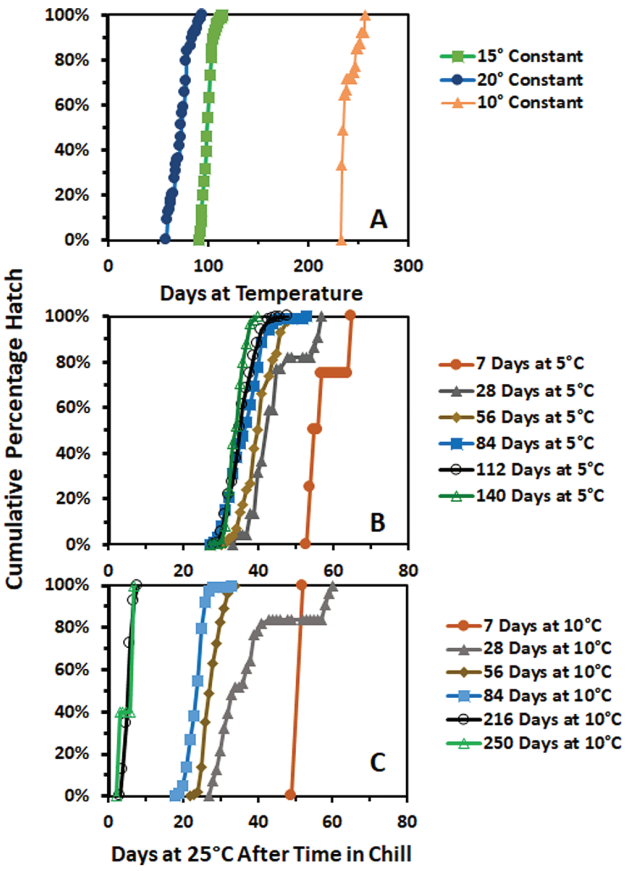

Fig. 1. Cumulative percentage hatch curves for *Lycorma delicatula* eggs (A) held at three constant temperatures 10, 15, and 20°C or exposed to different durations at 5 (B) or 10°C (C) followed by incubation at 25°C.

Table 1. Mean [± SE (*n*)] for various *Lycorma delicatula* egg hatch parameters under different temperature regimes

Temperature regime	Individual egg time to hatch (days)[1]	Percentage hatch for an egg mass	Time to first hatch for an egg mass (days)[1]	Duration of hatch for an egg mass (days)	Calculated DD (base 7.39°C)
10° Constant	241.5 ± 10.6a (33)	2.0 ± 1.0d (40)	239 ± 12.3a (8)	11.1 ± 3ab (8)	631.0 ± 21.0c (33)
15° Constant	93.4 ± 1.8b (3)	58.4 ± 6.1a (40)	91.6 ± 2.1b (39)	6.5 ± 0.8abcd (39)	711.1 ± 10.3b (1004)
20° Constant	69.3 ± 1.5c (56)	10.8 ± 2.9cd (40)	68.1 ± 1.7c (36)	7 ± 0.9ab (36)	875.2 ± 13.9a (208)
7 d at 10°C followed by 25°C	41.0 ± 3.8de (4)	0.2 ± 0.3bcd (40)	41.0 ± 4.2def (2)	1.0 ± 0.5f (2)	740.4 ± 51.3ab (591)
28 d at 10°C followed by 25°C	32.0 ± 1.2ef (976)	2.4 ± 1.2d (40)	28.3 ± 1.2egh (12)	12.8 ± 2.8a (12)	637.7 ± 17.5b (4)
56 d at 10°C followed by 25°C	21.5 ± 0.4g (591)	34.3 ± 5.5b (40)	20.6 ± 0.5i (35)	3.7 ± 0.5cdef (35)	526.3 ± 8.2c (976)
84 d at 10°C followed by 25°C	17.3 ± 0.3h (563)	52.7 ± 6.0a (40)	16.4 ± 0.4j (37)	4.1 ± 0.5bcd (37)	525.6 ± 7.8c (563)
216 d at 10°C followed by 25°C	5.8 ± 0.1i (1004)	30.6 ± 5.6b (40)	5.1 ± 0.1k (37)	2.8 ± 0.4def (37)	666.7 ± 10.0b (3)
250 d at 10°C followed by 25°C	5.5 ± 0.4i (208)	1.3 ± 1.1cd (20)	6 ± 0.4k (4)	1.0 ± 0.4ef (4)	667.4 ± 33.6b (56)
7 d at 5°C followed by 25°C	48.48 ± 3.344d (4)	0.2 ± 0.3bcd (40)	48.8 ± 3.5d (4)	1 ± 0.4f (4)	853.7 ± 43.7a (4)
28 d at 5°C followed by 25°C	36.74 ± 1.6de (22)	1.2 ± 0.8d (40)	35.7 ± 1.7f (9)	4.0 ± 1.0bcdef (9)	647.0 ± 21.2b (362)
56 d at 5°C followed by 25°C	33.104 ± 0.865e (142)	8.8 ± 2.5cd (40)	30.7 ± 1.0efh (22)	5.5 ± 0.9abcd (22)	582.9 ± 11.6b (213)
84 d at 5°C followed by 25°C	29.395 ± 0.639f (362)	19.6 ± 4.2bcd (40)	28.2 ± 0.7h (31)	4.9 ± 0.7bcd (31)	517.9 ± 8.6c (22)
112 d at 5°C followed by 25°C	29.904 ± 0.635ef (481)	20.9 ± 4.5bc (40)	28.3 ± 0.7gh (32)	5.5 ± 0.7abc (32)	527.0 ± 8.6c (481)
140 d at 5°C followed by 25°C	28.312 ± 0.746f (213)	10.6 ± 2.9cd (40)	26.9 ± 0.9h (21)	4.2 ± 0.7bcd (21)	499.0 ± 10.0c (142)
Statistical analyses	F = 1176.83; df = 14, 338.3; P < 0.0001	F = 24.29; df = 14, 559.7; P < 0.0001	F = 852.2; df = 14, 314; P < 0.0001	F = 8.1; df = 14, 314; P < 0.0001	F = 85.36; df = 14, 329.2; P < 0.0001

