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# Population growth and development of the psocid *Liposcelis obscura* (Psocodea: Liposcelididae) at constant temperatures and relative humidities

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## ABSTRACT

The effects of nine temperatures (22.5, 25.0, 27.5, 30.0, 32.5, 35.0, 37.5, 40.0, and 42.5 °C) and four relative humidities (RHs; 43, 55, 63, and 75%) on the population growth and development of the parthenogenetic *Liposcelis obscura* Broadhead (Psocodea: Liposcelididae) were investigated in laboratory studies. Results showed that *L. obscura* did not survive at 43% RH at all temperatures tested. At 55% RH, *L. obscura* survived at 22.5, 25, and 27.5 °C; none survived at 42.5 °C and ≤63% RH. Population growth was highest at 40.0 °C and 75% RH, where population increase was 216-fold from an initial population of five adult females. *Liposcelis obscura* has three-to-five nymphal instars, and the percentages of individuals with three, four, and five instars were 52, 41, and 7%, respectively. Temperature-dependent developmental equations were developed for *L. obscura* eggs, individual nymphal, combined nymphal, and combined immature stages. *Liposcelis obscura* populations grew much faster at 30–42.5 °C and 75% RH. Based on the equation for total developmental time, the predicted optimal development temperature is 39.2 °C and development was completed in 14.0 d. The upper developmental threshold was estimated as 47.1 °C. The lower developmental threshold was estimated as 14.7 °C. These data provide a better understanding of *L. obscura* population dynamics and can be used to develop effective management strategies for this psocid.

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## 1. Introduction

Psocids of the genus *Liposcelis* (Psocodea: Liposcelididae) have emerged as important pests of stored products worldwide over the last two-to-three decades (Phillips and Throne, 2010; Nayak et al., 2014). Psocids are mostly found in stores containing durable food commodities, such as grains, rice, pulses, and nuts, food processing facilities, and they thrive on a variety of food products (Opit and Throne, 2008a; Athanassiou et al., 2010). Psocid infestations do not only affect grain weight loss but also cause significant germination failure by feeding on the germ and endosperm (Kučerová, 2002; Gautam et al., 2013). Psocids have a short generation time at elevated temperatures which allows them to rapidly colonize new habitats (Nayak et al., 2014). The economic importance of psocids in a commodity is not just limited to direct feeding and

contamination but also the fact that their presence can lead to rejection of infested commodities from domestic and international markets (Nayak, 2006). Psocids are difficult to control using standard practices of protection and disinfestation that are used to manage other stored product insects, including various insecticides (Wang et al., 1999; Beckett and Morton, 2003; Athanassiou et al., 2009; Huang et al., 2009).

In the US, *Liposcelis* and *Lepinotus* are two genera of psocids that are found in large numbers in grain storages and are of economic importance (Gautam et al., 2010; Opit et al., 2011). Four *Liposcelis* species of notable economic importance worldwide are *L. bostrychophila* Badonnel, *L. entomophila* (Enderlein), *L. decolor* (Pearman), and *L. paeta* Pearman, but examples of the other species with limited significance in the US include *L. corrodens* Heymons, *L. brunnea* Motschulsky, *L. fusciceps* Badonnel, *L. obscura* Broadhead, and *L. rufa* Broadhead (Gautam et al., 2010, 2016; Lienhard and Smithers, 2002; Opit et al., 2018).

The number of studies that have been conducted on the biology of psocids has been steadily increasing over the last few decades

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(Knülle and Spadafora, 1969; Khalafalla, 1990; Opit and Throne, 2008b, 2009; Gautam et al., 2010, 2016; Aminatou et al., 2011). These studies investigated the effects of physical conditions such as temperature and relative humidity (RH) on the development and population growth of psocids; they showed that psocids survive at RHs  $\geq 55\%$  and thrive at optimum temperatures of 30–37.5 °C. Development of an effective management program for any pest depends on sound knowledge of its biology and ecology. Increased knowledge of a pest's biology and ecology helps to develop effective integrated pest management (IPM) programs to mitigate its economic impact.

*L. obscura* is an obligate parthenogen (Mockford, 1993). Outside the US, the only ecological study conducted on *L. obscura* published in the scientific literature investigated its biology, i.e., the effects of temperature and food on the reproductive parameters of *L. obscura* (Khalafalla, 1990). The present two studies were initiated to investigate the population growth and development of *L. obscura* in order to provide data for the development of management strategies for this pest. The hypothesis tested was that 30 or 32.5 °C and 70% RH are optimal for survival, multiplication, and development of *L. obscura*. Objectives of these studies were to determine the effects of constant temperatures and RHs on the population growth of *L. obscura* and to quantify the effects of temperature on the development of *L. obscura*.

