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## Abstract

To understand the role that temperature and humidity play in the population dynamics of the beet webworm, *Loxostege sticticalis* L. (Lepidoptera: Crambidae), egg hatch, survival of first–fifth instars, survival of the full larval stage, survival curves, and larval development rates were investigated at combinations of four temperatures (18, 22, 26, and 30°C) and five relative humidities (RH; 20%, 40%, 60%, 80%, and 100%). We found that greatest egg hatch rate, survival rates of the first and second instars, and survival rate of the complete larval stage occurred at 22°C and 60–80% RH; the lowest values for these parameters were observed at 30°C and 20% RH. Survival of first instars was significantly affected by the interaction of temperature and relative humidity. However, survival of third and fourth instars was neither affected by temperature nor relative humidity, and that of fifth instars was significantly affected only by relative humidity level. The survival curve for larvae was well described by a type III Weibull distribution. Duration of larval stage decreased as temperature increased, but was not affected by relative humidity. We therefore conclude that eggs and early instars are the most critical stages for survival to the pupal stage, and 22–26°C and 60–80% RH are the optimum conditions for their survival and development. These findings confirm that temperature and relative humidity are the critical environmental factors affecting the population growth of *L. sticticalis*, with temperature being more important.

## Keywords

*Loxostege sticticalis*, survival rate, development, temperature, relative humidity

## Disciplines

Ecology and Evolutionary Biology | Entomology | Population Biology

## Comments

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# Egg Hatch and Survival and Development of Beet Webworm (*Lepidoptera: Crambidae*) Larvae at Different Combinations of Temperature and Relative Humidity

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## Abstract

To understand the role that temperature and humidity play in the population dynamics of the beet webworm, *Loxostege sticticalis* L. (Lepidoptera: Crambidae), egg hatch, survival of first–fifth instars, survival of the full larval stage, survival curves, and larval development rates were investigated at combinations of four temperatures (18, 22, 26, and 30°C) and five relative humidities (RH; 20%, 40%, 60%, 80%, and 100%). We found that greatest egg hatch rate, survival rates of the first and second instars, and survival rate of the complete larval stage occurred at 22°C and 60–80% RH; the lowest values for these parameters were observed at 30°C and 20% RH. Survival of first instars was significantly affected by the interaction of temperature and relative humidity. However, survival of third and fourth instars was neither affected by temperature nor relative humidity, and that of fifth instars was significantly affected only by relative humidity level. The survival curve for larvae was well described by a type III Weibull distribution. Duration of larval stage decreased as temperature increased, but was not affected by relative humidity. We therefore conclude that eggs and early instars are the most critical stages for survival to the pupal stage, and 22–26°C and 60–80% RH are the optimum conditions for their survival and development. These findings confirm that temperature and relative humidity are the critical environmental factors affecting the population growth of *L. sticticalis*, with temperature being more important.

**Key words:** *Loxostege sticticalis*, survival rate, development, temperature, relative humidity

The beet webworm, *Loxostege sticticalis* L. (Lepidoptera: Crambidae), is a cosmopolitan migrant insect pest mainly inhabiting the temperate zone from 36 to 55°N (Pepper 1938, Luo and Li 1993a, Afonin et al. 2014). Crops and fodder plants throughout northern China are seriously damaged by outbreaks of larval populations during the breeding season, which usually result in significant yield and economic losses (Luo et al. 1998, 2009; Chen et al. 2016). To better understand outbreak dynamics and so reduce economic losses, the effects of temperature and humidity on *L. sticticalis* population dynamics have been studied. *L. sticticalis* is confined to areas southern to the limit of 8°C annual isotherm, while the eastern and western limits for the distribution are approximately between the 100- to 700-mm isohyetal lines (Pepper 1938, Luo and Li 1993a, Afonin et al. 2014). Outbreaks occur mostly in areas with prevailing temperatures of 22°C and relative humidity (RH) >60%, where adults emigrating from various source regions concentrate to oviposit (Luo et al. 1998, 2009; Huang et al. 2011). Lifetime fecundity at these ambient temperatures and humidities are maximal

(Meng et al. 1987, Wei et al. 1987, Luo and Li 1993b, Cheng et al. 2015). Finally, development, adult longevity, fecundity, and annual generation number (Luo and Li 1993a,b; Afonin et al. 2014), incidence of diapause (Huang et al. 2009), migration, and reproduction (Chen et al. 1992, Feng et al. 2004; Cheng et al. 2013, 2015), as well as the selection of oviposition sites by the female (Kang et al. 2007, Chen et al. 2010) are all highly related to ambient temperature and RH.

However, there is only one empirical study dealing with the effect of temperature and RH on oviposition and egg hatch rate (Meng et al. 1987), and egg hatch and larval survival of *L. sticticalis* under different combinations of temperature and RH are less well understood. This knowledge gap hinders our understanding of mechanisms underlying the life history strategy and population dynamics of this species. For example, although the distribution and outbreaks of *L. sticticalis* are confined to areas southern to the limit of annual 8°C isothermal, damage does not occur in areas further south, away from this line (Pepper 1938, Luo and Li 1993a, Afonin

et al. 2014). Why *L. sticticalis* is not distributed and causing damage in southern areas is not understood. Possibly the warmer and more humid climatological conditions in southern areas hinder survival of eggs and larvae, thus confining the distribution or damage of this species.

Meng et al. (1987) reported that egg hatch rate at 21°C was not significantly different within the range of 22–97% RH. Results from field investigations (Kang et al. 2007, Chen et al. 2010) showed that host plant species receiving eggs, and the position of eggs on a plant differed as ambient RH and temperature varied. For example, eggs are laid on the dicot weed lambsquarters, *Chenopodium album* L., when the local climate is dry or normal (<60% RH), but on the monocot weed stinkgrass, *Eragrostis ciliaris* (All.) Link ex Vignolo-Lutati, when local RH is >80% (Chen et al. 2010). Kang et al. (2007) and Chen et al. (2010) speculated that flexibility in oviposition site selection by females might increase egg hatch. This raises the question whether egg hatch is affected by temperature and RH.

