Environmental Entomology, 48(3), 2019, 655–666
doi: 10.1093/ee/nvz028
Advance Access Publication Date: 6 April 2019
Research

Effects of Temperature on First Instar *Lymantria* (Lepidoptera: Erebidae) Survival and Development With and Without Food

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Subject Editor: Rudolf Schilder

Received 22 October 2018; Editorial decision 27 February 2019

Abstract

Lymantria dispar L. and *Lymantria monacha* (L.) are Eurasian pests that have the potential for accidental introduction via trade into other world areas. Establishment of first instars of *Lymantria* depends on larvae surviving long enough to disperse and finding suitable hosts. The survival and development of newly hatched *Lymantria* larvae from nine geographic populations at seven temperatures (1–30°C) held without food, with summer foliage of a preferred or conifer host was determined. There was considerable variation both within and among the *Lymantria* populations in the survival of larvae at different temperatures when held with and without food. Without food survival declined from about a month at 5°C to a few days at 30°C, following a typical enzymatic kinetic rate function. At 1°C larval survival was less than at 5°C likely because the larvae were susceptible to freezing. Larvae from the one *L. monacha* population fed and gained weight on the summer foliage, particularly on the conifer, at 10–15°C but < 20% survived for 14 d at 20–30°C. The newly hatched *L. dispar* larvae from all eight populations fed (at 10–30°C) and developed (at 15–30°C) on the summer foliage of one or both of the hosts. This suggests that they may be able to find adequate food for establishment even if hatch is not synchronous with bud break in the invaded habitat. Survival on the conifer was highest for one Chinese and two European populations of *L. dispar*, suggesting the ability to utilize conifers is population and not subspecies specific.

Key words: establishment, *Lymantria dispar*, gypsy moth, *Lymantria monacha*, nun moth

Since the early 1990s, multiple incursions of Asian gypsy moth, *Lymantria dispar japonica* Motschulsky and *L. dispar asiatica* Vnukovskij (Lepidoptera: Erebidae), that have females capable of strong directed flight have occurred in North America (USDA-APHIS_PPQ 2014). *Lymantria dispar japonica* occurs in patches on all islands in Japan and *L. dispar asiatica* is found east of the Ural Mountains in Russia, and throughout China and Korea (Pogue and Schaefer 2007). The nun moth, *Lymantria monacha* (L.) (Lepidoptera: Erebidae), is a Eurasian pest that also has a high potential of being transported along trade routes and intercepted but no established populations outside its native range have been found to date. The most often intercepted stage of both Asian gypsy moth and nun moth is the egg mass. Females of both species are attracted to lights at night and will lay their egg masses near the lights on cargo and the superstructure of ships in Asian ports (Munson et al. 1995, Wallner et al. 1995). Although there is a ship inspection program in place at most Asian ports, egg masses are still found on vessels entering North American ports (USDA-APHIS_PPQ 2014). If the egg mass has been exposed to the right conditions for embryonation, has received enough chill to break diapause, and has completed post-diapause development, the larvae can hatch while the ship is in port in another country. Many countries that do not have nun moth or the gypsy moth females that are capable flight have both programs to prevent introductions and protocols for eradicating any established populations. These moths are of particular concern since they are capable of spreading quickly and are highly polyphagous (Sliwa 1987, Baranchikov 1988, Wallner et al. 1995, Shi et al. 2003, Keena et al. 2008, Wei et al. 2012). Females of the *Lymantria dispar dispar* L. that is already established in North America and native to Western Europe are incapable of flight (Keena et al. 2008) and the population only spreads by ballooning as first instars or crawling as larger larvae (McManus 1973).

Many spring folivorous Lepidoptera (e.g., *Lymantria dispar* L., *Choristoneura fumiferana* Clemens, *Malacosoma disstria* Hübner, *Lambdina fiscellaria* (Guenée), *Operophtera brumata* L.) experience reduced survival and establishment when their eggs hatch asynchronously with host bud break (Feeny 1970, Hunter 1992, Parry et al. 1998, Carroll 1999, Tikkanen et al. 2003, Uelmen et al. 2016, Fuentealba et al. 2017). If the larvae hatch too early they risk starvation before they can locate suitable foliage and if they hatch too late the foliage they find to feed on will be of declining nutritional quality. Since climate affects the phenology of both plants and insects, it can also alter the relationship between the herbivore and its host. To improve our ability to predict the survival and establishment of *Lymantria* in new habitats or under climate change scenarios the role that temperature plays in larval survival after hatch must be better understood.

When the *Lymantria* larvae hatch they have a limited amount of time to passively balloon on the wind to find a suitable host. The larvae do not climb trees when the weather is cool (<10°C) or rainy so host-seeking may be further limited after hatch (Leonard 1971, McManus 1973). Capinera and Barbosa (1976) found that if *L. dispar* larvae hatched before host bud break they could starve to death in as little as 5 d without food and Hunter (1993) found that at temperatures above 10°C the larval starvation rate for a Canadian population increased with temperature, while at temperatures below 10°C the starvation rate was constant. Maksimović (1963) found that *L. dispar* larvae starved to death without food in an average of 4 and 3 d at 20 and 30°C, respectively, with 100% RH and that at cooler temperatures the %RH had more impact on survival than at higher temperatures. There is no published information on *L. monacha* starvation rates in the literature. Also, although both moths are generalists and can survive on hundreds of hosts, larvae that hatch out of synchrony with suitable hosts may have trouble using the available leaves because they are tougher or contain more defensive compounds (Bejer 1988, Liebhold et al. 1995, Erelli and Elkinton 2000). Therefore the ability of *Lymantria* first instars to establish will depend on both surviving long enough under the weather conditions after hatch to disperse and finding suitable hosts in the right phenological state.

In this paper, we evaluate the effects of temperature on the survival of newly hatched *Lymantria* larvae without food. We also document the survival and development of newly hatched *Lymantria* larvae at different temperatures when offered a preferred host in the wrong phenological state (summer foliage) and an evergreen host (less preferred by some populations of *L. dispar*) for food. The significance of the results of these studies for the *Lymantria* exclusion and eradication programs is discussed.

Materials and Methods

Gypsy Moth Populations

Information on the source location and collection of the nine *Lymantria* geographical populations used in these studies are given in Table 1. Based on the Pogue and Schaefer (2007) review of *Lymantria*, the Japanese population (JN) is the *L. dispar japonica* subspecies, the Russian (RM and RS) and Chinese (CJ and CR) populations are the *L. dispar asiatica* subspecies, the remaining *L. dispar* populations (KG, LJ, and UC) are the *dispar* subspecies, and the single *L. monacha* population was from the Czech Republic (CZ). All populations were transported as eggs under permit to the USDA Forest Service quarantine facility in Ansonia, CT. Voucher specimens for each population were deposited at the Entomology Division, Yale Peabody Museum of Natural History, New Haven, CT.

The *L. dispar* that produced the egg masses used in the studies were reared in walk-in environmental chambers maintained at 25°C, 60% RH, a photoperiod of 16:8 (L:D) h. Larvae were reared, in cohorts of 8–10, as described in Keena (1996). The high wheat germ artificial diet (Bell et al., 1981) was optimized for the individual populations using Wesson salt mix without iron and by adding 0.27 g (Asian populations) or 0.10 g (European and North American populations) amorphous $FePO_4$ per liter of diet. Pupae were harvested, sexed, and stored by sex, and population. Adults were randomly mated in groups of 25 within each population. The females were allowed to oviposit on paper sheets; eggs were harvested 40 d after mating. Before the eggs were incubated for hatch, egg masses from 100 females from each population were scraped from the sheets and scrambled together to make packets of mixed eggs. The eggs of all the populations were chilled 116 d at 5°C except the CJ (179 d) before they were moved to 25°C for hatch.