## 2. Materials and methods

### 2.1. Insects

Cultures of *L. obscura* used in this study were started using insects collected from a peanut (*Arachis hypogaea*) warehouse in Oklahoma. Voucher specimens of 100 *L. obscura* preserved in 95% ethyl alcohol that were used in this study were deposited at the K.C. Emerson Entomology Museum at Oklahoma State University under lot numbers 119 (females). Psocids were reared on a mixture of 93% cracked wheat (*Triticum aestivum* L.; Duster variety), 5% Rice Krispies (Kellogg North America Company, Battle Creek, MI), and 2% wheat germ (The Quaker Oats Company, Chicago, IL) (wt/wt; referred to as psocid diet hereafter) in 360-ml glass canning jars with mite-proof lids (Opit and Throne, 2008b). The top one-third of the inner surface of each jar was coated with Fluon (polytetrafluoroethylene; Northern Products, Woonsocket, RI) to prevent psocids from accessing and gathering on the inside of the lid. Cultures were placed inside a growth chamber maintained at  $30.0 \pm 1$  °C in plastic boxes (42 × 29 × 24 cm high) painted black, which had saturated NaCl solution beneath perforated false floors to maintain a RH of  $75 \pm 5\%$  RH. The boxes were painted black to mimic dark conditions in which psocids are typically found.

### 2.2. Effects of temperature and relative humidity on population growth

#### 2.2.1. Experimental arenas and diet equilibration

Effects of nine temperatures (22.5, 25.0, 27.5, 30.0, 32.5, 35.0, 37.5, 40.0, and 42.5 °C) and four RHs (43, 55, 63, and 75%) on the population growth of *L. obscura* over a 30-d period were determined. The inner sides of 108 Petri dishes (90 × 25-mm high) were coated with Fluon to prevent psocids from escaping. Five g of red colored diet, 1 g of cracked wheat (Duster variety), and 0.5 g wheat germ (hereafter referred to as diet) were placed in each Petri dish. The mixture of red colored diet, cracked wheat, and wheat germ was used because *L. obscura* survived much better on this diet than on a mixture of 93% cracked wheat, 5% Rice Krispies, and 2% wheat germ. The plastic Petri dish lids were replaced, that is, Petri dishes closed. Red colored diet was made by mixing 100 g of Rice Krispies

with a solution of 5 ml of red food dye (Global Chem Sources Inc., Cedar Grove, NJ) in 300 ml of water, drying the mixture in a mechanical convection oven (model HTM 85, Precision Scientific, Inc., Chicago, IL) for 6 h, and then grinding the dried mixture in a Wiley Mill. A U.S. Standard #20 sieve (0.85-mm openings) (Scientific Apparatus, Philadelphia, PA) was used to sieve the colored diet. Twenty-seven Petri dishes with diet were randomly placed in each of the four plastic boxes (42 × 29 × 24-cm high) containing saturated solutions of K<sub>2</sub>CO<sub>3</sub> (43%), NaBr (55%), NaNO<sub>2</sub> (63%), or NaCl (75%) (Greenspan, 1977) beneath perforated false floors to maintain the required RH. Petri dishes were kept at the four RHs to equilibrate the diet in them at room temperature for 4 wk.

#### 2.2.2. Obtaining 1- to 2-wk-old psocids

To obtain 1- to 2-wk-old *L. obscura* adult females required for the experiment, 300 female late-instar nymphs of *L. obscura* were picked from culture jars and placed in each of six 9-cm Petri dishes with Fluon-coated sides. Each Petri dish contained 5 g of colored psocid diet, 1 g of cracked wheat, and 0.5 g of wheat germ. The Petri dishes were placed on perforated false floors of one black Rubbermaid plastic box (32 × 18 × 13 cm). Late instar nymphs were maintained at  $75 \pm 5\%$  RH for 2 wk. After 2-wk, adult females in the six Petri dishes were 1- to 2-wk old.

#### 2.2.3. Experimental set up

After 4 wk of diet equilibration, five 1- to 2-wk-old adult *L. obscura* were placed in each of the 108 Petri dishes containing equilibrated diet. Nine incubators (Thermo Fisher Scientific; Waltham, MA) were set at temperatures of 22.5, 25.0, 27.5, 30.0, 32.5, 35.0, 37.5, 40.0, and 42.5 °C, where four plastic boxes (17 × 17 × 12-cm high) containing saturated solutions of K<sub>2</sub>CO<sub>3</sub>, NaBr, NaNO<sub>2</sub>, or NaCl were placed. Three Petri dishes containing diet, equilibrated at room temperature and each RH, were randomly assigned to the corresponding RH box in all incubators. Four locations were established in every incubator for the boxes to occupy. To counteract any temperature variability that may have existed in the incubators, every 7 d the boxes in each incubator were shuffled so that each box spent a total of at least 7 d in each location during the course of the experiment. During shuffling, the boxes were also checked to ensure that the salt solutions were still saturated. This was done by making sure that the desired amounts of solute and solution of the saturated solution were present in each box. Environmental conditions in each incubator were monitored using a temperature and RH sensor (HOBO U12, Onset Computer Corporation, Bourne, MA). Live insects in each Petri dish were counted after 30 d by spreading a portion of the contents of a vial on a 9-cm Petri dish, which had a coat of Fluon on the inner walls, and removing all mobile *L. obscura* using a moist brush under a stereomicroscope (Zeiss Stemi, 2000-C; Thornwood, NY).