We investigated egg hatch and survival of instars at different combinations of temperature and RH in the laboratory. The survival curves for larvae and their individual instars were determined and fitted to a Weibull distribution equation. The developmental period of the larval stage under the same experimental conditions was also determined. The information on temperature and RH effects help us interpret population dynamics and outbreaks in the species. Ultimately it will help use develop forecasting tools to improve management.

## Materials and Methods

### Experimental Insect

A laboratory colony of *L. sticticalis* was established with larvae collected from the alfalfa field in Kangbao County (114.63° E, 41.87° N) of Hebei province, China, in 2014. Larvae were reared at a density of 20 larvae per 850-ml glass jar and fed daily with fresh leaves of *C. album* until they were mature and stopped feeding. They were then transferred into another jar containing sterilized fine sandy loam (10% moisture content) at a depth of 6–8 cm to provide a substrate for cocoon formation, pupation, and adult emergence. Adults emerging on the same day were collected and held together in a 2-liter plastic cage for mating and oviposition. Adults were provided daily with 10% glucose solution (w/v). Eggs laid on gauze were collected daily and transferred to another plastic cage. All stages of the colony were maintained at 22 ± 1°C, 75 ± 10% RH, and a photoperiod of 16:8 (L:D) h. These conditions are optimum for growth, development, and reproduction of *L. sticticalis* (Luo and Li 1993a,b; Huang et al. 2009; Kong et al. 2010). The insect had been reared for two generations when they were being tested.

### Experimental Design and Treatment

#### Treatment and Control of Temperature and Relative Humidity

The experimental treatments consisted of four temperatures (18, 22, 26, and 30°C) and five relative humidity (20, 40, 60, 80, and 100%) regimes, which represent the range of climatological conditions that *L. sticticalis* may encounter during the breeding season (Luo and Li 1993a). Temperature was controlled by an environmental incubator (Ningbo, China), accurate to ± 1°C. Relative humidity was controlled by saturating water with selected salts (analytical reagent grade; Table 1). The salt and pure water were added to about 1/3 of a one-liter glass desiccator to form a saturated solution or slurry. The salt layer of the slurry was around half of the solution in depth.

The lid of the closed desiccator was sealed using vaseline. RH of the atmosphere within each desiccator could be controlled by the selected salt solution, accurate to ± 5% across the temperatures tested (Winston and Bates 1960; Table 1). Once a day, the relative humidities were checked by a thermo-hygrometer (TES-1360A, Beijing Detector Instrument Co., China) to ensure tight control of RH. In cases where deviation from the target was >10%, the slurry was substituted with a new one.

### Egg Hatch

Eggs laid on the same day were put into a 450-ml plastic jar and covered tightly by a nylon net. The jars were then placed into the corresponding desiccators containing the designated salt slurry for controlling RH. This method guaranteed that the eggs and larvae were fully suffused by atmosphere at the correct RH inside the desiccator while preventing neonates from escaping the jar. Each desiccator containing eggs was held in the corresponding thermal incubator. The number of larvae hatching from the eggs was checked and recorded daily until no new larvae emerged. Egg hatching rate was calculated as the number of larvae found/number of eggs placed in the jar. Each combination of temperature and humidity treatment was replicated five times and each replication consisted of >40 eggs.

### Survival Rate of Larvae

In general, the mortality of early instars of Lepidoptera is high and varies greatly (Zalucki et al. 2002). The larvae of *L. sticticalis* develop through five instars, and their respective feeding and living habitats vary to some extent (Gu et al. 1987). It is therefore necessary to discriminate the effect of temperature and humidity on survival rate of each instar separately and determine the instar most sensitive to these parameters. The procedure and container used to test the survival of larvae were similar to those used for the eggs. Each treatment combination of temperature and humidity was replicated 10 times. The experiment began with neonates, which were fed daily with fresh *C. album* leaves. The number of dead or molted larvae were recorded daily until they entered the prepupal stage. The survival rate of the larvae was calculated following the method for egg hatchability rate. For the first instar, each replication consisted of 20 larvae, but for the second to fifth instars, the number of larvae tested in each replication was the number of previous instars that survived to the current instar. The survival rate of the entire larval stage was determined by summarizing the survival rate from the first instar to the prepupal stage.

### Survival Curve and Developmental Duration of the Larval Stage

To determine the instar most sensitive to the different combinations of temperature and relative humidity, survival curves were fitted by the two-parameter Weibull frequency distribution according to Deevey (1947), Pinder et al. (1978), Tingle and Copland (1989), and Aghdam et al. (2009). The probability that an individual lives to at least instar  $t$  was expressed as  $S(t)$  [ $S(t) = N(t)/N(\text{number of neonates})$ ] and described by the following equation:

$$S(t) = \exp(-(t/b)^c) \quad b, c, \text{ and } t > 0$$

where  $b$  is the scale parameter that is inversely related to the mortality rate, and  $c$  is the shape parameter that allows the model to produce survival distributions of different forms, from exponential to an extreme inverted shape. Type I, II, and III survivorship curves correspond to the values of the shape parameter:  $c > 1$ ,  $c = 1$ , or  $c < 1$  (Deevey 1947, Pinder et al. 1978, Tingle and Copland 1989).