The *L. monacha* that produced the egg masses used in the studies were reared in walk-in environmental chambers maintained at 25°C, 60% RH, a photoperiod of 16:8 (L:D) h. Larvae were reared using the methods and artificial diet described in Keena et al. (2010). Pupae were harvested, sexed, and stored by sex. Adults were removed daily and randomly mated as single pairs. Egg clumps laid in the single-sided corrugated cardboard were harvested 20 d after

Table 1. Approximate location (latitude and longitude) of source populations, species, and designations for populations evaluated in this study, arranged by population designation

Species	Population	Country	Closest city, region	Collection date[a]	Egg masses received	Latitude	Longitude
Lymantria dispar asiatica	CJ	China	Yanzikou, Beijng	Aug. 2011 (Oct. 2012)	4 individual	40.32°N	116.15°E
Lymantria dispar asiatica	CR	China	Harbin, Heilongjiang	Aug. 2012 (Oct. 2012)	6 individual	45.78°N	126.61°E
Lymantria monacha	CZ	Czech Republic	Předín, Třebíč District	Aug. 1996	30 egg clumps	49.20°N	15.66°E
Lymantria dispar japonica	JN	Japan	Nagoya, Honshu	Mar. 1996	4 individual	35.15°N	137.08°E
Lymantria dispar dispar	KG	Greece	Kavála, Macedonia	Feb. 1997	58 individual	41.00°N	24.25°E
Lymantria dispar dispar	LJ	Lithuania	Juodkrante, Kuzsin Nezijos	Aug. 1994	47 individual	55.31°N	21.06°E
Lymantria dispar asiatica	RM	Russia	Mineralni, Primorski	Aug. 1992	20 individual	44.10°N	133.15°E
Lymantria dispar asiatica	RS	Russia	Shira, Khakassia	Aug. 1994	6 individual	54.41°N	90.00°E
Lymantria dispar dispar	UC	United States	Bethany, New Haven County, CT	Mar. 1994	12 individual	41.25°N	73.00°W

[a]Date in parentheses is when the population was received at the Forest Service Quarantine Laboratory in Ansonia, CT.

mating and held together in a 473.2-ml paper cup at 5°C for 158 d. Egg packets were made from the mixed eggs and incubated at 25°C for hatch.

Temperature Treatments and Experimental Setup

Larval survival and development were assessed at seven constant temperatures: 1, 5, 10, 15, 20, 25, and 30°C and a 16:8 (L:D) h photoperiod. The temperature fluctuations within the environmental chambers typically remained within 1°C of the set point. Humidity was passively maintained by placing open buckets (with 896 cm^2 of surface area) of water in the bottom of each chamber. Two chambers (15 and 25°C), which had full humidity controls, did not require the open humidity source. The humidity in the chambers averaged 55 ± 5 at 1°C, 80 ± 10 at 5°C, 85 ± 15 at 10°C, 65 ± 2 at 15°C, 80 ± 5 at 20°C, 60 ± 5 at 25°C, and 45 ± 5% RH at 30°C. Humidity and temperature were monitored using the chart recorders that were part of the chambers and calibrated regularly using a Traceable 6400 humidity/temperature/dew-point meter (Calibration Control Company, Webster, TX).

At each temperature, 50 newly hatched larvae (0 d old) from each of the nine populations (Table 1) were reared using each of the following food treatments: no food, coastal Douglas fir foliage (*Pseudotsuga menziesii* var. *menziesii* (Mirb.) Franco, Pinaceae), and summer black oak foliage (*Quercus velutina* Lam., Fagaceae). Coastal Douglas fir was chosen to represent conifer foliage that larvae might encounter on the west coast when ballooning from vessels or cargo in port areas where most of the Asian gypsy moth interceptions have occurred. The black oak was chosen to represent a preferred host (both insects) in a less desirable phenological state (summer) and also it had a similar leaf texture to that of the evergreen oaks found on the west coast. The *L. monacha* larvae should find both hosts acceptable since they utilize conifers like *Larrix gmelinii* (Rupr.) Rupr. (Pinaceae) and *Pinus* sps L. in their native habitat (J. S. unpublished data, Bejer 1988, Keena 2003). Some of the *L. dispar* populations should find the Douglas fir foliage acceptable since Asian populations use non-deciduous conifers and even European gypsy moth in Spain defoliate exotic *Pinus radiata* D. Don (Castedo-Dorado et al. 2016). The study was intentionally started 5 August 2013 so only summer foliage, with no new growth, was available on either host. This mimicked a worst case scenario for larvae that hatch from egg masses out of synchrony with leaf bud break in port areas after arriving on vessels or cargo.

Larvae with no food were held individually in 1.5 ml clear microcentrifuge tubes (Daigger EF4268D, Vernon Hills, IL) with a single pinhole in the lid. All the tubes for each population and temperature combination were kept together in a 355 ml clear plastic cup (Dart Solo TP12-0090 and 662TS lid, Lake Forest, IL), and the cups were kept in large clear plastic boxes on a screen over water. Holding the larvae without food in these boxes maintained the humidity at 100% RH regardless of the humidity in the chamber. The actual humidity inside the boxes was measured using a Traceable 6400 humidity/temperature/dew-point meter (Calibration Control Company, Webster, TX).

The containers used for the foliage rearing for 1–15°C were tight fitting Petri dishes (Falcon 50 mm diameter × 9 mm deep, Fig. 1A and B) and for 20–30°C were 473 ml double-poly-coated squat paper cups (Chinet 71840, Huhtamaki, Espoo, Finland) with translucent vented plastic lids (Chinet 89107, Huhtamaki, Espoo, Finland) (Fig. 1C and D). Each Petri dish had either two 2-cm-diameter discs of black oak foliage cut from large leaves using a cork-hole borer or a 1 cm long twig section of Douglas fir. The paper cups had either one large leaf of black oak or two 9-cm-long sections of Douglas fir twig. We obtained the foliage from full-grown trees planted on the Forest Service property (3–10 per host), rotating between the trees but always using the same tree for all foliage changes done on the same day. The foliage was inspected and gently washed with water and dried to remove any debris or other insects, if needed, before it was used. The Douglas fir foliage was changed at least every 3–4 d at 20–30°C and at least weekly at 1–15°C. The oak foliage was changed at least every 2–3 d at 20–30°C and at least weekly at 1–15°C. If at any time the foliage in a container looked dry or the larvae had eaten most of it, it was changed. All of the containers that had foliage in them were placed in the chambers and experience the chamber humidity levels.

At 14 d the larval feeding in each container was assessed and assigned one of the following feeding codes: 0 = no feeding and no green frass, 1 = some green frass but no obvious feeding, 2 = obvious feeding but less than half of the larvae feeding, 3 = patches of foliage eaten and more than half of the larvae feeding, and 4 = large patches of foliage eaten and most or all of the larvae feeding. Larvae will pass an almost black frass pellet after hatch that is not the result of feeding, so this type of frass was not considered evidence of feeding. An average (±SE) feeding code was calculated for each population and foliage type combination.

Each larva was checked daily to determine whether it was still alive and whether or not it had molted to the next instar. The containers with larvae were only out of their respective temperatures long enough to make these checks. A dissecting microscope was used to check the larvae reared in microcentrifuge tubes and a fine tipped paint brush was used to gently probe larvae reared with foliage. If a larva was able to move any part of its body it was considered to be alive. If a larva held at 1–10°C was presumed dead it was held for 10 min at room temperature and then rechecked. The larvae held without food were checked until death and those held with foliage were checked for 14 d and then any live larvae were individually weighed and frozen. The instars of the larvae on foliage at 14 d were also recorded. If a larva was close to a molt, as evidenced by the current head capsule being extended and the new larger head capsule could be seen to be forming behind it, then 0.75 was added to the instar number when it was recorded. An overall percentage survival was determined for each population and foliage type combination.