#### 2.2.4. Data analysis

The experiment had three temporal replications. Experimental design was a randomized complete block (RCBD) with sub-sampling. Statistical procedures were done by using Statistical Analysis System software version 9.4 (SAS, Institute 2014). PROC MIXED was used for analysis of variance (ANOVA) to determine the effects of temperature and RH on the number of psocids in the Petri dishes. We used the least significant difference (LSD) test to determine differences among mean numbers of psocids produced at the various temperatures and RHs despite the quantitative independent variables, because we were not able to quantify the relationship using a biologically meaningful equation (TableCurve 3D) (Systat Software, Inc., 2007a). A mathematical expression that adequately describes the relationship in a biologically meaningful way (in this case, relationship between temperature and RH to

psocid population increase) is known as a biologically meaningful equation.

### 2.3. Effects of temperature on development

#### 2.3.1. Obtaining eggs

Eggs were obtained by placing 1 g of red colored diet, 5 particles of wheat germ, and 30 adult-female psocids of unknown age from our psocid cultures in each of eighty 35-mm-diameter Petri dishes (Greiner Bio-One, Kaysville, UT), which had a coat of Fluon on the sides. Procedures used to obtain the red colored diet were similar to those described above for the effects of temperature and RH on population growth of *L. obscura*. Red colored diet was used because psocids prefer to lay eggs between diet particles. Additionally, the red colored diet made it easy to see eggs and helped us to assess whether numbers of eggs were sufficient for the experiment. The Petri dishes were placed in two black Rubbermaid plastic boxes (30 × 23 × 9-cm high) that contained saturated NaCl solution (75 ± 5% RH) beneath a perforated false floor. Boxes were placed in an incubator maintained at 40 ± 1 °C. After 2 d, adult females were removed, and the diet in each Petri dish was examined for eggs by using a dissecting microscope at 25x magnification.

#### 2.3.2. Determination of incubation period

Each egg was carefully transferred into a flat cap of 1.5-ml centrifuge vial (LabSource Inc., Willowbrook, IL) with the help of a moist camel's-hair brush. A particle of cracked wheat kernel was placed in the centrifuge cap. The centrifuge cap was then placed inside a 25-mm diameter vial cap. The vial cap had three cracked wheat kernel particles (with a centrifuge cap inside it) and was placed inside a 35-mm Petri dish. The inner sides of centrifuge caps, vial caps, and 35-mm Petri dishes were coated with Fluon. One egg transferred from the colored diet was placed on the floor of each of the 270 centrifuge caps, and the 35-mm Petri dish lids replaced. Thirty centrifuge caps (associated with vial caps and Petri dishes) were randomly placed in each of nine Rubbermaid plastic boxes (37 × 22 × 13-cm high; 270 centrifuge caps total) that were painted black and contained saturated NaCl solution to maintain 75% RH. One box was placed in each of the nine incubators set to maintain treatment temperatures of 22.5, 25.0, 27.5, 30.0, 32.5, 35.0, 37.5, 40.0, and 42.5 °C. Temperatures above 42.5 °C were not tested because preliminary experiments had shown that *L. obscura* eggs did not hatch at temperatures above 42.5 °C. The experiment had three temporal replications. To estimate the incubation period of eggs, development of eggs was monitored daily with the aid of a dissecting microscope at 25x magnification until eggs hatched.

#### 2.3.3. Determination of nymphal stadia

To mark insects after egg hatch to determine when one developmental stage ended and the next began, rocket red fluorescent powder (Day-Glo Color Corp., Cleveland, OH) was used. A fine camel-hair brush altered by decreasing the number of hairs on it to one, and further shortening the length of that hair to 7 mm was used to apply the powder on the insects. The single hair of the modified camel-hair brush was gently dipped in a 35-mm Petri dish half-full of fluorescent powder in such a way as to obtain as little an amount of powder as possible, and the powder on the brush was gently rubbed against the body of each psocid under observation.

After egg hatch, either the abdomen, thorax or both of each first instar (N1) was dusted with fluorescent powder. N1 psocids were not transferred to the vial caps until they had molted into second instar (N2). At the N2 stage, psocids were developed enough to endure handling. Each N2 was then transferred from the centrifuge

cap into the larger vial cap, and the centrifuge cap together with the cracked wheat particle in it was removed. Nymphs in vial caps were examined daily, using a dissecting microscope at 25x magnification, to monitor development. The absence of fluorescent powder on the abdomen, thorax, or both on the nymphs indicated a molt had occurred. After each molt, psocids were immediately marked again. Additionally, vial caps were examined daily for exuvium to determine when a molt had occurred and whether the exuvium had been consumed or not. Any exuvium found was removed from the vial cap immediately. The psocid was not removed from the vial cap throughout the marking process. Any fluorescent powder that dropped on the vial cap floor and or on any cracked wheat particle was completely cleaned up using a moist camel-hair brush.