**Table 1.** Selected salts used to make saturated solutions in water, and corresponding range of relative humidity expected and detected in the atmosphere above the solutions

Desired RH (%)	Salt	Expected RH (%) between 10–30°C <sup>a</sup>	Observed RH (%) (mean ± SE) at 18–30°C			
			18°C	22°C	26°C	30°C
20	Potassium acetate (KC <sub>2</sub> H <sub>3</sub> O <sub>2</sub> )	20.0–22.5	20.50 ± 0.26	21.40 ± 0.50	21.93 ± 0.19	22.00 ± 0.26
40	Potassium carbonate (K <sub>2</sub> CO <sub>3</sub> )	43.0–47.0	43.47 ± 0.03	45.03 ± 0.47	44.17 ± 0.09	43.13 ± 0.13
60	Sodium nitrite (Na <sub>2</sub> NO <sub>2</sub> )	63.0–65.5	67.00 ± 1.00	64.60 ± 1.03	63.43 ± 0.58	62.47 ± 0.03
80	Potassium chloride (KCl)	84.5–88.0	85.87 ± 0.09	84.77 ± 0.07	84.07 ± 0.09	83.10 ± 0.06
100	Potassium sulfate (K <sub>2</sub> SO <sub>4</sub> )	96.5–99.0	99.37 ± 0.07	98.63 ± 0.32	97.03 ± 0.52	96.97 ± 0.09

<sup>a</sup>From Winston and Bates (1960).

The Weibull distribution parameters were estimated by the Marquardt iteration method using the nonlinear regression procedure in SAS (Proc NLIN). All the figures were drawn by OriginPro 2015 (OriginLab, Northampton, MA).

To test the effects of temperature and humidity on larval development, the duration of the full larval stage was investigated under the same combinations of temperature and humidity. Duration of the full larval stage was defined as the average period from the day of hatch to the day when they entered the prepupal stage. The number of replications and the initial number of larvae used for each replication were similar to those of the instar survival test. However, larvae that died prior to the prepupal stage were excluded from analysis.

### Data Analysis

All numeric values are presented as means ± SE. All data were checked for normality before they were subjected to analysis. Data that were not normally distributed were arcsine transformed. Differences between temperature, relative humidity treatments, and their interactions were first evaluated by two-way analysis of variance (ANOVA). If the interaction was significant, simple effect comparisons were made, i.e. comparing temperature effects at each level of humidity and/or humidity effects at each temperature level separately. If the interaction was not significant, then only main effects of each factor were compared. Significant differences among multiple means were separated using Tukey's Studentized Range Test (HSD) at the 0.05 level. All statistical analyses were performed using the SAS statistical package V9.0 (SAS Institute 1999).

## Results

### Egg Hatch

Egg hatch varied greatly and was significantly affected by temperature ( $F_{3,80} = 24.05$ ;  $P < 0.0001$ ) and relative humidity ( $F_{4,80} = 12.58$ ;  $P < 0.0001$ ) but not by their interaction (Fig. 1;  $F_{12,80} = 1.28$ ;  $P = 0.24$ ). Highest hatch rate of eggs was at 22 and 26°C, and lowest at 18 and 30°C (Fig. 1A). Egg hatch rate at 22 and 26°C did not differ significantly, and both were significantly greater than at 18 and 30°C. For relative humidity, egg hatch rate was lowest at 20% RH, and increased as the humidity level rose to 80% (Fig. 1B).

### Survival Rate of Larvae

#### First Instars

The survival rate of first instars varied greatly at different combinations of temperature and relative humidity (Fig. 2), and was significantly affected by temperature ( $F_{3,180} = 29.69$ ;  $P < 0.0001$ ), relative

humidity ( $F_{4,180} = 32.65$ ;  $P < 0.0001$ ), and their interaction ( $F_{12,180} = 2.12$ ;  $P < 0.0001$ ). Within temperature treatments, survival rate differed significantly as the relative humidity level varied (Fig. 2). At 18°C, survival rate at 80% RH was significantly greater than those at other RH levels, which did not differ from one another ( $F_{4,45} = 13.5$ ;  $P < 0.0001$ ; Fig. 2). At 22°C, survival rate at 80% RH was significantly greater than at 20, 40, and 100% RH levels, while that at 20% RH was significantly lower than all other treatments ( $F_{4,45} = 15.13$ ;  $P < 0.0001$ ). At 26°C, survival rate at 20% RH was significantly lower than at 60 and 80% RH ( $F_{4,45} = 5.74$ ;  $P = 0.0008$ ). At 30°C, first instar survival rate at 80 and 60% RH were significantly greater than at 20 and 40% RH ( $F_{4,45} = 4.9$ ;  $P = 0.0023$ ).

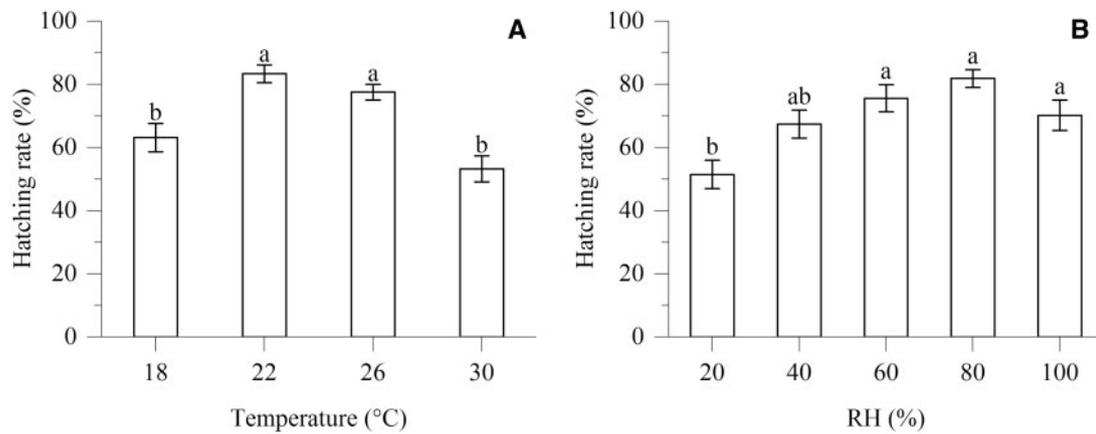
Within the relative humidity treatments, survival rate of the first instars was significantly affected by temperature, except for 20% RH where survival rate was uniformly low at all temperatures tested ( $F_{3,36} = 2.00$ ;  $P = 0.13$ ; Fig. 2). At 40% RH, survival rates varied greatly. Survival rate at 22°C (80.5%) was significantly greater than at 18 and 30°C, and survival at 26°C was significantly higher than that at 30°C ( $F_{3,36} = 6.02$ ;  $P = 0.002$ ). At 60% RH, survival rate at 22°C was significantly greater than at 18 and 30°C ( $F_{3,36} = 8.30$ ;  $P = 0.0003$ ). At 80% RH, survival rate was uniformly highest at 18, 22, and 26°C, and was significantly greater than that at 30°C ( $F_{3,36} = 12.35$ ;  $P < 0.0001$ ). At 100% RH, survival rate of first instars at 18, 22, and 26°C were similar and significantly greater than at 30°C ( $F_{3,36} = 8.19$ ;  $P = 0.0003$ ).