Statistical Analyses

PROC UNIVARIATE (SAS Institute 2015) was used to assess the fit of the data for each parameter that was measured to various distributions and the Shapiro–Wilk and the Anderson–Darling tests were used to assess normality. The following dependent continuous variables were analyzed in PROC GLIMMIX (SAS Institute 2015): number of days larvae survived without food, weight of larvae after 14 d on the foliage, and instar of the larvae after 14 d on the foliage. For the larvae without food a completely randomized design was used to evaluate the effects of population, temperature and the interaction between the two on the listed variables. The number of days larvae survived without food was also analyzed by grouping populations to assess the effects of temperature, subspecies/species, and the interaction between the two on survival. For the foliage reared larvae a completely randomized design was used to assess the effects of foliage type, temperature, population and the interaction between the three on the same three variables listed above and setup date and container were included as random effects. The gamma distribution with a log link was used for all response variables that were evaluated. For each model, residuals were evaluated for normality and the homogeneity of variance was assessed using Levene's test. Differences among means were determined by the least squares

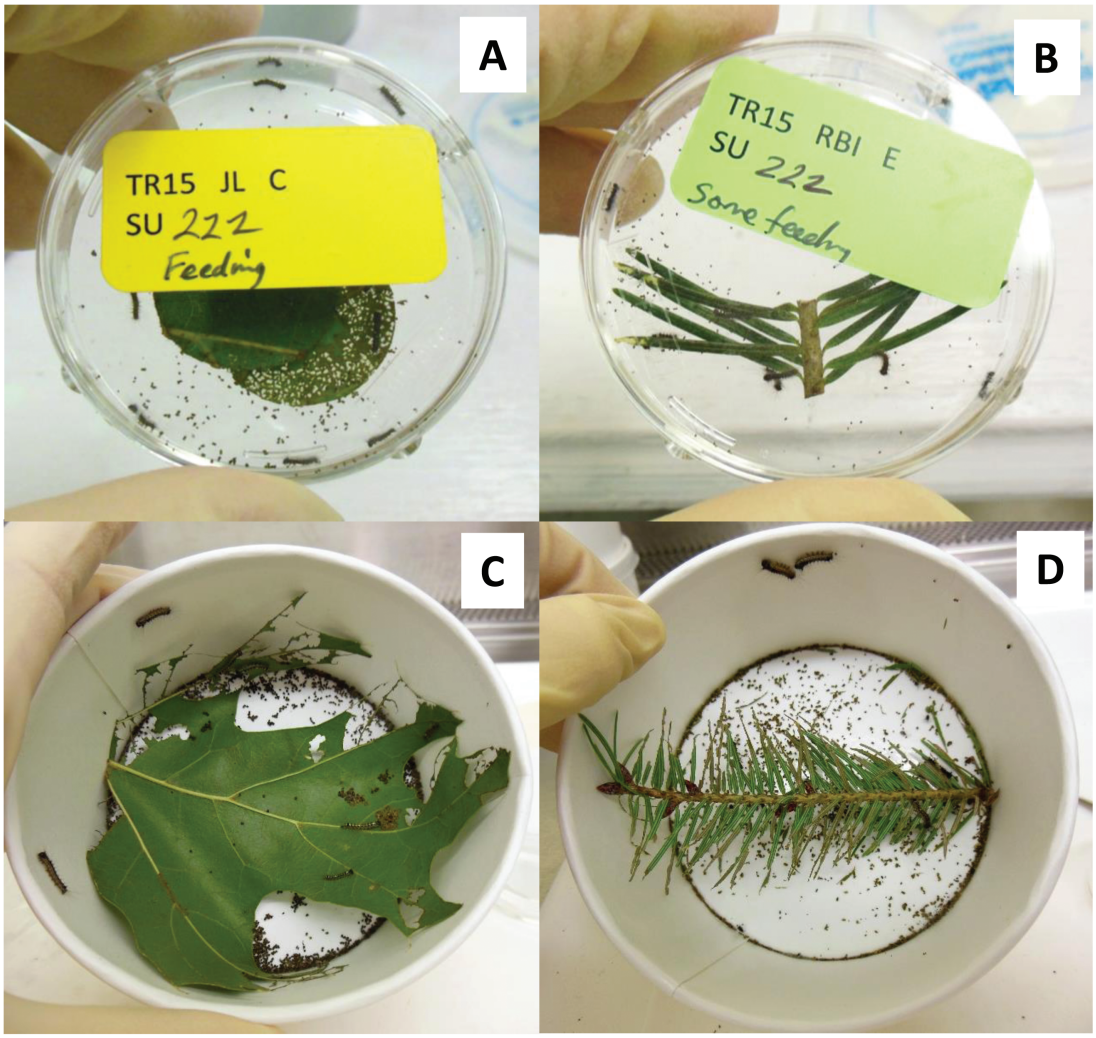

Fig. 1. Methods used for rearing at the different temperatures and examples of the type of feeding observed. A: first instar LJ larvae in a Petri dish with black oak leaf discs at 15°C, B: first instar RS larvae in a Petri dish with Douglas fir twig section at 15°C, C: third instar CR larvae in a paper cup with a large black oak leaf at 25°C, and D: third instar KG larvae in a paper cup on a Douglas fir twig section at 25°C.

means test with $\alpha = 0.05$ and a conservative Tukey–Kramer grouping (SAS Institute 2015).

For each population and temperature combination, the cumulative percentage mortality distributions over days since hatch for the larvae held without food was described using a Gompertz function,

$$P = \exp[-\exp(bD + a)]$$

in which D, a and b are the number of days, lag and the rate of increase, respectively (Brown and Mayer 1988), PROC NLIN and Marquardt convergence method, SAS Institute 2015). The one exception was the KG population at 30°C where a plot of the data indicated that it would fit a straight line better than the Gompertz function. Predicted days for 10, 50, and 90% mortality for each population were calculated. For each *L. dispar* subspecies (appropriate populations combined) and the *L. monacha* population the average days the larvae survived over the temperatures evaluated (5–30°C) was described by a separate Morgan-Mercer-Flodin function,

$$D = \frac{ab + cT^d}{b + T^d}$$

in which D is days, T is temperature (°C), a is the estimated number of days an insect would survive when the temperature = 0, b is a parameter that controls the inflection point of the curve, c rate at which survival approaches zero days, and d is the survival rate as T approaches zero (Morgan et al. 1975), PROC NLIN and Marquardt convergence method, SAS Institute 2015). This function is normally used to model growth rate in response to nutrient intake but here we use it to model survival in response to nutrient depletion. When the larvae hatch, they have limited nutrient resources that are depleted faster as temperature increases thus resulting in larvae surviving a shorter number of days. In this case, the larvae only had the nutrient resources they hatched with and so at higher temperatures those were used up faster than at lower temperatures. The response at 1°C (and 5°C for JN) was not included when the function was fit since a different process is likely involved in the mortality of the larvae at temperatures near 0°C.

Results

Survival Without Food

The population ($F = 57.8$; df = 6, 3087; $P < 0.0001$), temperature ($F = 3367.4$; df = 8, 3087; $P < 0.0001$) and the interaction between population source and temperature ($F = 23.91$; df = 48, 3087; $P < 0.0001$) all had significant effects on larval survival when held without food. The number of days a newly hatched larva could survive without food decreased with increasing temperature between 5

and 30°C (Tables 2 and 3; Fig. 2). The one exception was that larvae from one Japanese population (JN) had significantly lower survival at 5°C than did the other populations or the larvae from the same population held at 10°C. At 5°C >50% of the larvae survived more than a month, while at 30°C most were dead within the first week. Larvae from all the populations had significantly lower survival at 1°C than at 5°C. The longest any single newly hatched larva survived without food was 47 d at 5°C (a larva from the Siberian Russia population [RS]). When survival time was assessed by species/subspecies group, the temperature by species/subspecies interaction was significant ($F = 42.66$; df = 18, 3122; $P < 0.0001$). The *L. dispar asiatica* larvae survived significantly longer than *L. dispar dispar* larvae at 5 and 20°C (Fig. 2). The *L. monacha* larvae from the one population evaluated survived a longer time than all the subspecies of *L. dispar* at 1°C, but survived a shorter time at 10 and 15°C (Fig. 2).

Survival and Development With Food

When held with food >70% of the larvae from most populations survived the 2-wk period at 1°C (Fig. 3, the exceptions were one Japanese population (JN) both hosts ~20% survival and one Chinese population (CJ) on Douglas fir 56% survival). At 1°C there were no signs of feeding or green frass (feeding code 0, Table 4). At 5°C ≥82% of the larvae survived 14 d and some green frass was present (feeding code 1). There was some obvious larval feeding on both foliage types at 10°C by all populations except one Russian population (RM) on both foliage and one Chinese population (CJ) on Douglas fir. The *L. monacha* larvae (CZ) fed the most at 10°C, especially on the Douglas fir. Most of the *L. monacha* larvae died when held with foliage in the 473 ml paper cups at temperature ≥20°C.