### 2.4. Data analysis

In the determination of the effects of temperature on the duration of development of *L. obscura*, PROC MIXED was used for analysis of variance (ANOVA). The experimental design for the analysis of the proportions of viable eggs and nymphs that developed to the adult stage was a RCBD. Regression (TableCurve 2D; Systat Software Inc, 2007b) was used to describe the relationship between temperature and development time for the egg, individual nymphal, combined nymphal, and combined immature stages. Fitting curves with nonlinear regression showing the relationship between temperature and development time for the individual developmental stages were constructed using SigmaPlot version 14.0 (Systat Software Inc, 2018). The selection of an equation used to describe the data was based on the magnitude and pattern of residuals, lack-of-fit tests, and whether the curve had a reasonable shape to describe the data. In the analysis of the proportions of viable eggs and nymphs that developed to the adult stage, the design for analysis was RCBD. To analyze the proportions of viable eggs and nymphs, PROC MIXED was used for ANOVA after arcsine square-root transformation to stabilize variances.

The lower developmental threshold for *L. obscura* was determined by fitting a linear equation to development rate (reciprocal of development time) and temperature data using TableCurve 2D (Systat Software Inc, 2007b). The upper developmental threshold for *L. obscura* combined immature stage was found by determining the temperature at which the rate of development begins to decrease (Zilahi-Balogh and Pfeiffer, 1998). The upper developmental threshold was obtained by fitting the appropriate equation to the development rate and temperature data and by using the "EVALUATION" procedure in TableCurve 2D (Systat Software Inc, 2007b) to determine the upper developmental threshold.

## 3. Results

### 3.1. Effects of temperature and relative humidity on population growth

The nine temperatures and four RHs tested affected *L. obscura* population growth (Table 1). No live *L. obscura* were found in the following conditions: at 43% RH for all temperatures; at 55% RH and 30–42.5°C; and 63% RH at 42.5 °C. Numbers of *L. obscura* at 35 and 37.5 °C and 75% RH were very similar, i.e., approximately a 143-fold increase in population in 30 d, for each temperature. Population growth was highest at 40.0 °C and 75% RH, where population increase was 216-fold (Table 1). At 42.5 °C and 75% RH, *L. obscura* populations was not as high as at 40.0 °C but nonetheless increased 56-fold.

**Table 1**  
Number ( $\pm$ SE) of mobile *Liposcelis obscura* present in Petri dishes after 30 d (n = 9).

Temp (°C)	Relative humidity (%)			
	43	55	63	75
22.5	0.0 $\pm$ 0.0 a	5.89 $\pm$ 0.70 a	9.33 $\pm$ 1.44 a	6.78 $\pm$ 0.76 a
25.0	0.0 $\pm$ 0.0 a	13.67 $\pm$ 2.73 a	31.89 $\pm$ 3.65 ab	25.0 $\pm$ 3.09 a
27.5	0.0 $\pm$ 0.0 a	16.56 $\pm$ 4.9 a	47.67 $\pm$ 9.02 abc	51.67 $\pm$ 7.0 abc
30.0	0.0 $\pm$ 0.0 a	0.0 $\pm$ 0.0 a	112.0 $\pm$ 16.11 bcd	81.44 $\pm$ 9.3 abc
32.5	0.0 $\pm$ 0.0 a	0.0 $\pm$ 0.0 a	180.11 $\pm$ 22.95 de	293.44 $\pm$ 40.51 f
35.0	0.0 $\pm$ 0.0 a	0.0 $\pm$ 0.0 a	430.67 $\pm$ 80.0 g	714.56 $\pm$ 95.1 h
37.5	0.0 $\pm$ 0.0 a	0.0 $\pm$ 0.0 a	223.67 $\pm$ 41.55 ef	714.67 $\pm$ 65.85 h
40.0	0.0 $\pm$ 0.0 a	0.0 $\pm$ 0.0 a	122 $\pm$ 41.57 cd	1077.89 $\pm$ 79.36 i
42.5	0.0 $\pm$ 0.0 a	0.0 $\pm$ 0.0 a	0.0 $\pm$ 0.0 a	281.33 $\pm$ 50.54 f

ANOVA results for temperature and relative humidity interaction were  $F = 34.5$ ;  $df = 24, 70$ ;  $P < 0.0001$ . Means within a column or row followed by the same letter(s) are not significantly different ( $P > 0.05$ ).

### 3.2. Effects of temperature on *L. obscura* development

#### 3.2.1. Eggs

Incubation varied with temperature and the relationship between temperature and incubation time was well described by a quadratic equation (Fig. 1A; Tables 2 and 3). The optimal incubation temperature is 41.8 °C, and development is completed in 4.5 d.