#### Second–Fourth Instars

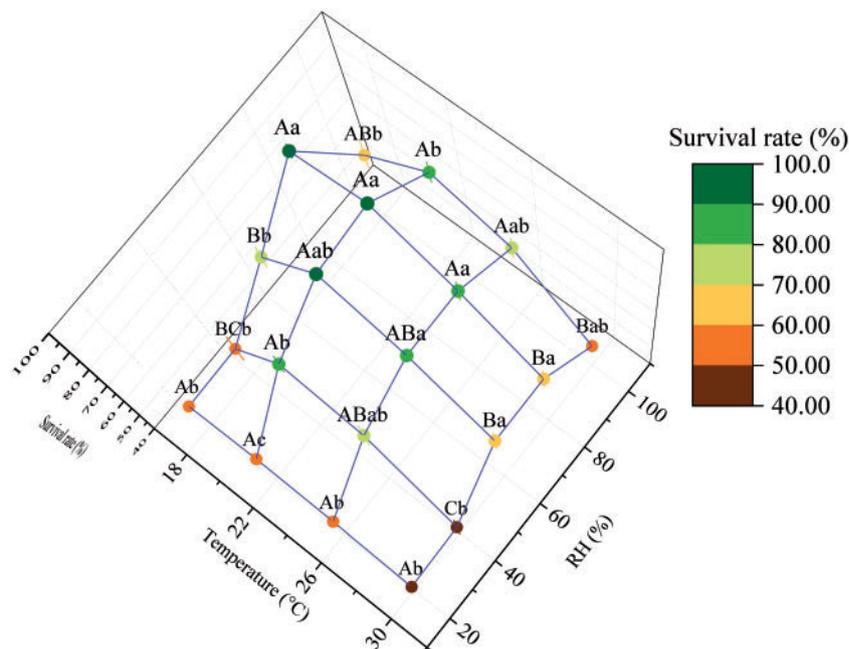
Survival rate of second instars was significantly affected by temperature ( $F_{3,180} = 6.35$ ;  $P = 0.0004$ ) and relative humidity ( $F_{4,180} = 4.77$ ;  $P = 0.0011$ ) but not by their interaction ( $F_{12,180} = 0.66$ ;  $P = 0.79$ ). Within temperature treatments, survival rate at 22 and 26°C was significantly greater than at 30°C (Fig. 3A). Survival rate of second instars at 20% RH was significantly lower than that at 60 and 80% RH (Fig. 3B). Neither temperature ( $F_{3,180} = 0.06$ ;  $P = 0.98$ , and  $F_{3,180} = 0.72$ ;  $P = 0.54$ ) nor relative humidity ( $F_{4,180} = 0.28$ ;  $P = 0.89$ , and  $F_{4,180} = 1.78$ ;  $P = 0.13$ ) regimes had any effect on survival rate of third and fourth instars, which was always > 90% (Data were not showed).

#### Fifth Instars

Survival rate of fifth instars was not significantly affected by temperature ( $F_{3,180} = 0.75$ ;  $P = 0.52$ ), but was significantly affected by relative humidity ( $F_{4,180} = 9.28$ ;  $P < 0.0001$ ). Survival rates were all around 90% across temperature regimes (Fig. 4A). Survival rate at 100% RH was significantly lower than that in the 40, 60, and 80% RH treatments (Fig. 4B).



**Fig. 1.** The hatching rate of *L. sticticalis* eggs at different (A) temperatures and (B) relative humidities. Data are presented as mean  $\pm$  SE. Sample sizes for each treatment were (A) 25 and (B) 20. Sample size for each replication was  $>40$ . Means sharing the same letter are not significantly different as determined by Tukey's HSD test ( $\alpha=0.05$ ).



**Fig. 2.** Survival rate of *L. sticticalis* first instars reared at different combinations of temperature and relative humidity. Sample size for each treatment was 10. Means sharing the same lower case letter are not significantly different between humidity treatments at the same temperature; means sharing the same capital letter are not significantly different between temperature treatments at the same humidity, as determined by Tukey's HSD test ( $\alpha=0.05$ ).