The population ($F = 113.9$; df = 8, 3929; $P < 0.0001$), temperature ($F = 1351.1$; df = 6, 3929; $P < 0.0001$), foliage type ($F = 16.41$; df = 1, 3929; $P < 0.0001$) and the interaction between the three ($F = 24.84$; df = 103, 3929; $P < 0.0001$) all had significant effects on larval weight at 14 d (Table 4). For most populations larvae were significantly heavier at temperatures >20°C than those reared at temperatures <20°C on both foliage types. The population ($F = 101.1$; df = 8, 3927; $P < 0.0001$), temperature ($F = 1571.9$; df = 6, 3927; $P < 0.0001$), foliage type ($F = 51.18$; df = 1, 3926; $P < 0.0001$) and the interaction between the three ($F = 21.34$; df = 103, 3926; $P < 0.0001$) all also had significant effects on mean larval instar at 14 d (Table 4). At the end of the 14 d at 15°C, seven of the *L. monacha* larvae on Douglas fir reached the pre-molt to the second instar and several larvae from all but the *L. monacha* (CZ), one Japanese (JN) and the Connecticut populations had reached the pre-molt to the second instar on the oak. Two Lithuanian (LJ) and one Chinese (CJ) larvae molted to the second at 15°C on the oak foliage and feeding was evident for all populations on both hosts (Fig. 3). At the higher temperatures larvae that fed generally grew and molted successfully on both foliage types, many reaching the fourth instar at 30°C. The larvae that grew the best on the Douglas fir in the 473 ml paper cups (20–30°C) were one Chinese strain (CR) and two European strains (LJ and KG). However, 14 d larval survival was generally lower and the larvae developed slower on the summer foliage at temperatures ≥15°C in this study compared previous studies where larvae were reared on artificial diet (Fig. 3, Limbu et al. 2017, Keena et al. 2010).

Discussion

There was considerable variation both within and among the *Lymantria* populations from different world areas in the survival of newly hatched larvae at different temperatures when held without food. The ability of newly hatched larvae to feed and develop on less than ideal host foliage at different temperatures also varied among *Lymantria* populations. Even with this variation, these results suggest that egg masses that are moved through the transportation system and hatch when the temperatures are between 10 and 20°C will have a week or more to find food and could establish if the foliage they find is adequate. Below 10°C larvae will also survive a week or more but may be unable to locate a host in time since they don't disperse at those temperatures. At temperatures above 20°C larvae will have a shorter period of time to find adequate food but there may be a greater variety of foliage types available during the time of the year when those temperatures occur. Survival without food may not be as long as predicted here since 100% RH rarely occurs in nature, especially at the higher temperatures evaluated.

Newly hatched *Lymantria* larvae survived longer without food in this study than has been previously reported for *L. dispar dispar* larvae at the same temperatures and %RH. At 20°C and near 100% RH larvae from a Connecticut population survived an average of 6 d and larvae from a Yugoslavian population survived an average of 4 d (maximum of 7 d) (Maksimovic 1963, Diss et al. 1996). The *Lymantria* populations we evaluated had average survival times of 7–10 d and maximum survival of 13 d at 20°C. Similarly, at 30°C larvae from the Yugoslavian population (Ada Huja near Belgrade in modern-day Serbia) survived an average of 2.7 d (Maksimovic 1963) while the populations we evaluated averaged 3.8–5.0 d. It is possible that the differences may in part be due to the conditions the eggs were exposed to before hatch, since the only nutrition available to the newly hatched larvae is their reserves from the egg and prolonged time in post-diapause development can burn up more reserves. Lower relative humidity has also been shown to decrease survival time (about 50% reduction going from 100 to 20% RH at the same temperature, Maksimovic 1963) but should not be responsible for these differences since all three studies held the larvae at near 100% RH. What is not known is if the prolonged starvation might have irreversible adverse effects on the larvae that compromise their development and result in less fit individuals.

The reduced survival of the newly hatched larvae of all populations held without food at 1°C and the JN larvae held at 5°C was unexpected. At the higher temperatures the response of the larvae fits well a starvation curve that is mediated by the rate at which the nutritional reserves are consumed and fits a typical enzymatic kinetic rate function. At these lower temperatures a different mechanism may be at work that drives the mortality. At 1°C, the minor fluctuations in the chamber's temperature may have exposed the larvae to near 0°C causing the formation of ice crystals in their bodies. Although *Lymantria dispar* eggs can supercool to temperatures of −20°C without freezing (Madrid and Stewart 1981, Waggoner 1985), the larvae are susceptible to freezing during hard frosts in the spring just after hatching Lyamtsev et al. 2000. Newly hatched larvae in nature can avoid freezing by moving to sunny spots where their black color allows them to attain temperatures 6–10°C above ambient (Mason and McManus 1981) but the larvae in the tubes would not have had this advantage. In addition, there seems to be some variation between populations in sensitivity to the cold as seen by the reduced survival of larvae from the JN population at even 5°C. The cold sensitivity of JN larvae seems to extend to larvae held with foliage, but larvae from all populations held with foliage at 1°C survived longer than those without food even though feeding was not observed. In addition to the presence of foliage, larvae reared in groups instead of singly and held in larger heavier plastic containers than used for the no food treatments, all of which could have helped

Table 2. Average (±SE) and range of days 50 newly hatched larvae from different source populations survived without food at various temperatures