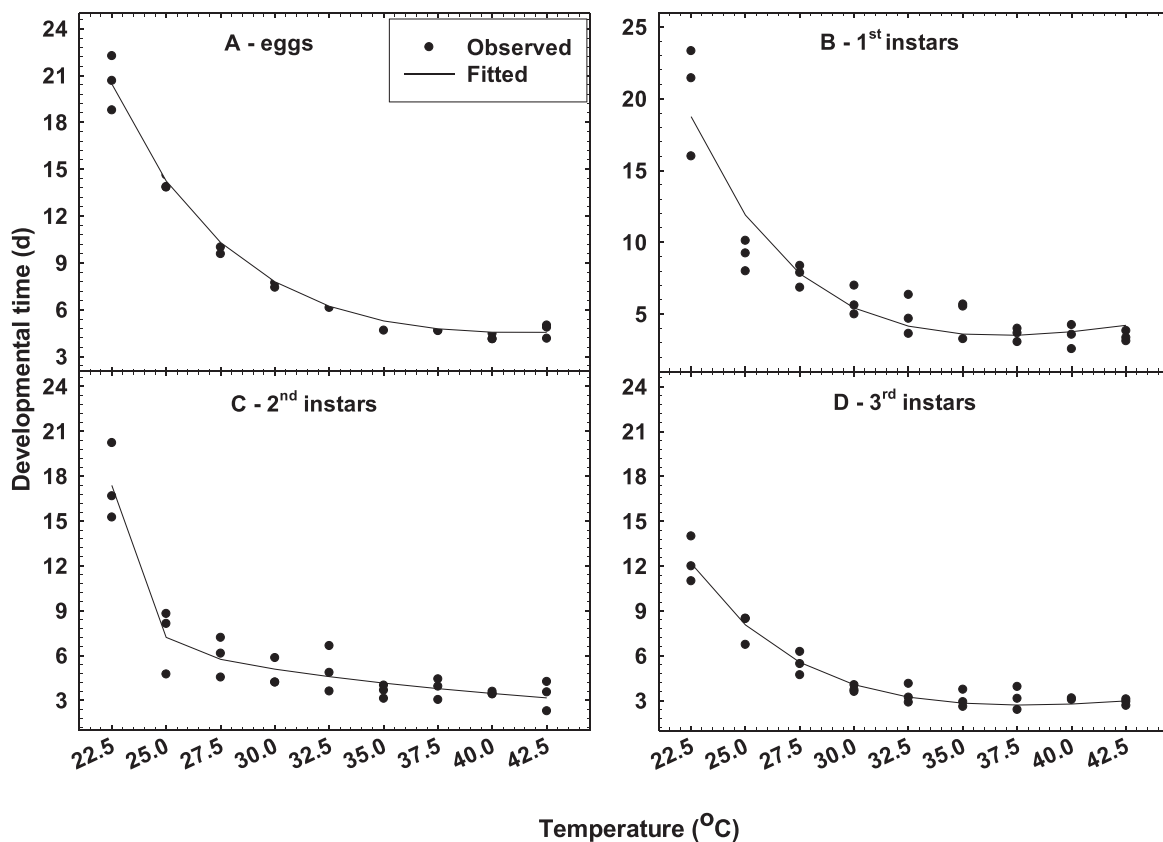
#### 3.2.2. Nymphal and combined nymphal stages

Duration of the nymphal and combined nymphal stages varied with temperature (Fig. 1B–D and 2A and B; Tables 2 and 3).

Quadratic equations described the relationship between temperature and development time well for individual nymphal and combined nymphal stages (Table 3). Temperature had a significant effect on development time for N1 (first instar), N2 (second instar), N3 (third instar) (Fig. 1B–D), and N4 (fourth instar) (Fig. 2A); where development time decreased with increasing temperature. Based on analysis of data for all nymphs that developed to adults, combined nymphal development time averaged 42.6 d at 22.5 °C and declined to 10.0 d at 37.5 and 40.0 °C. However, developmental time increased slightly at 42.5 °C and development is completed in 10.7 d (Table 2). Based on the quadratic equation for the combined nymphal stages, the predicted optimal developmental temperature is 39.0 °C and development is completed in 9.5 d.

### 3.3. Effects of temperature on egg viability and nymphal survivorship

Analysis of data for all individuals that developed to adults showed that temperature had a significant effect on total developmental time from egg to adult (Table 2), and a quadratic equation fit the data well (Fig. 2C; Table 3). Total developmental time from egg to adults averaged 58.8 d at 22.5 °C and declined to 14.3 d at 40.0 °C. However, developmental time increased slightly at 42.5 °C and development is completed in 15.3 d (Table 2). Based on the quadratic equation for total developmental time, the predicted optimal development temperature is 39.2 °C and development was completed in 14.0 d. The upper developmental threshold was estimated as 47.1 °C. The lower developmental threshold was



**Fig. 1.** Development of *L. obscura* at constant temperatures and 75% RH: (A) eggs, (B) first, (C) second, and (D) third instars. The quadratic equation  $y = a + b/x + c/x^2$  described the effects of constant temperatures on the development rate of the eggs, first instars, and third instars. In the case of second instars, the quadratic equation  $y = a + b/x + ce^{-x}$  described the effects of constant temperatures on the development rate.