[1]This is the number of days at 25°C until egg hatch for treatments held at 5 or 10°C for various number of days before incubation.

Means within a column followed by a different letter are significantly different from each other at $P < 0.05$ using Tukey–Kramer post-hoc test. Sample size (*n*) is the number of egg masses for each parameter.

15, and 20°C, respectively. Over this temperature range, there were no significant differences in the duration of hatch within an egg mass. The parameter values for the relationship between developmental rate (1/d) and temperature for eggs held at constant temperatures is given in Table 2 and shown in Fig. 2. The estimated lower threshold for egg development was calculated to be 7.39°C.

Time to first hatch within an egg mass after transfer to 25°C decreased significantly as time at 5°C increased up to 56 d, then stayed constant to 140 d of exposure (Table 1, Fig. 1B). For those eggs held at 10°C, time to first hatch at 25°C decreased significantly as chill length increased to 12 wk, but reached a lower limit of just less than a week to hatch at 25°C after 216 d at 5°C (Table 1, Fig. 1C). Mean time to individual egg hatch decreased significantly as exposure to 5 or 10°C increased from 28 to 84 d. The mean days to individual egg hatch after exposure to 10°C reached a minimum of just under 1 wk when exposure exceeded 200 d, but the minimum for eggs exposed 5°C remained at about 4 wk for eggs held for ≥ 84 d. There were some significant differences in duration of hatch for eggs within a mass but no clear trends.

When modeled, average percentage hatch of the eggs in each mass was predicted to increase from 0 to 60% as time at 10°C increased from 14 to 139 d then decrease again to 0% at 264 d (Tables 1 and 2, Fig. 3). Holding egg masses at 5°C resulted in a similar pattern but the peak percentage hatch of 21% was predicted to occur at 105 d and percentage hatch returned to 0% much earlier at 151 d. Associated with the decrease in average percentage hatch were increasing numbers of individuals that only partially hatched (actual counts of these were not made) or died soon after hatch when egg masses were held for 216 or 250 d at 10°C or 140 d at 5°C. The proportion of egg masses with some hatch was predicted to reach a maximum at about 100 d for eggs held at both 5 and 10°C, but >80% of those held at 10°C had hatch for exposure durations of 56–216 d while only the 56–84 d treatments at 5°C approached 80% (Table 2, Fig. 4).

The average number of cumulative DD required for hatch increased with increasing constant temperature (Table 1, Fig. 5). For eggs held at 10°C, the minimum required DD for hatch was obtained with eggs held at 10°C for 56–84 d. Eggs held at 10°C for less than or more than this length of time required increased HDD accumulation. Eggs exposed to ≥ 84 d at 5°C required less DD for hatch than did eggs held for shorter times at that temperature. These DD differences along with the plateaus seen in the cumulative percentage hatch of eggs exposed to 28 d of chill suggested that DD to hatch for some treatments included the DD to complete both diapause (which was completed in other treatments by longer exposure to temperatures below the development threshold) and post-diapause development at temperatures above the lower threshold. Therefore, the relationships between the proportion of hatch and DD (base 7.39°C)