Pop.	Temp. °C	Average ± SE survival (d)	Survival range (d)	Curve predicted days to % mortality			Curve fit adjusted R^2	Curve parameter values	
				10	50	90		a ± SE	b ± SE
JN	1	4.80 ± 0.17zABC	2–7	2.06	4.14	7.42	0.948	0.58 ± 0.09	2.02 ± 0.37
CJ	1	6.30 ± 0.22vwx	1–9	3.66	5.61	8.66	0.953	0.62 ± 0.13	3.09 ± 0.79
CR	1	8.36 ± 0.29rst	2–14	4.24	7.41	12.38	0.979	0.38 ± 0.03	2.44 ± 0.26
RS	1	11.24 ± 0.39lmno	7–14	6.70	10.65	16.86	0.779	0.30 ± 0.08	2.87 ± 0.81
RM	1	11.50 ± 0.40lmno	5–15	7.57	11.03	16.47	0.926	0.35 ± 0.05	3.46 ± 0.57
UC	1	6.72 ± 0.23uvw	4–10	4.21	5.64	7.88	0.972	0.84 ± 0.13	4.37 ± 0.81
LJ	1	10.58 ± 0.37nop	7–15	6.47	9.73	14.84	0.945	0.37 ± 0.04	3.22 ± 0.44
KG	1	11.12 ± 0.39lmno	3–15	8.24	10.75	14.68	0.940	0.48 ± 0.08	4.78 ± 0.85
CZ	1	13.36 ± 0.46jkl	1–18	8.00	12.93	20.67	0.870	0.24 ± 0.05	2.78 ± 0.57
JN	5	17.72 ± 0.62ghi	2–34	8.08	17.00	31.00	0.970	0.13 ± 0.01	1.92 ± 0.15
CJ	5	30.58 ± 1.06ab	12–42	19.58	30.28	47.06	0.936	0.11 ± 0.01	3.03 ± 0.29
CR	5	32.12 ± 1.12ab	7–45	24.88	32.14	43.54	0.965	0.17 ± 0.01	4.95 ± 0.41
RS	5	34.58 ± 1.20a	16–47	27.45	34.22	44.84	0.967	0.18 ± 0.01	5.70 ± 0.47
RM	5	34.74 ± 1.21a	9–46	28.94	34.78	43.94	0.971	0.21 ± 0.02	6.79 ± 0.61
UC	5	26.68 ± 0.93bcd	7–40	19.01	26.22	37.55	0.933	0.17 ± 0.02	4.00 ± 0.53
LJ	5	30.46 ± 1.06ab	4–44	25.50	30.53	38.43	0.976	0.24 ± 0.02	6.91 ± 0.53
KG	5	28.10 ± 0.98bc	3–40	25.44	29.74	36.48	0.931	0.28 ± 0.05	7.95 ± 1.55
CZ	5	27.88 ± 0.97bc	4–43	18.33	28.22	43.74	0.927	0.12 ± 0.01	3.06 ± 0.40
JN	10	21.70 ± 0.75ef	12–29	18.98	21.23	24.76	0.993	0.53 ± 0.03	10.97 ± 0.71
CJ	10	19.12 ± 0.66fg	12–27	15.72	18.42	22.66	0.991	0.44 ± 0.03	7.82 ± 0.50
CR	10	24.60 ± 0.85cde	3–31	20.90	24.21	29.39	0.985	0.36 ± 0.03	8.43 ± 0.64
RS	10	21.82 ± 0.76def	16–28	18.19	21.04	25.49	0.990	0.42 ± 0.03	8.52 ± 0.55
RM	10	22.16 ± 0.77def	11–34	17.59	21.41	27.40	0.979	0.31 ± 0.03	6.37 ± 0.54
UC	10	17.66 ± 0.61ghi	4–26	13.22	17.08	23.13	0.973	0.31 ± 0.03	4.95 ± 0.50
LJ	10	18.60 ± 0.65fgh	8–26	15.34	18.07	22.36	0.977	0.44 ± 0.04	7.57 ± 0.79
KG	10	20.60 ± 0.72efg	8–28	16.74	20.01	25.15	0.989	0.37 ± 0.02	6.97 ± 0.45
CZ	10	15.26 ± 0.53hij	9–22	10.57	14.45	20.54	0.983	0.31 ± 0.02	4.11 ± 0.29
JN	15	14.58 ± 0.51ijk	4–19	11.82	14.25	18.05	0.980	0.50 ± 0.05	6.69 ± 0.71
CJ	15	11.58 ± 0.40lmno	4–18	8.85	10.87	14.05	0.990	0.59 ± 0.05	6.09 ± 0.49
CR	15	14.84 ± 0.52ij	4–24	10.52	14.36	20.38	0.979	0.31 ± 0.03	4.13 ± 0.40
RS	15	13.20 ± 0.46jklm	6–17	10.96	12.60	15.17	0.995	0.73 ± 0.05	8.85 ± 0.59
RM	15	15.56 ± 0.54hij	11–20	12.54	14.90	18.60	0.985	0.51 ± 0.04	7.22 ± 0.61
UC	15	10.88 ± 0.38mno	3–14	8.08	10.35	13.92	0.973	0.53 ± 0.06	5.11 ± 0.66
LJ	15	12.10 ± 0.42klmn	7–17	9.30	11.42	14.73	0.993	0.57 ± 0.04	6.12 ± 0.40
KG	15	13.56 ± 0.47jkl	8–20	10.54	12.80	16.35	0.995	0.53 ± 0.03	6.43 ± 0.35
CZ	15	9.62 ± 0.33opqrs	5–14	6.24	8.75	12.68	0.987	0.48 ± 0.04	3.82 ± 0.34
JN	20	10.32 ± 0.36nopq	8–13	8.20	9.69	12.01	0.985	0.81 ± 0.08	7.48 ± 0.78
CJ	20	8.04 ± 0.28stu	6–10	6.20	7.44	9.38	0.979	0.97 ± 0.13	6.86 ± 0.98
CR	20	10.16 ± 0.35nopqr	1–12	8.14	9.72	12.20	0.994	0.76 ± 0.06	7.01 ± 0.53
RS	20	8.66 ± 0.30pqrst	7–11	7.23	8.06	9.36	0.998	1.44 ± 0.09	11.25 ± 0.69
RM	20	10.18 ± 0.35nopqr	7–13	8.33	9.59	11.57	0.984	0.95 ± 0.12	8.76 ± 1.13
UC	20	7.48 ± 0.26tuv	5–10	5.47	6.86	9.04	0.984	0.87 ± 0.10	5.58 ± 0.67
LJ	20	8.10 ± 0.28stu	6–11	6.23	7.37	9.17	0.996	1.05 ± 0.07	7.35 ± 0.50
KG	20	8.54 ± 0.30qrst	6–11	6.78	7.90	9.66	0.995	1.07 ± 0.08	8.09 ± 0.64
CZ	20	7.68 ± 0.27tuv	2–12	4.99	7.02	10.21	0.986	0.59 ± 0.05	3.78 ± 0.36
JN	25	6.44 ± 0.22vwx	4–9	4.80	5.85	7.49	0.993	1.15 ± 0.10	6.35 ± 0.60
CJ	25	5.06 ± 0.18zyAB	4–7	3.76	4.44	5.50	0.998	1.77 ± 0.08	7.49 ± 0.32
CR	25	5.96 ± 0.21wxyz	5–10	4.59	5.29	6.38	0.998	1.73 ± 0.07	8.78 ± 0.36
RS	25	5.32 ± 0.18xyzAB	4–7	4.11	4.76	5.78	0.991	1.85 ± 0.24	8.42 ± 1.15
RM	25	5.92 ± 0.21wxy	4–8	4.51	5.35	6.66	0.993	1.44 ± 0.16	7.32 ± 0.83
UC	25	4.56 ± 0.16ABCDE	1–6	2.95	4.02	5.70	0.980	1.12 ± 0.18	4.14 ± 0.70
LJ	25	4.90 ± 0.17yzABC	2–7	3.79	4.40	5.38	0.992	1.94 ± 0.25	8.18 ± 1.08
KG	25	5.44 ± 0.19xyzA	3–8	4.16	4.87	5.99	0.997	1.69 ± 0.13	7.86 ± 0.66
CZ	25	5.80 ± 0.20wxyz	2–9	4.17	5.18	6.77	0.997	1.19 ± 0.08	5.78 ± 0.41
JN	30	4.70 ± 0.16ABCD	3–6	3.25	4.07	5.37	0.990	1.46 ± 0.2	5.56 ± 0.83
CJ	30	4.08 ± 0.14CDE	2–5	3.00	3.51	4.31	0.998	2.37 ± 0.14	7.94 ± 0.49
CR	30	4.58 ± 0.16ABCDE	1–7	3.65	4.10	4.81	0.995	2.65 ± 0.54	10.53 ± 2.18
RS	30	4.62 ± 0.16ABCDE	4–6	3.79	4.15	4.71	1.000	3.33 ± 0.04	13.46 ± 0.15
RM	30	4.72 ± 0.16ABCD	4–6	3.79	4.15	4.71	1.000	3.33 ± 0.04	13.46 ± 0.15
UC	30	3.86 ± 0.13DE	1–6	2.72	3.34	4.32	0.997	1.94 ± 0.15	6.11 ± 0.49
LJ	30	3.80 ± 0.13E	2–6	2.68	3.23	4.09	0.999	2.17 ± 0.11	6.65 ± 0.33
KG	30	4.38 ± 0.15BCDE	3–5	3.11	3.94	4.78	0.995	0.48 ± 0.01[a]	−1.39 ± 0.05[a]
CZ	30	5.02 ± 0.17yzAB	3–7	3.18	4.36	6.19	0.989	1.03 ± 0.11	4.1 ± 0.47

Means in the same column followed by the same letter and case are not significantly different when analyzed by PROC GIMMIX (SAS Institute 2015) followed by Tukey–Kramer least squares mean test with $\alpha = 0.05$. Days to various larval percentage mortality and parameters for the Gompertz function used to make the predictions.

[a]This instance was fit a line ($y = a + bx$) and not the Gompertz curve [$y = \exp(-\exp(-ax + b))$] which was used for the other prediction curves.

Table 3. Fit of the Morgan–Mercer–Flodin function and parameter values for survival time (d) versus temperature for each species/subspecies

Species/subspecies	Populations	Curve fit adjusted R^2	Curve parameter values			
			a ± SE	b ± SE	c ± SE	d ± SE
Lymantria dispar japonica	JN	0.6990	4.09 ± 0.9	0.0000 ± 0.0000	19.62 ± 0.46	−5.61 ± 0.99
Lymantria dispar asiatica	CJ, CR, RM, RS	0.8629	−0.93 ± 1.31	0.0092 ± 0.0049	39.62 ± 1.79	−1.95 ± 0.21
Lymantria dispar dispar	UC, LJ, KG	0.8286	0.65 ± 1.39	0.0027 ± 0.0023	30.59 ± 1.34	−2.363 ± 0.33
Lymantria monacha	CZ	0.7618	2.51 ± 2.99	0.0509 ± 0.1464	48.01 ± 30.19	−1.71 ± 0.93

a is the estimated number of days an insect would survive when the temperature = 0, b is a parameter that controls the inflection point of the curve, c rate at which survival approaches zero days, and d is the survival rate as T approaches zero. Actual values for b for JN are 0.00000009 ± 0.00000024.