**Table 2**  
Duration (d ± SE) of immature stages of female *L. obscura* at nine constant temperatures and 75% RH.

Temp (°C)	n	Duration (d)						
		Eggs	N1	N2	N3	N4 <sup>a</sup>	Nymphs	Eggs + nymphs
22.5	16	20.0 ± 0.63	20.4 ± 1.40	18.3 ± 1.32	12.8 ± 1.14	8.5 ± 1.66	42.6 ± 5.57	58.8 ± 7.51
25.0	40	14.0 ± 0.22	9.5 ± 0.52	7.85 ± 0.58	8.1 ± 0.43	5.4 ± 0.84	27.4 ± 0.86	41.3 ± 0.87
27.5	49	9.9 ± 0.16	7.9 ± 0.34	6.3 ± 0.44	5.6 ± 0.32	2.9 ± 0.53	18.7 ± 0.57	27.7 ± 0.77
30.0	63	7.7 ± 0.09	5.9 ± 0.29	4.9 ± 0.23	3.8 ± 0.27	3.1 ± 0.49	15.6 ± 0.55	23.3 ± 0.55
32.5	61	6.4 ± 0.08	4.7 ± 0.21	5.1 ± 0.42	3.4 ± 0.24	3.1 ± 0.46	13.7 ± 0.73	19.7 ± 0.80
35.0	58	5.6 ± 0.10	4.9 ± 0.24	3.6 ± 0.23	3.2 ± 0.26	3.2 ± 0.68	12.1 ± 0.71	17.7 ± 0.77
37.5	60	4.7 ± 0.07	3.5 ± 0.19	3.8 ± 0.26	3.3 ± 0.24	2.0 ± 0.59	10.0 ± 0.45	14.7 ± 0.47
40.0	57	4.2 ± 0.06	3.6 ± 0.18	3.5 ± 0.18	2.8 ± 0.21	1.8 ± 0.34	10.0 ± 0.45	14.3 ± 0.43
42.5	43	4.6 ± 0.10	3.6 ± 0.15	3.6 ± 0.21	3.0 ± 0.18	3.0 ± 0.52	10.7 ± 0.51	15.3 ± 0.49

ANOVA for: eggs, N1, N2, N3, N4, combined nymphs, and combined immature stages were  $F = 254.4, P < 0.0001$ ;  $F = 46.04, P < 0.0001$ ;  $F = 26.84, P < 0.0001$ ;  $F = 39.9, P < 0.0001$ ;  $F = 5.3, P = 0.0145$ ;  $F = 2.2, P = 0.0833$ ; and  $F = 2.35, P = 0.0690$ , respectively. In all cases,  $df = 8, 16$  except for N4 where  $df = 8, 8$ .

<sup>a</sup> Values of  $n$  for N4 at 22.5, 25, 27.5, 30, 32.5, 35, 37.5, 40, and 42.5 were 4, 13, 16, 24, 24, 19, 8, 20, and 11, respectively.

**Table 3**

Parameters ( $\pm$ SE) for quadratic equations describing the duration of the egg, individual nymphal, combined nymphal, and combined immature stages of *Liposcelis obscura* at constant temperatures. The quadratic equation  $y = a + b/x + c/x^2$  adequately described the effects of constant temperatures on the development rate of the eggs, first instars, third instars, and fourth instars. In the case of second instars, equation was  $y = a + b/x + ce^{-x}$ , and for combined nymphal stage and the combined immature stage, it was the equation  $y = a + bx + cx^2$ .

Subject	Max. R <sup>2</sup>	Adjusted R <sup>2</sup>	F	A	b	c
Egg duration	0.99	0.98	843.4	27.16 ± 3.4	-1873.10 ± 212.3	38,752.93 ± 3139.9
N1 duration	0.94	0.87	93.4	41.73 ± 9.9	-2805.07 ± 603.4	51,483.17 ± 8923.1
N2 duration	0.93	0.92	148.2	-1.42 ± 1.6	195.2 ± 50.5	5.99e <sup>+10</sup> ± 6.4e <sup>+9</sup>
N3 duration	0.95	0.93	187.4	23.55 ± 4.4	-1570.26 ± 266.7	29,576.87 ± 3943.9
N4 duration	0.94	0.80	39.0	17.17 ± 5.0	-1075.57 ± 312.6	19,555.43 ± 4719.6
Nymphal duration	0.48	0.41	11.0	148.28 ± 50.1	-7.13 ± 3.2	0.09 ± 0.1
Egg + nymphal duration	0.50	0.43	11.7	205.81 ± 68.4	-9.78 ± 4.3	0.12 ± 0.1

N1, N2, N3, and N4 represent the first, second, third, and fourth instar, respectively.

In all cases,  $df = 2, 26$  and  $P = 0.0001$  except for N4, combined nymphal, and combined immature stages where  $P = 0.0126, 0.0004, \text{ and } 0.0003$ , respectively.

Lack-of-fit  $P$  values for the duration of the egg, N1, N2, N3, N4, combined nymphal, and combined immature stages were 0.956, 0.052, 0.979, 0.876, 0.075, 0.999, and 0.999, respectively.

estimated as 14.7 °C using a linear equation that best described the development rate and temperature relationship. Based on this study, *L. obscura* has three to five nymphal instars, and the percentages of individuals with three, four, and five instars were 52, 41, and 7%, respectively.