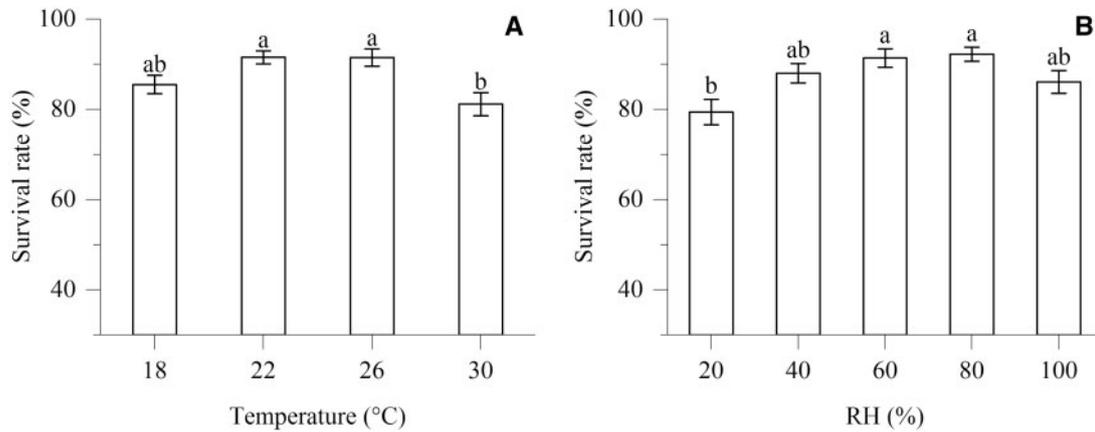
### Full Larval Stage

Temperature ( $F_{3,180}=32.72$ ;  $P<0.0001$ ) and relative humidity ( $F_{4,180}=40.99$ ;  $P<0.0001$ ) both significantly affected the overall survival rate of larvae, but their interaction was not significant ( $F_{12,180}=32.72$ ;  $P=0.13$ ). Larval survival rate was highest at 22°C, followed by that at 26, 18, and 30°C (Fig. 5A). Survival rate at 30°C was significantly less than those at temperatures  $<26^\circ\text{C}$ , and survival at 22°C was significantly greater than at 18°C. Survival at 20% RH was significantly lower than at RH levels  $>40\%$  (Fig. 5B). Survival at 80 and 60% RH was significantly greater than at 40 and 100% RH.

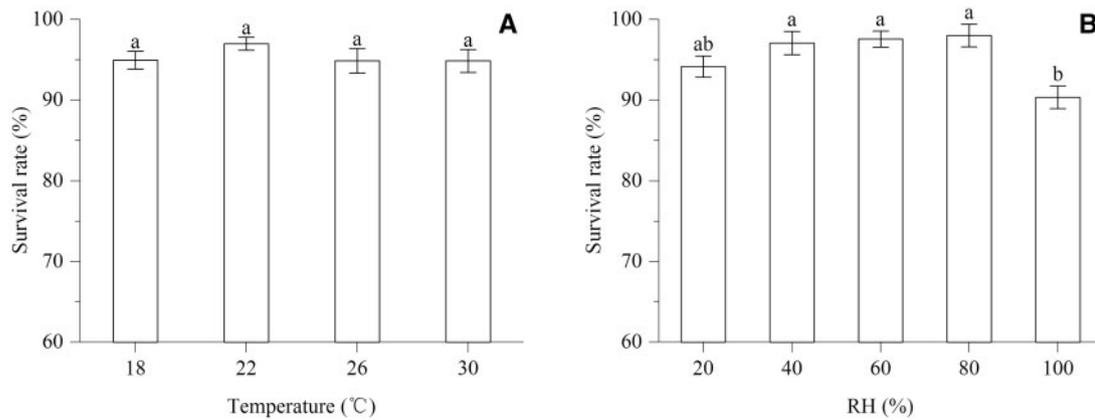
### Survival Curves and Development Time of Larvae

Survival curves of larval *L. sticticalis* at different combinations of temperature and relative humidity at each instar interval, as

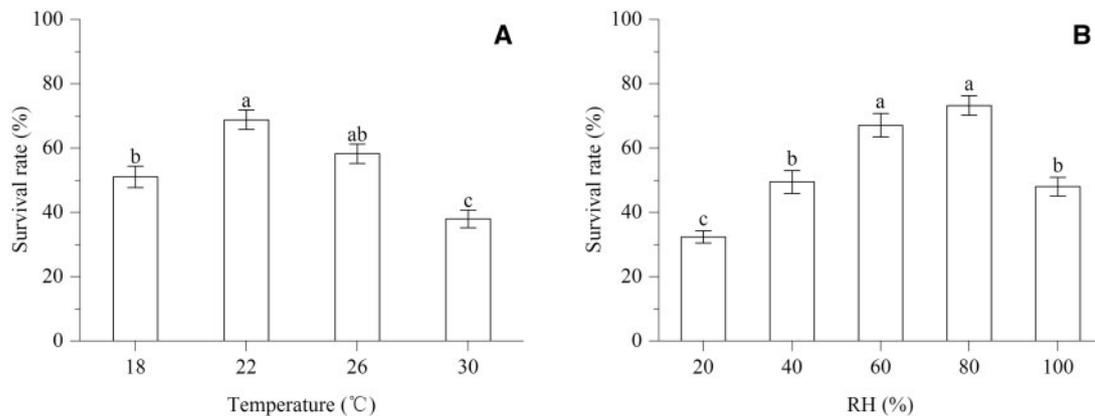
described by the two-parameter Weibull distribution equation, is shown in Fig. 6. Values of the Weibull parameters are presented in Table 2. Chi-square goodness-of-fit tests indicated good agreement between the Weibull distribution and the survival data (Fig. 7; Table 2). They are all type III survival curves (Deevey 1947, Pinder et al. 1978) because all values of the shape parameter ( $c$ ) were  $<1$  (Table 2). The value of the scale parameter ( $b$ ) was greatest for the combination of 22°C and 80% RH, followed by that at 22°C and 60% RH (Table 2); those for 20% RH across all temperatures, and at 30°C across all relative humidity levels were the smallest (Table 2). These results are consistent with our observations that the greatest survival rates of larvae occurred at the combination of 22°C and 60–80% RH, and the lowest at the combination of 30°C and 20% RH. This was expected because the scale parameter is inversely related to mortality rate.