Table 2. Parameter values for nonlinear models used to describe the relationship between various *Lycorma delicatula* egg hatch characteristics

Relationship modeled (y vs x)	Temperature	Model	a	b	c	d	*n*	R^2_{Adj}
% Eggs Masses Hatching vs. Days at Temperature	5°C	$Y = a + bx + cx^2$	−0.0922 ± 0.102	0.01739 ± 0.00339	−0.00009 ± 0.000022	NA	6	0.8853
	10°C	$Y = a + bx + cx^2$	−0.1423 ± 0.1376	0.0205 ± 0.00342	−0.00008 ± 0.000013	NA	6	0.8723
Average % Hatch vs Days at Temperature	5°C	$Y = a + bx + cx^2$	0.0142 ± 0.0130	−0.00252 ± 0.000856	0.000102 ± 0.000014	−0.000000569 ± 0.000000062	6	0.9890
	10°C	$Y = a + bx + cx^2 + dx^3$	−0.1456 ± 0.0737	0.0111 ± 0.00183	−0.00004 ± 0.0000068	NA	6	0.8765
Egg Developmental Rate vs Temperature	10–20°C	$Y = a + bx + cx^2$	−0.0154 ± 0.000953	0.00238 ± 0.000116	−0.00004 ± 0.00000348	NA	1251	0.8332
Cumulative % Hatch vs DD Base 7.39°C	All temperature regimes	$Y = \exp[- \exp(- bx + a]$.	0.00979 ± 0.000191	5.2885 ± 0.112	NA	NA	4670	0.9838

See Methods for details of the analysis.

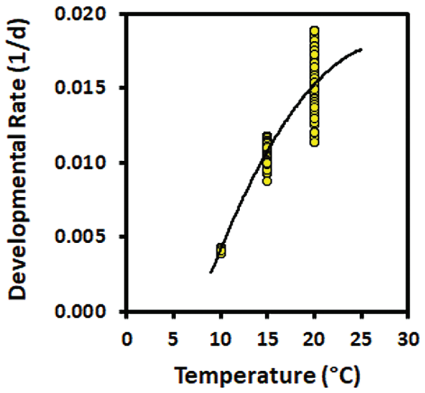

Fig. 2. Relationship between temperature and the developmental rate (1/d) for *Lycorma delicatula* eggs held at three constant temperatures. Dots represent the observed individual values for each egg and the solid line is the polynomial predicted relationships between developmental rate and temperature.

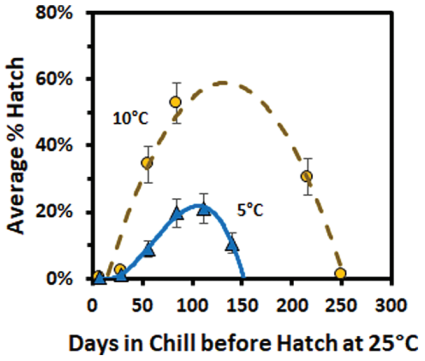

Fig. 3. Comparison of mean percentage hatch of *Lycorma delicatula* eggs exposed to different durations at 5 or 10°C, followed by incubation at 25°C. Bars represent standard errors. Lines represent the fitted expected polynomial curves for 5°C (solid line) and 10°C (dashed line).

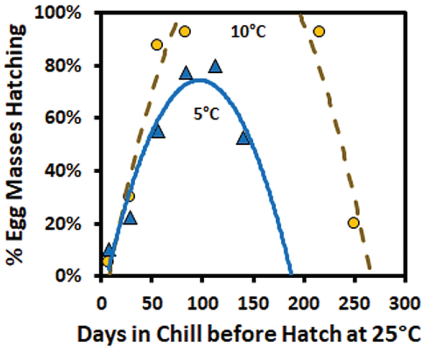

Fig. 4. Comparison of the percentage of *Lycorma delicatula* egg masses that hatched after exposure to different durations at 5 or 10°C, followed by incubation at 25°C. Lines represent the fitted expected polynomial curves for 5°C (solid line) and 10°C (dashed line).

for both diapause and post-diapause development were estimated (Table 2, Fig. 6). The average (95% confidence interval [CI]) number of DD for 10, 50, and 90% of the eggs to complete diapause development was predicted to be 170.4 (170.2–179.6), 181.06 (181.04–181.08), and 197.8 (197.4–198.2), respectively. The average (95% CI) DD for 10, 50, and 90% of the eggs to complete post-diapause