#### 3.4. Effects of temperature on egg viability and nymphal survivorship

Temperature affected egg viability ( $F = 3.8$ ;  $df = 7, 7$ ;  $P = 0.049$ ), which ranged from 83% to 100% and averaged 91.5% for all temperatures. Temperature had no effect on nymphal survivorship ( $F = 1.0$ ;  $df = 1, 1$ ;  $P = 0.50$ ). Proportions of nymphs surviving to adults at the nine different temperatures ranged from 65 to 73%.

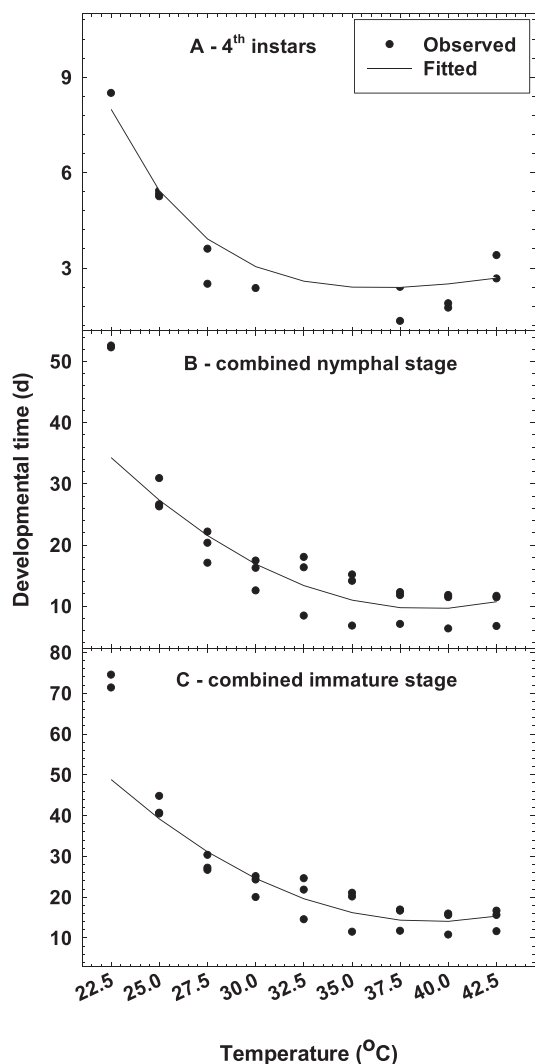
#### 4. Discussion

Results from this study show that *L. obscura* did not survive at 43% RH at any of the temperatures tested, at 55% RH and 30.0–42.5 °C, and at 63% RH and 42.5 °C. The optimal temperature and RH conditions for population growth of *L. obscura* are 40.0 °C and 75% RH. *Liposcelis obscura* survived and barely multiplied at 55% RH and 22.5–27.5 °C over 30 d indicating it will not thrive at this low RH. At 63% RH, a low temperature of 22.5 °C results in virtually no increase in population. All psocids get killed at 42.5 °C and RH of 63% and RHs of 43 and 55% had a detrimental effect on the survival and population growth of *L. obscura*. At 75% RH, a low temperature of 22.5 °C results in the *L. obscura* population barely increasing.

Based on observed data, the optimal temperature for incubation of *L. obscura* eggs was 40.0 °C and incubation time averaged 4.2 d. In the case of development from egg to adult, based on observed data,

the optimal temperature was 40.0 °C and total developmental time averaged 14.3 d. Temperature-dependent equations for the development of *L. obscura* eggs, individual nymphal, combined nymphal, and combined immature stages developed in this study describe the relationship between temperature and development time for these life stages. The predicted optimal temperatures for eggs, combined nymphal, and combined immature development were 41.8, 39.0, and 39.2 °C, respectively. At these temperatures, development is completed in 4.5, 9.5, and 14.0 d, respectively. Based on the developed equations, predicted time for development of *L. obscura* decreased with increasing temperature. The temperatures observed (40.0 °C) and predicted (41.8 °C) as optimal for incubation of *L. obscura* eggs, and the observed (4.2 d) and predicted (4.5 d) times for incubation were numerically almost similar. In the case of development from egg to adult, the temperatures observed (40.0 °C) and predicted (39.2 °C) as optimal for development, and the observed (14.3 d) and predicted (14.0 d) times for development were numerically nearly similar. We found the lower and upper developmental thresholds for *L. obscura* combined immature stages were 14.7 and 47.1 °C, respectively.