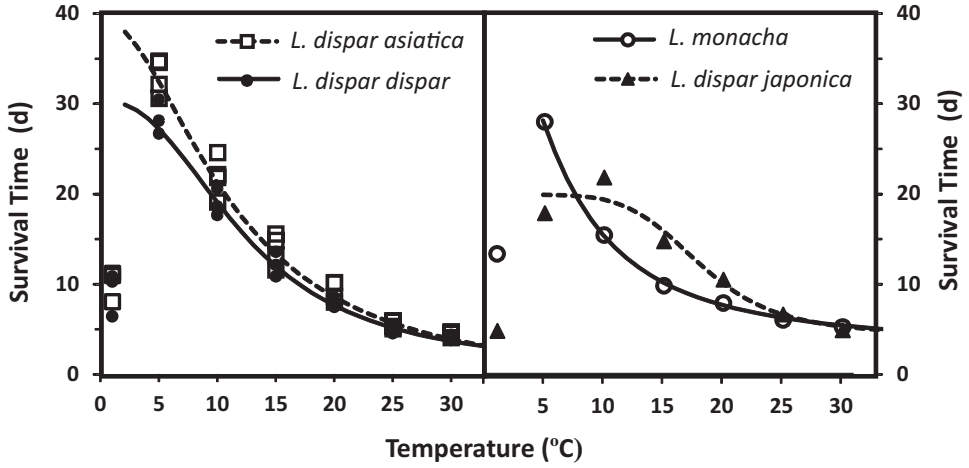

Fig. 2. Observed average first instar survival time (d) for each population at various temperatures without food are shown. Estimated Morgan–Mercer–Flodin curves for each *L. dispar* subspecies (groups of populations) and *L. monacha* are plotted.

protect them from freezing. Previous work has shown that early instar gypsy moth reared in groups survive better and develop faster than those reared individually (Ponomarev et al. 2009).

Foliage maturity at the time of *Lymantria* egg hatch has been shown to affect larval survival and development (Raupp et al. 1988, Stoyenoff et al. 1994, Erelli and Elkinton 2000). Younger expanding leaves are better food sources because they have higher nutrient and water content, and lower toughness and secondary compounds (Feeny 1970, Haukioja et al. 1978, Mattson 1980, Scriber 1984, Rossiter et al. 1988). Despite these issues the newly hatched *L. dispar* larvae from the eight populations were able to feed (10–30°C) and develop (15–30°C) on the summer foliage of one or both of the hosts offered. This suggests that they should be able to find adequate food for establishment even if hatch is not synchronous with bud break for the invaded habitat. However, if the larvae are developing later than normal in the season they would likely face other obstacles that would impact survival to the next generation, such as insufficient heating degree days to complete the lifecycle before winter and increased susceptibility to pathogens due to feeding on older foliage (Martemyanov et al. 2015). Mismatched phenology may increase susceptibility to natural enemies as it does for *Malacosoma disstria* in established habitats (Parry et al. 1998). However, they may experience a release from parasitism due to both asynchrony with the parasitoids and a lack of parasitoids in newly invaded habitats where *Lymantria* larvae are at low density or there is no alternate host to harbor parasitoids that can host switch (Hunter and Elkinton 2000).

Lymantria dispar asiatica and *L. dispar japonica* are generally considered to have a broader host range, including conifers, than *L. dispar dispar* (Baranchikov 1988, Turova 1992). Previous laboratory studies generally showed that the Asian populations (from Russia and China) evaluated could utilize many North American hosts, and that they developed faster and survived better on hosts considered marginal for North American gypsy moths (Baranchikov and Montgomery 2009, Matsuki et al. 2011). Other studies have shown Chinese populations could only use some *Pinus* sp. to complete development and died before reaching the second instar on others (Wei et al. 2012). Based on our results for Douglas fir the ability to utilize other conifers may also vary between populations across all subspecies of *L. dispar* and between conifer hosts. Further research to evaluate the utilization of conifers by *L. dispar* populations from several different geographic regions is needed to confirm this. It is already known that *L. monacha* can utilize many North American conifers and it is known to outbreak on conifers in Europe (Keena 2003) but it is not known if survival rate and host utilization varies among populations as it does in *L. dispar*. Since the current findings are based on one *L. monacha* population they should not be generalized to be the case for all populations of this species.

Although survival of this population of *L. monacha* larvae without food was similar to that of *L. dispar* they had lower survival and developed more slowly on mature foliage under warm dry conditions. The CZ *L. monacha* larvae fed more than *L. dispar* larvae at 10°C, particularly on the Douglas fir, but at 20–30°C on black oak their survival was lowest of all the populations. This population of *L. monacha* likely would have more difficulty establishing in warmer dry climates but could do well in cooler moist climates even if conifers are the only available hosts.

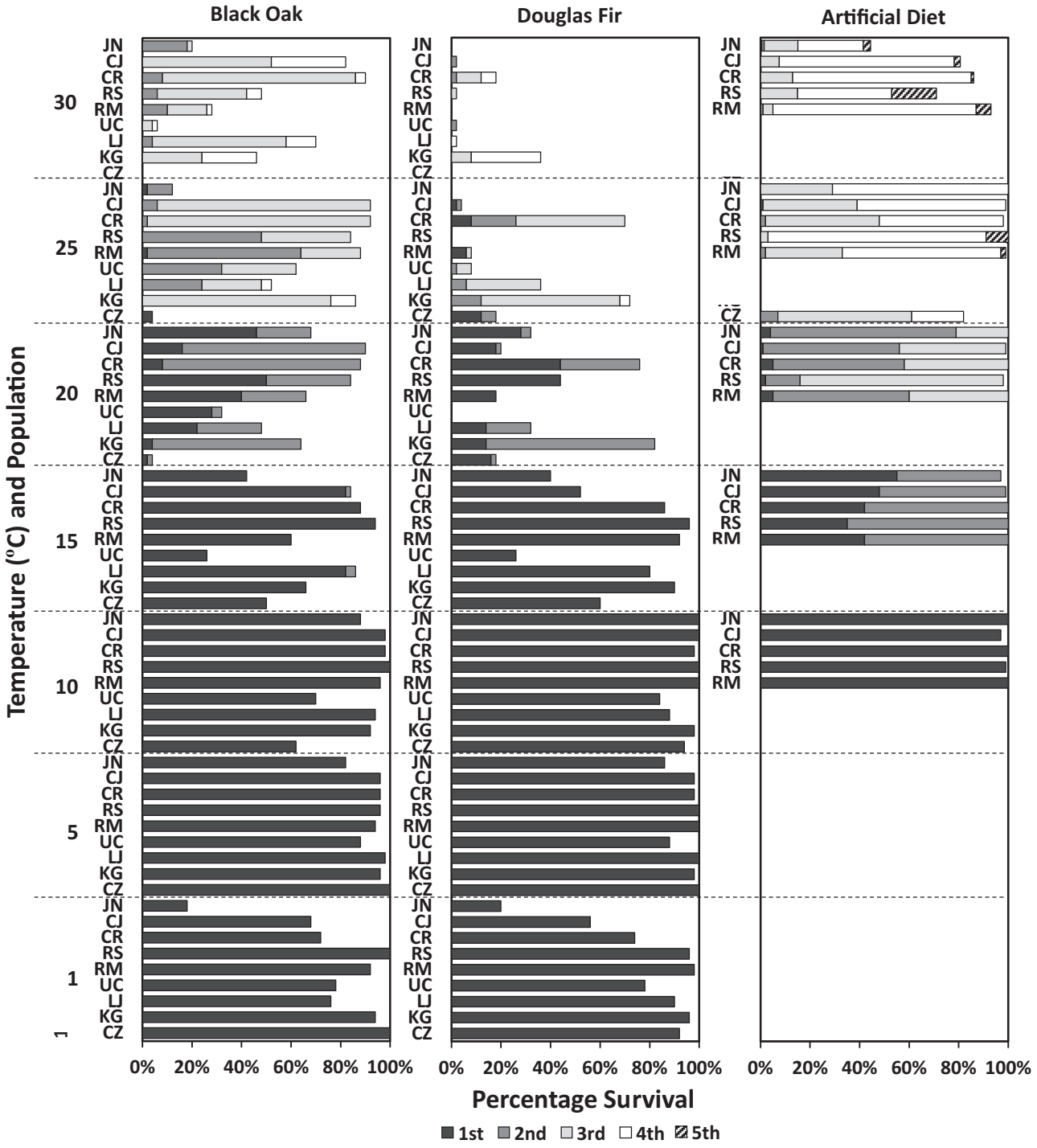

Fig. 3. Percentage survival of newly hatch *Lymantria* larvae from nine geographic populations at different constant temperatures after 14 d on black oak or Douglas fir summer foliage. For comparison the data for larvae from some of populations reared on artificial diet at some of the temperature that is available in the published literature is provided (Keena et al. 2010, Limbu et al. 2017). The proportion of the surviving larvae in each instar is also shown for each population. Population designations are given in Table 1.