**Fig. 3.** Survival rate of second instars of *L. sticticalis* reared at different (A) temperatures and (B) relative humidities. Data are presented as mean  $\pm$  SE. Sample sizes for each treatment were (A) 50 and (B) 40. Means sharing the same letter are not significantly different, as determined by Tukey's HSD test ( $\alpha = 0.05$ ).



**Fig. 4.** Survival rate of fifth instars of *L. sticticalis* reared at different (A) temperatures and (B) relative humidities. Data are presented as mean  $\pm$  SE. Sample sizes for each temperature and humidity treatment were 50 and 40, respectively. Means sharing the same letter are not significantly different, as determined by Tukey's HSD test ( $\alpha = 0.05$ ).



**Fig. 5.** Survival rate of larval *L. sticticalis* reared at different (A) temperatures and (B) relative humidities. Data are presented as mean  $\pm$  SE. Sample sizes for each temperature and humidity treatment were 50 and 40, respectively. Means sharing the same letter are not significantly different, as determined by Tukey's HSD test ( $\alpha = 0.05$ ).

#### Developmental Period

Duration of the larval stage was significantly affected by temperature ( $F_{3,180} = 1549.12$ ;  $P < 0.0001$ ), but not by relative humidity ( $F_{4,180} = 1.11$ ;  $P = 0.35$ ) or their interaction ( $F_{4,180} = 1.41$ ;  $P = 0.17$ ; Fig. 7). Larval development period decreased as temperature increased (Fig. 7A), dropping from 17.8 d at 18°C to 7.9 d at 30°C.

#### Discussion

##### Egg Hatch

Egg hatch of *L. sticticalis* is significantly affected by temperature and relative humidity conditions, but not by their interactions. These results are similar to those obtained in the oriental

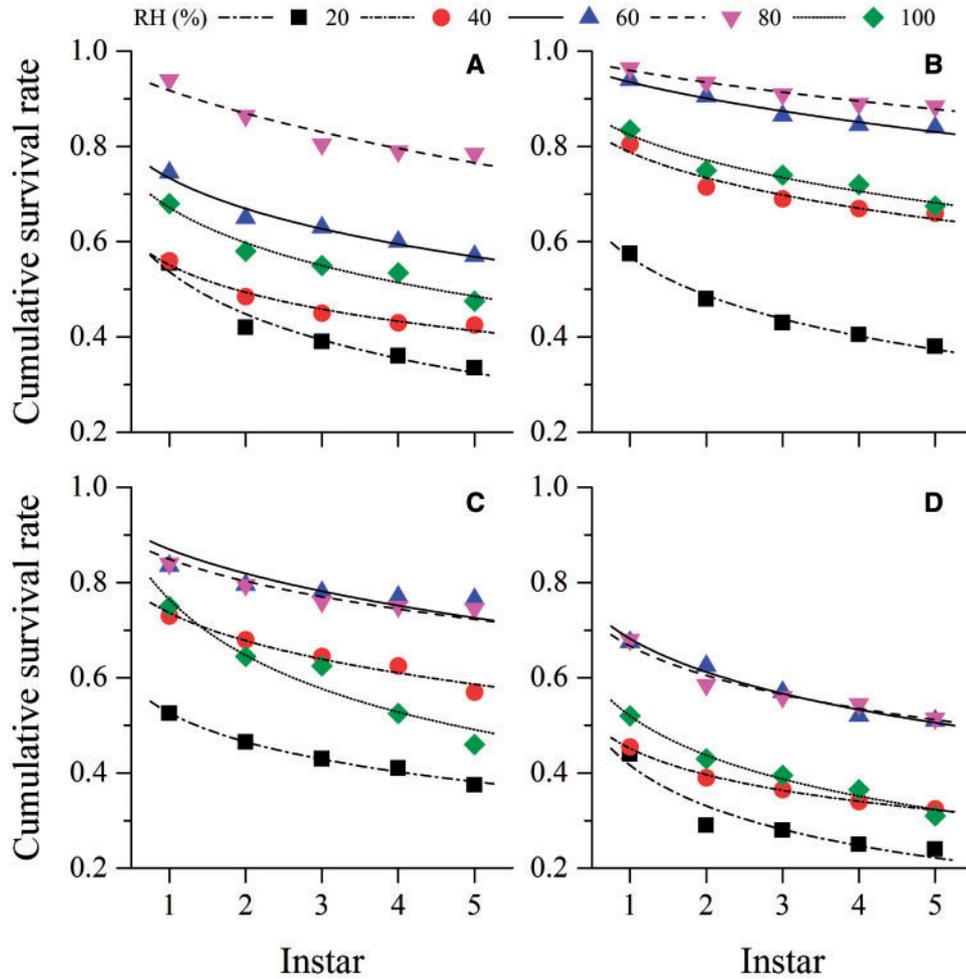


Fig. 6. Survival curves for *L. sticticalis* larvae at different combinations of temperature and relative humidity regimes. A, B, C, and D represent temperature treatments of 18, 22, 26, and 30°C, respectively. Symbol, observed; line, estimated.