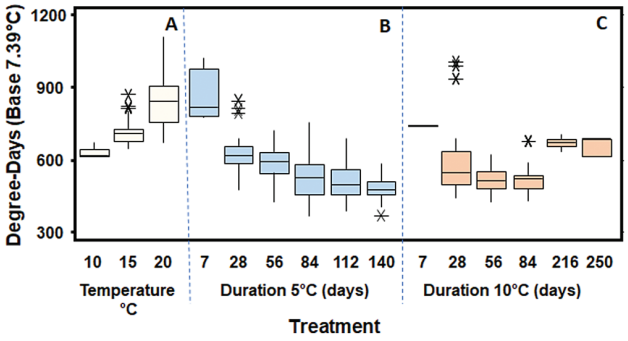

Fig. 5. Box plot comparison of the number of DD required for hatch by individual eggs of *Lycorma delicatula* after exposure to different constant temperatures (A), or durations at 5°C (B) or 10°C (C), followed by incubation at 25°C.

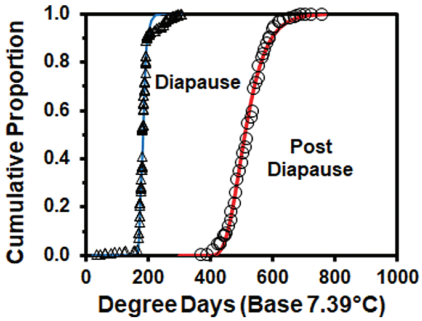

Fig. 6. Cumulative proportion of *Lycorma delicatula* completing diapause (open triangles) or post-diapause (open circles) development over accumulated DD using a base of 7.39°C. Markers are individual eggs and the solid lines are the fitted Gompertz curves, $P = \exp[-\exp(-bDD + a)]$.

development was predicted to be 455.0 (452.4–457.5), 577.6 (577.5–577.8), and 779.1 (766.5–773.7), respectively.

Discussion

Both spotted lanternfly egg masses as a whole as well as individual eggs within an egg mass varied in their requirements to complete diapause and post-diapause development and hatch. Almost all eggs entered diapause and 7 d of chill at either 5 or 10°C was insufficient to complete diapause, since very few eggs with this little exposure to chill were able to complete diapause and post-diapause development after being moved to 25°C. The potential roles of other cues, such as photoperiod during the nymphal stage, that may be required to predispose eggs to this temperature diapause induction cue remain to be identified and described.

As exposure to 5 or 10°C increased up to about 100 d both more eggs and more egg masses had their diapause requirements met (possibly removal of some inhibitor as in gypsy moth (Gray et al. 2001) and were able to complete post-diapause development once moved to 25°C. Eggs within an egg mass hatched more synchronously than did egg masses within a treatment for all treatments except those that involved > 120 d chill at 5 or 10°C. Synchronous hatch of egg masses may buffer against stage-specific predation through predator satiation, while variation in the hatch timing among egg masses provides a way to bet hedge against mortality resulting from stochastic conditions such as rain or adverse temperatures, or phenological asynchrony with hosts. Egg hatch in the field occurs over a 2-mo time period in Pennsylvania (Liu 2019b) which is consistent with the laboratory findings of variation between egg masses in hatch timing, but suggests that the timing of egg laying coupled with microclimate differences with exist between the locations where egg masses are actually laid also may contribute to the variation observed in hatch timing.

Post-diapause development was able to occur at temperatures ≥ 10°C (up to 25°C) as evidenced by decreasing time to first hatch at 10°C. The lower threshold for egg development was estimated to be 7.39°C and the lack of a decrease in the number of days to first hatch with increasing time at 5°C confirmed that no post diapause development occurred 5°C. The viability of eggs (ability to hatch) also varied when either post-diapause development was completed at 10°C (>140 d) or diapause development was complete at 5°C (>110 d) but post-diapause development could not proceed because it was below 7.39°C. One key area of clarification is that we did not conduct tests to determine the respiratory rate (a surrogate for metabolic rate) or hormone levels of the eggs that may indicate true physiological diapause was occurring, rather egg hatch was used as a proxy for diapause termination followed by post-diapause development. We suspect that there is variation in both the duration and depth of chill required for spotted lanternfly egg to complete diapause, which could serve as a bet hedging strategy in climates that are variable and may allow this insect to adapt to novel climates.