In summary, the window of opportunity for establishment of non-native *Lymantria* may be larger than previously thought. The larvae survived longer without food than previously reported, especially at cooler temperatures, giving them longer to disperse to find food. The larvae from most populations could utilize foliage of their preferred host in a more mature state or of a conifer to survive and develop over most of the temperature range evaluated. This suggests that they may be able to establish outside the normal timeframe when their preferred hosts leaf out, if they can complete development, lay eggs, and the embryos are able to enter diapause before winter. This makes the prediction of hatch for eggs, which have traveled along trade routes, critically important for efficacious biosecurity inspection of vessels and cargo in port areas. Currently, the gypsy moth model for egg hatch (Gray et al. 2001) is only calibrated for the North American gypsy moth so additional work to recalibrate it to predict hatch for eggs from other world areas is needed.

Table 4. Percentage survival, mean (±SE) weight, mean (±SE) larval instar, and mean (±SE) feeding code at 14 d for newly hatched larvae from different source populations on black oak (*Quercus velutina*) and Douglas fir (*Pseudotsuga menziesii)* foliage at various temperatures

Pop.	Temp. °C	14 d on black oak foliage					14 d on Douglas fir foliage				
		% Survival	Mean feeding code	Mean larval weight (mg)	Mean larval instar	*N*	% Survival	Mean feeding code	Mean larval weight (mg)	Mean larval instar	*n*
JN	1	18%	0.0 ± 0.0	0.93 ± 0.11efg	1.00 ± 0.04ef	9	20%	0.0 ± 0.0	0.87 ± 0.10efgh	1.00 ± 0.02ef	10
CJ	1	68%	0.0 ± 0.0	0.72 ± 0.04fghi	1.00 ± 0.02ef	34	56%	0.0 ± 0.0	0.69 ± 0.05fghi	1.00 ± 0.02ef	28
CR	1	72%	0.0 ± 0.0	0.93 ± 0.06ef	1.00 ± 0.02ef	36	74%	0.0 ± 0.0	0.99 ± 0.06ef	1.00 ± 0.02ef	37
RS	1	100%	0.0 ± 0.0	0.72 ± 0.04fghi	1.00 ± 0.02ef	50	96%	0.0 ± 0.0	0.72 ± 0.04fghi	1.00 ± 0.02ef	48
RM	1	92%	0.0 ± 0.0	0.75 ± 0.04fghi	1.00 ± 0.02ef	46	98%	0.0 ± 0.0	0.69 ± 0.04fghi	1.00 ± 0.02ef	49
UC	1	78%	0.0 ± 0.0	0.40 ± 0.02i	1.00 ± 0.02ef	39	78%	0.0 ± 0.0	0.62 ± 0.04fghi	1.00 ± 0.02ef	39
LJ	1	76%	0.0 ± 0.0	0.68 ± 0.04fghi	1.00 ± 0.02ef	38	90%	0.0 ± 0.0	0.65 ± 0.03fghi	1.00 ± 0.02ef	45
KG	1	94%	0.0 ± 0.0	0.72 ± 0.04fghi	1.00 ± 0.02ef	47	96%	0.0 ± 0.0	0.74 ± 0.04fghi	1.00 ± 0.02ef	48
CZ	1	100%	0.0 ± 0.0	0.55 ± 0.03fghi	1.00 ± 0.02ef	50	92%	0.0 ± 0.0	0.40 ± 0.02i	1.00 ± 0.02ef	46
JN	5	82%	0.6 ± 0.2	0.77 ± 0.04fghi	1.00 ± 0.02ef	41	86%	0.6 ± 0.2	0.73 ± 0.04fghi	1.00 ± 0.02ef	43
CJ	5	96%	0.0 ± 0.0	0.77 ± 0.04fghi	1.00 ± 0.02ef	48	98%	0.0 ± 0.0	0.79 ± 0.04efgh	1.00 ± 0.02ef	49
CR	5	96%	0.6 ± 0.2	0.64 ± 0.03fghi	1.00 ± 0.02ef	48	98%	0.2 ± 0.2	0.60 ± 0.03fghi	1.00 ± 0.02ef	49
RS	5	96%	0.2 ± 0.2	0.76 ± 0.04fghi	1.00 ± 0.02ef	48	100%	0.2 ± 0.2	0.77 ± 0.04fghi	1.00 ± 0.02ef	50
RM	5	94%	0.0 ± 0.0	0.72 ± 0.04fghi	1.00 ± 0.02ef	47	100%	0.0 ± 0.0	0.72 ± 0.04fghi	1.00 ± 0.02ef	50
UC	5	88%	0.0 ± 0.0	0.60 ± 0.03fghi	1.00 ± 0.02ef	44	88%	0.0 ± 0.0	0.38 ± 0.02i	1.00 ± 0.02ef	44
LJ	5	98%	0.0 ± 0.0	0.62 ± 0.03fghi	1.00 ± 0.02ef	49	100%	0.0 ± 0.0	0.63 ± 0.03fghi	1.00 ± 0.02ef	50
KG	5	96%	0.6 ± 0.2	0.72 ± 0.04fghi	1.00 ± 0.02ef	48	98%	0.6 ± 0.2	0.69 ± 0.04fghi	1.00 ± 0.02ef	49
KG	5	96%	0.6 ± 0.2	0.72 ± 0.04fghi	1.00 ± 0.02ef	48	98%	0.6 ± 0.2	0.69 ± 0.04fghi	1.00 ± 0.02ef	49
CZ	5	100%	0.2 ± 0.2	0.50 ± 0.03i	1.00 ± 0.02ef	50	100%	1.0 ± 0.0	0.52 ± 0.03hi	1.00 ± 0.02ef	50
JN	10	88%	1.4 ± 0.2	0.57 ± 0.03fghi	1.00 ± 0.02ef	44	100%	1.2 ± 0.2	0.66 ± 0.03fghi	1.00 ± 0.02ef	50
CJ	10	98%	1.6 ± 0.2	0.67 ± 0.03fghi	1.00 ± 0.02ef	49	100%	1.0 ± 0.0	0.66 ± 0.03fghi	1.00 ± 0.02ef	50
CR	10	98%	1.4 ± 0.2	0.60 ± 0.03fghi	1.00 ± 0.02ef	49	98%	1.4 ± 0.2	0.54 ± 0.03fghi	1.00 ± 0.02ef	49
RS	10	100%	2.0 ± 0.0	0.72 ± 0.04fghi	1.00 ± 0.02ef	50	100%	1.6 ± 0.2	0.68 ± 0.03fghi	1.00 ± 0.02ef	50
RM	10	96%	1.0 ± 0.0	0.53 ± 0.03ghi	1.00 ± 0.02ef	48	100%	1.0 ± 0.0	0.49 ± 0.03i	1.00 ± 0.02ef	50
UC	10	70%	1.8 ± 0.2	0.45 ± 0.03i	1.00 ± 0.02ef	35	84%	1.4 ± 0.2	0.38 ± 0.02i	1.00 ± 0.02ef	42
LJ	10	94%	2.0 ± 0.0	0.74 ± 0.04fghi	1.00 ± 0.02ef	47	88%	1.2 ± 0.2	0.59 ± 0.03fghi	1.00 ± 0.02f	44
KG	10	92%	1.8 ± 0.2	0.55 ± 0.03fghi	1.00 ± 0.02ef	46	98%	1.8 ± 0.4	0.76 ± 0.04fghi	1.00 ± 0.02ef	49
CZ	10	62%	3.0 ± 0.0	0.63 ± 0.04fghi	1.00 ± 0.02ef	31	94%	4.0 ± 0.0	0.96 ± 0.05ef	1.00 ± 0.02ef	47
JN	15	42%	2.2 ± 0.2	0.73 ± 0.06fghi	1.00 ± 0.03ef	21	40%	2.0 ± 0.0	0.58 ± 0.05fghi	1.00 ± 0.03ef	20
CJ	15	84%	4.0 ± 0.0	1.49 ± 0.08ef	1.17 ± 0.02de	42	52%	3.0 ± 0.0	0.60 ± 0.04fghi	1.00 ± 0.02f	26
CR	15	88%	3.8 ± 0.2	1.64 ± 0.09ef	1.20 ± 0.02de	44	86%	3.2 ± 0.2	0.77 ± 0.04fghi	1.00 ± 0.02ef	43
RS	15	94%	4.0 ± 0.0	1.24 ± 0.06ef	1.05 ± 0.02ef	47	96%	4.0 ± 0.0	0.80 ± 0.04efgh	1.00 ± 0.02ef	48
RM	15	60%	3.0 ± 0.0	0.94 ± 0.06ef	1.02 ± 0.02ef	30	92%	2.0 ± 0.0	0.67 ± 0.04fghi	1.00 ± 0.02ef	46
UC	15	26%	2.0 ± 0.0	0.95 ± 0.09ef	1.00 ± 0.03ef	13	26%	2.0 ± 0.0	0.59 ± 0.06fghi	1.00 ± 0.03ef	13
LJ	15	86%	4.0 ± 0.0	1.24 ± 0.07ef	1.10 ± 0.02ef	43	80%	4.0 ± 0.0	0.82 ± 0.05efgh	1.00 ± 0.02ef	40
KG	15	66%	3.6 ± 0.2	1.40 ± 0.09ef	1.11 ± 0.02ef	33	90%	3.6 ± 0.2	0.92 ± 0.05efg	1.00 ± 0.02ef	45
CZ	15	50%	3.4 ± 0.2	0.88 ± 0.06efg	1.00 ± 0.02ef	25	60%	3.8 ± 0.2	1.41 ± 0.09ef	1.17 ± 0.03de	30
JN	20	68%	2.8 ± 0.2	1.87 ± 0.11def	1.43 ± 0.03de	34	32%	2.0 ± 0.0	2.34 ± 0.21def	1.50 ± 0.05de	16
CJ	20	90%	4.0 ± 0.0	4.25 ± 0.23cde	1.97 ± 0.04bcd	45	20%	1.8 ± 0.2	1.10 ± 0.12ef	1.10 ± 0.04ef	10
CR	20	88%	3.8 ± 0.2	4.61 ± 0.25bcd	1.94 ± 0.04bcd	44	76%	2.6 ± 0.4	2.16 ± 0.12def	1.62 ± 0.03cd	38
RS	20	84%	4.0 ± 0.0	2.32 ± 0.13def	1.46 ± 0.03de	42	44%	1.6 ± 0.2	1.10 ± 0.08ef	1.03 ± 0.03ef	22
RM	20	66%	2.8 ± 0.2	2.30 ± 0.14def	1.55 ± 0.03cd	33	18%	1.2 ± 0.2	0.96 ± 0.11ef	1.00 ± 0.04ef	9