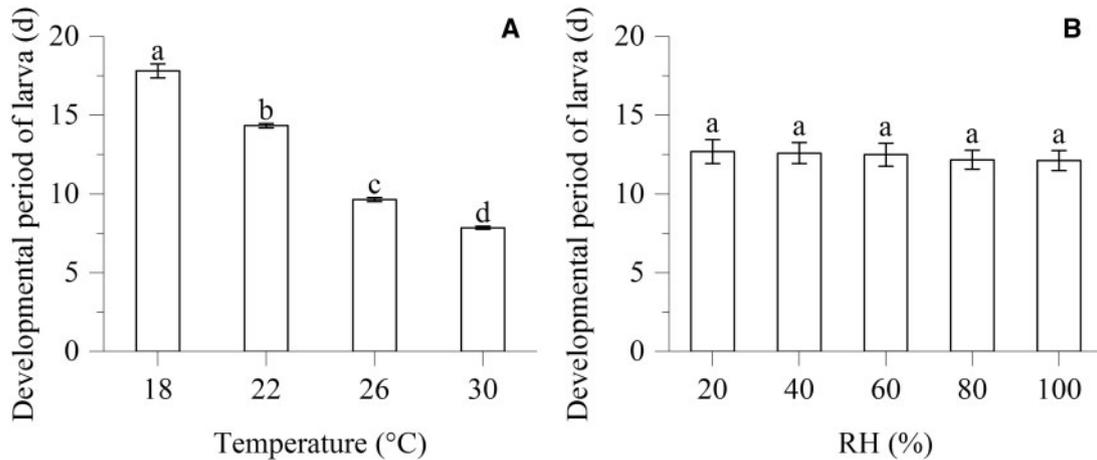


Fig. 7. Developmental period of larval *L. sticticalis* reared at (A) different temperatures and (B) relative humidities. Data are presented as mean  $\pm$  SE. Sample sizes for each temperature and humidity treatment were 50 and 40, respectively. Means sharing the same letter are not significantly different, as determined by Tukey's HSD test ( $\alpha = 0.05$ ).

armyworm, *Mythimna seperata* (Walker) (Chen et al. 1965, Jing et al. 1964) and the rice leaf folder, *Cnaphalocrocis medinalis* (Guenée) (Fang et al. 2013), in which their egg hatchability was also significantly affected by temperature and relative humidity

conditions. Although the optimum temperature and relative humidity conditions for egg hatch rate varies with species, a common feature in these three species is that egg hatch rate decreased under high temperature and low relative humidity conditions. Low hatch under

**Table 2.** Estimated Weibull frequency distribution parameters and  $\chi^2$  goodness-of-fit tests for the instar-specific survival curves of *L. sticticalis* larvae at different combinations of temperature and relative humidity

T (°C)	RH (%)	<i>b</i>	<i>c</i>	$\chi^2$	$\chi^2_{0.05(n-3)}$	Goodness of fit
18	20	3.64 ± 0.29	0.37 ± 0.05	0.5619	5.99	*
	40	8.26 ± 1.07	0.25 ± 0.03	0.1618	5.99	*
	60	23.17 ± 5.40	0.37 ± 0.04	0.1618	5.99	*
	80	32.46 ± 16.46	0.71 ± 0.16	0.3704	5.99	*
	100	12.01 ± 2.30	0.37 ± 0.05	0.3407	5.99	*
22	20	5.25 ± 0.26	0.34 ± 0.02	0.09604	5.99	*
	40	46.78 ± 21.64	0.37 ± 0.06	0.2439	5.99	*
	60	71.86 ± 28.35	0.63 ± 0.08	0.05786	5.99	*
	80	86.46 ± 31.94	0.72 ± 0.08	0.02528	5.99	*
	100	47.70 ± 21.44	0.43 ± 0.07	0.2251	5.99	*
26	20	5.82 ± 0.29	0.25 ± 0.01	0.05411	5.99	*
	40	30.81 ± 9.09	0.35 ± 0.04	0.1871	5.99	*
	60	45.30 ± 0.01	0.52 ± 0.03	0.03112	5.99	*
	80	70.16 ± 0.01	0.43 ± 0.01	0.5439	5.99	*
	100	8.27 ± 1.25	0.61 ± 0.09	0.8049	5.99	*
30	20	1.48 ± 0.22	0.34 ± 0.06	1.5774	5.99	*
	40	2.85 ± 0.07	0.22 ± 0.01	0.03018	5.99	*
	60	14.54 ± 2.17	0.36 ± 0.03	0.1419	5.99	*
	80	18.09 ± 4.47	0.31 ± 0.04	0.2262	5.99	*
	100	3.51 ± 0.18	0.34 ± 0.03	0.2771	5.99	*

\*Asterisk indicates that the survival data fit the model [ $\chi^2 < \chi^2_{0.05(n-3)}$ ].

such circumstances might result from evaporation of moisture from the eggs (Fang et al. 2013). Such desiccation could directly result in embryo death, or perhaps prevent neonates from exiting the hardened egg shell. However, the egg hatch of *M. separata* and *C. medicinalis* at 100% RH were over 90% (Chen et al. 1965, Jing et al. 1964, Fang et al. 2013) while that of *L. sticticalis* under same circumstance was only 70% and much lower than that at 60–80% RH regimes (Fig. 1). These results indicated that egg hatch of *L. sticticalis* under saturated humidity was detrimentally affected.

Meng et al. (1987) reported that egg hatchability of *L. sticticalis* at 21°C across five relative humidity levels from 22 to 97% was around 95% and not significantly different. In contrast, we found that egg hatch rate at 22°C differed greatly across the five relative humidity levels, being significantly lowest at 20% (67.0 ± 6.80%), and significantly greatest at 80% RH (91.5 ± 3.5%). The differences in egg hatch rate between the two studies can be explained by a difference in methods. Meng et al. (1987) placed the eggs inside finger tubes (25 by 100 mm), which were plugged tightly with cotton; the eggs in the tube were then put inside the desiccator. In our study, eggs were fully exposed to the controlled atmosphere inside the desiccator (see Materials and Methods). Our result was consistent with the field investigations that oviposition site selection by females was determined by relative humidity (Kang et al. 2007, Chen et al. 2010; see also below).