The egg stage in *L. delicatula* appears to go through three phases: pre-diapause, diapause and post-diapause. Microscopic observation of *L. delicatula* eggs has shown that they diapause after some development has occurred and they reach the blastokinesis stage (Shim and Lee 2015). The exact temperature requirements are not known, but this apparently occurs rapidly since 1 wk at 20°C before being placed in the treatments was enough to complete this pre-diapause phase. The requirements for diapause completion were shown to be either met by enough days spent at temperatures below (or near) the developmental threshold, or by extra DD spent at temperatures above the developmental threshold. After about 100 d of chill at 5°C (below the lower threshold) then being moved to 25°C or about 200 $DD_{7.39}$ (90% of individuals completed diapause development), the majority of egg masses and the highest percentage of the eggs were able to complete post-diapause development and hatch. This is similar to what has been seen for both spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae), and gypsy moth, *L. dispar*, where post-diapause developmental rates are negatively temperature dependent at early physiological ages and become more positively temperature dependent at later ages (Regniere 1990, Gray et al. 1995). This change in response to temperature and the post diapause development process may be mediated by the heat shock proteins since the heat shock cognate 70 (*hsc*70) gene expression level in *L. delicatula* eggs has been shown to increase with post-diapause development (Shim and Lee 2015). The timing of the increase in the *hsc*70 in field-collected eggs also fits with the about 100-d chill requirement for diapause completion (Shim and Lee 2015).

This is the first study that has followed egg development from oviposition to hatch under controlled temperatures for spotted lanternfly; previous work used egg masses collected in the field after exposure to a month or more of late fall or winter temperatures, which could affect the estimated DD required for hatch and predictions of percentage hatch. For example, one study collected eggs in February and both the percentage hatch and days to hatch were equivalent to our 56 d exposure at 5°C treatment where several of the eggs had already completed diapause

(Choi et al. 2012). Since field temperatures may have exceeded 5°C, some of their field-collected eggs may have begun post-diapause, so the DD estimate of 355 using a lower developmental threshold of 8.14°C may not capture all post-diapause development, making it lower than our estimate. It is also difficult to compare our data—which covered the entire egg period since oviposition in October—with field-collected data on egg hatch when the estimated accumulation of growing DD above 10°C starting in January was used and found that 210 DD_{10} were required for peak hatch in Pennsylvania (Liu 2019b). Without estimating when diapause would actually be complete or taking into account the more complicated effects of temperature on the three phases of egg diapause these DD estimates will not be able to fully predict when egg hatch will occur for all climatic regimes to which the insect may be exposed. Even with the data presented here there are still some important questions that need to be answered: what are the temperature effects on pre-diapause, what are the effects of temperatures below 5°C on diapause, and how do fluctuating temperatures affect the three stages of egg development? There is some indication that under fluctuating temperatures in the field, average percentage hatch has the potential to be higher (88.8% hatch for March collected eggs) than in any of the treatments in this study (Shim and Lee 2015).

Effects of extended chill on the viability of eggs also need to be further evaluated. As time in chill at 5 or 10°C exceeding about 100 d increased the percentage of eggs that were able to hatch decreased. This may be due to eggs running out of energy when post-diapause has been completed at 10°C, or perhaps eggs cannot tolerate extended chill once diapause has been completed at 5°C (temperatures below the developmental threshold). Previously, complete winter mortality of eggs in the field has been correlated with a mean daily temperature from December to February of –3.44°C or a mean daily minimum temperature in January of –12.72°C (Lee et al. 2011). But, since no estimate of the lower lethal temperature for eggs has been made, these data remain unconfirmed. The possibility exists that part of the observed winter mortality may have simply been due to extended exposure to low temperatures as seen at 5°C. Alternatively, the increased egg mortality seen with extended chill at 5 or 10°C may have been due to moisture issues or the substrates the eggs were laid on, as hatch of field-collected egg masses has been shown to vary with the tree they were laid on (Liu 2019b). Additionally, moisture availability has been shown to be critical to post-diapause development in *Apolygus lucorum* Meyer-Dür (Hemiptera: Miridae) (Jin et al. 2016). The humidity in the chambers where the treatments were held was maintained at or above 65% RH, but the egg masses were on dry pieces of bark and not exposed to periods of rain or snow as they would be in the field.

It remains to be determined what effects the temperature responses of eggs may have on the potential range of *L. delicatula* or on its potential to have multiple generations in a year. Modeling using MaxEnt has suggested that *L. delicatula* should be able to establish in most of New England, the Mid Atlantic states, the central United States, and Pacific coastal states (Wakie et al. 2020). However, the ability of *L. delicatula* eggs to complete development at 15 or 20°C without receiving chill at a lower temperature may allow it to hatch where winters are milder. This may also make it possible for this insect to complete more than one generation a year if the rest of its life stages can complete development quickly enough and under the higher temperatures found further south in the United States. These data could be used to develop a hatch phenology model that could predict when hatch will occur based on local temperature data and what will happen in novel habitats across the landscape, which will help determine the potential range.

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