Table 4. *Continued*

Pop.	Temp. °C	14 d on black oak foliage					14 d on Douglas fir foliage				
		% Survival	Mean feeding code	Mean larval weight (mg)	Mean larval instar	*N*	% Survival	Mean feeding code	Mean larval weight (mg)	Mean larval instar	*n*
UC	20	32%	1.6 ± 0.2	1.22 ± 0.11ef	1.12 ± 0.03ef	16	0%	0.0 ± 0.0			0
LJ	20	48%	3.4 ± 0.4	2.56 ± 0.19def	1.73 ± 0.04bcd	24	32%	2.0 ± 0.5	2.99 ± 0.27cde	1.8 ± 0.06bcd	16
KG	20	64%	3 ± 0.3	4.35 ± 0.27cde	2.01 ± 0.04abc	32	82%	3.0 ± 0.0	5.04 ± 0.28bcd	1.96 ± 0.04bcd	41
CZ	20	4%	1.4 ± 0.2	1.65 ± 0.41def	1.50 ± 0.13cde	2	18%	2.0 ± 0.0	1.37 ± 0.16ef	1.11 ± 0.05ef	9
JN	25	12%	2.2 ± 0.2	2.52 ± 0.37def	1.96 ± 0.10bcd	6	0%	0.0 ± 0.0			0
CJ	25	92%	4.0 ± 0.0	19.31 ± 1.01ab	3.20 ± 0.06a	46	4%	0.6 ± 0.2	3.52 ± 0.88cde	1.88 ± 0.16bcd	2
CR	25	92%	4.0 ± 0.0	15.34 ± 0.80abc	3.26 ± 0.06a	46	70%	3.2 ± 0.2	7.19 ± 0.43bcd	2.62 ± 0.05abc	35
RS	25	84%	4.0 ± 0.0	5.42 ± 0.30bcd	2.54 ± 0.05abc	42	0%	1.0 ± 0.0			0
RM	25	88%	4.0 ± 0.0	6.17 ± 0.33bcd	2.42 ± 0.05abc	44	8%	1.0 ± 0.3	3.27 ± 0.58cde	1.50 ± 0.09de	4
UC	25	62%	3.0 ± 0.0	5.26 ± 0.34bcd	2.60 ± 0.06abc	31	8%	0.8 ± 0.5	7.75 ± 1.38bcd	2.75 ± 0.17abc	4
LJ	25	52%	4.0 ± 0.0	7.72 ± 0.54bcd	2.76 ± 0.07ab	26	36%	2.6 ± 0.2	13.64 ± 1.14abc	2.92 ± 0.08ab	18
KG	25	86%	4.0 ± 0.0	16.09 ± 0.87abc	3.27 ± 0.06a	43	72%	3.8 ± 0.2	15.27 ± 0.90abc	3.24 ± 0.07a	36
CZ	25	4%	2.0 ± 0.0	0.78 ± 0.20efgh	1.00 ± 0.09ef	2	18%	1.6 ± 0.4	5.06 ± 0.60bcd	1.42 ± 0.06de	9
JN	30	20%	3.0 ± 0.0	2.94 ± 0.33cde	2.10 ± 0.08abc	10	0%	0.0 ± 0.0			0
CJ	30	82%	4.0 ± 0.0	14.53 ± 0.81abc	3.64 ± 0.07a	41	2%	0.6 ± 0.4	2.60cdef	2.00abcd	1
CR	30	90%	4.0 ± 0.0	10.4 ± 0.55bcd	3.34 ± 0.06a	45	18%	1.4 ± 0.5	26.61 ± 3.15a	3.55 ± 0.15a	9
RS	30	48%	3.0 ± 0.3	7.50 ± 0.54bcd	3.09 ± 0.08a	24	2%	0.6 ± 0.4	11.00abcd	3.00ab	1
RM	30	28%	2.4 ± 0.2	6.24 ± 0.59bcd	2.82 ± 0.09ab	14	0%	0.4 ± 0.2			0
UC	30	6%	2.0 ± 0.0	6.63 ± 1.36bcd	3.33 ± 0.24a	3	2%	0.8 ± 0.5	4.40bcde	2.75abc	1
LJ	30	70%	3.6 ± 0.2	10.45 ± 0.63bcd	3.37 ± 0.07a	35	2%	0.0 ± 0.0	16.10abc	4.00a	1
KG	30	46%	3.6 ± 0.2	12.83 ± 0.95abc	3.67 ± 0.09a	23	36%	2.6 ± 0.4	31.58 ± 2.64a	3.90 ± 0.11a	18
CZ	30	0%	1.8 ± 0.2			0	0%	1.8 ± 0.2			0

Means in the same column for both hosts followed by the same letter are not significantly different when analyzed by PROC GIMMIX (SAS Institute 2015) followed by Tukey–Kramer least squares mean test with $\alpha = 0.05$.

Acknowledgments

We thank N. Bloomfield, D. Gray, and Y. Wu and the two anonymous reviewers for their critical review of this article. We thank G. Bradford, C. Keena, P. Moore, V. Sánchez, A. Vandel and D. Yu who provided technical assistance. This study was funded in part by a grant from the National Science Foundation of The People's Republic of China (31770687).

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