### Larval Survival

Survival of first and second instars of *L. sticticalis* was significantly affected by temperature and relative humidity levels. Survival of first instars was greatest at temperatures of 22–26°C and 60–80% RH. Survival decreased more or less at temperatures and relative humidities outside these ranges: the greater the deviation the lower the survival. Numerically, the trend was for higher survival of first instars at 22 than at 26°C at each humidity treatment, but the differences were not statistically significant. Thus, the combinations of 22–26°C and 60–80% RH are optimum conditions for survival of first-instar *L. sticticalis*. Second instars are a bit more robust, with

optimum conditions ranging from 18–26°C and ≥40%. These results are consistent with that of the oriental tobacco budworm, *Helicoverpa assulta* (Guenée), in which the impact of temperature and relative humidity on survival of first and second instars is greater than on later instars (Xie et al. 1998).

Although the survival of both first- and second-instar *L. sticticalis* was significantly affected by temperature and relative humidity, their interaction was significant only for the first instars. Consequently, the temperature and relative humidity levels required for first instar survival are stricter than those required by second instars, as noted above. This result is consistent with findings for 80 other species of Lepidoptera, in which the natural mortality rate of the first instars ranges from 25–75% (Zalucki et al. 2002). The epidermis of second instars is thicker than that of first instars, and is likely the reason for their ability to withstand a greater range of temperature and humidity conditions.

In contrast to first and second instars, survival rates of third to fifth instars were less influenced by temperature and relative humidity regimes. Likewise, survival rates of *H. assulta* instars 3–5 were not significantly affected by temperature and relative humidity (Xie et al. 1998). The survival rate of fifth-instar *L. sticticalis* was not significantly affected by temperature, but was significantly less at 100% RH than at 40–80%, indicating greater sensitivity than third and fourth instars to saturated humidity condition. The process of metamorphosis from last (fifth) instar to pupa may leave the insect more vulnerable to adverse temperature and humidity conditions. Survival rate of the last (sixth) instar in *H. assulta* was greatly affected by temperature and relative humidity (Xie et al. 1998).

Although temperature and relative humidity had only minor effects on survival of third to fifth instars, their impact on survival rate through the full larval stage was significant. The highest larval stage survival rate occurred also at 22–26°C, and 60–80% RH, declining outside this range. The congruence of these results with those for early instars demonstrates that survival rate over the entire larval stage is mainly determined by that of young larvae.

## Survival Curve and Development Period of Total Larval Stage

Survival rates of larval *L. sticticalis* at different combinations of temperature and relative humidity at each instar interval were well-fitted by the two-parameter Weibull equation. The type III survival curve reflects high mortality of early instars, decreasing over time or at higher instars. This differs from the type I survival curve, which commonly describes mortality of adult insects that increases as they age (Tingle and Copland 1989, Aghdam et al. 2009). Results from analysis of scale parameter (*b*) in the Weibull distributions support the combination of 22°C, 60–80% RH as the optimum climatological conditions for survival of *L. sticticalis* larvae. The poorest conditions are at 30°C, across all relative humidity treatments, and 20% RH across all temperature treatments. In other insects, the greatest *b* value also occurs in treatments with high survival rates (Tingle and Copland 1989, Aghdam et al. 2009). The consistency of the observed and simulated results confirms that most larval mortality occurs in early instars, and that 22°C and 60–80% RH are optimal for survival of *L. sticticalis* larvae.

In contrast to survival, rate of development of *L. sticticalis* larvae is significantly affected by temperature, but not by relative humidity (Fig. 7). This finding is consistent with the models that the development rate of insects is mainly determined by temperature (Wigglesworth 1965, Bursell 1974) and results in earlier studies of *L. sticticalis* (Wei et al. 1987, Luo and Li 1993a). Lack of sensitivity to relative humidity coincides with similar results for the jasmine bud borer, *Trichophyesis cretacea* Butler (Yang et al. 2008), and *H. assulta* (Xie et al. 1998) but differs from that of Angoumois grain moth, *Sitotroga cerealella* (Olivier) (Wu et al. 1994), and sod webworm, *Herpetogramma basalis* (Walker) (Xie et al. 2012). Physiological mechanism underlying this phenomenon is unknown and needs further study.

Based on these findings, several aspects underlying population dynamics and life history strategy in *L. sticticalis* can be clarified. First, low survival rate of eggs and larvae at 30°C is a major reason this species is seldom found in regions south of the annual 8°C isotherm (Pepper 1938, Luo and Li 1993a, Afonin et al. 2014). Similarly, one may predict that outbreaks of *L. sticticalis* larvae are unlikely in areas where the prevailing climatological conditions during the breeding season do not favor survival of eggs and early instars. Second, outbreaks of larval populations are observed to always occur in areas averaging 22°C, and where relative humidity is ≥60% during the period (usually a week) of peak adult abundance (Luo et al. 1998, 2009; Huang et al. 2011; Chen et al. 2006). Thus, outbreaks are not only the result of adults immigrating to oviposit, or the maximum expression of reproductive potential or lifetime fecundity (Meng et al. 1987, Wei et al. 1987, Luo and Li 1993b, Cheng et al., 2015), but also of maximal survival rate of eggs and larvae under optimal climatological conditions (Figs. 1–2 and 6). Finally, decreased survival rates of eggs and early instars at low or saturated relative humidity levels may modulate the observed choice of host plant species for oviposition as well as positioning of eggs on the plant by the female. This is evidenced by field data showing that eggs are laid on the leaf nearest the soil surface on the dicot weed, *C. album*, when the local climate is dry (<60% RH), whereas eggs are laid at the top of the monocot weed, *E. ciliaris*, when the climate is humid (81–99% RH; Kang et al. 2007, Chen et al. 2010). In both cases, survival of eggs and early instars would benefit, because the ambient relative humidity can be adjusted to decrease (first case) water loss from the eggs and neonates, or to protect them (second case) from the detrimental effects of condensed

water common in a humid environment (i.e., >90% RH). Thus, it seems likely that survival of eggs and early instars, as modulated by prevailing climatological conditions especially temperature and relative humidity, is a pivotal driver in the incidence of facultative migratory flight, expression of reproductive potential, and oviposition site selection behavior by *L. sticticalis*.

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