Having a temperature of 40.0 °C for optimal development from egg to adult is remarkable for *L. obscura* which is a small insect of ~1-mm length. The optimal temperature for development of 40.0 °C for *L. obscura* is the highest reported to date for any psocid of genus *Liposcelis*. The optimal temperature for development of female *Liposcelis badia* Wang, Wang, and Lienhard, *L. bostrychophila*, *L. reticulatus*, *L. pearmani*, and *Liposcelis tricolor* Badonnel was 32.5 °C and times to complete development were between 17 and 31 d (Wang et al., 2000; Dong et al., 2007; Jiang et al., 2008; Opit and Throne, 2008b; Aminatou et al., 2011). For *L. brunnea*, *L. entomophila*, *L. decolor*, *Liposcelis yunnaniensis* Li & Li, and *L. fusciceps*, the optimal temperature for development was



**Fig. 2.** Development of *L. obscura* at constant temperatures and 75% RH: (A) fourth instars, (B) combined nymphal, and (C) combined immature stages. The quadratic equation  $y = a + b/x + c/x^2$  described the effects of constant temperatures on the development rate of the fourth instars. In the case of the combined nymphal stage and the combined immature stage, the quadratic equation  $y = a + bx + cx^2$  described the effects of constant temperatures on the development rate.

35.0 °C, and times to complete development were between 16 and 28 d (Wang et al., 1998; Tang et al., 2008; Opit and Throne, 2009; Hassan et al., 2011; Gautam et al., 2016). The optimal temperature for development of *L. paeta* and *L. rufa* was 37.5 °C and times to complete development were 11.5 and 21.6 d, respectively (Wang et al., 2009; Gautam et al., 2010). At the optimal temperature of 40.0 °C, development of *L. obscura* from egg to adult takes a slightly shorter time of 14.3 d compared with other psocids that have been studied, with the exception of *L. paeta* (11.5 d).

*Liposcelis decolor*, *L. brunnea*, *L. entomophila*, *L. paeta*, *L. fusciceps*, *L. yunnaniensis*, and *L. rufa* have relatively high optimal temperatures for development of 35.0 and 37.5 °C (Wang et al., 1998, 2009; Tang et al., 2008; Opit and Throne, 2009; Gautam et al., 2010, 2016; Hassan et al., 2011), but based on this study, *L. obscura* has even a much higher optimal temperature for development (40.0 °C).

Guedes et al. (2008) found *L. entomophila* to have greater tolerance to heat shock stress than *L. reticulatus*. Although a heat-shock protein of 70 kDa (HSP 70) was not identified in

*L. entomophila*, small heat-inducible proteins (23; mainly 27 kDa) having a common epitope with HSP 70 were found. The higher optimal temperatures for development for *L. decolor*, *L. brunnea*, *L. entomophila*, *L. paeta*, *L. fusciceps*, *L. yunnaniensis*, and *L. rufa* may be due to the presence of small heat-inducible proteins which enable heat tolerance. This could explain why *L. decolor*, *L. entomophila*, *L. paeta*, and *L. rufa* are commonly found in large numbers in grain storage structures during the summer and fall months of the year when temperatures are high, whereas *L. reticulatus* is rarely found (Opit et al., 2011). By inference, *L. obscura* with the highest optimal temperature (40.0 °C) for development among psocids of genus *Liposcelis* so far studied would be expected to have higher levels of heat-shock proteins or small heat-inducible proteins which may enable heat tolerance.

The upper developmental threshold of *L. obscura* was 47.1 °C and is higher than that of *L. bostrychophila*, *L. rufa*, *L. pearmani*, *L. yunnaniensis*, *L. paeta*, *L. tricolor*, and *L. decolor*, whose upper developmental thresholds were between 33 and 42 °C, respectively (Wang et al., 2000, 2009; Dong et al., 2007; Tang et al., 2008; Gautam et al., 2010; Aminatou et al., 2011; Hassan et al., 2011). The lower developmental threshold of *L. obscura* was lower than that of *L. bostrychophila* (15.5 °C) and *L. paeta* (20.2 °C). However, the lower developmental threshold of *L. obscura* was higher than that of *L. rufa* (8.5 °C), *L. badia* (10.0 °C), *L. tricolor* (11.3 °C), *L. fusciceps* (11.9 °C), *L. decolor* (13.0 °C), *L. yunnaniensis* (13.02 °C) and *L. pearmani* (13.9 °C) (Wang et al., 2000, 2009; Dong et al., 2007; Jiang et al., 2008; Tang et al., 2008; Gautam et al., 2010, 2016; Hassan et al., 2011; Aminatou et al., 2011). The relatively high lower and upper developmental thresholds for *L. obscura* may indicate this species thrives at higher temperatures compared to the other psocid species.

Temperature, relative humidity (RH), and commodity moisture content (MC) have a profound effect on the proliferation of stored product pest populations (Rees, 2004). The highest population growth for *L. obscura* occurred at 40.0 °C and 75% RH. A RH of 75% has also been found to be optimal for the population growth of *L. reticulatus*, *L. rufa*, *L. pearmani*, and *L. fusciceps* but 63% RH was optimal for *L. brunnea* (Opit and Throne, 2008b, 2009; Gautam et al., 2010, 2016; Aminatou et al., 2011). Optimal RH for *L. brunnea* (63%) explains why it mainly occurs in the relatively drier parts of US compared with other species (Mockford, 1993; Gautam et al., 2010). However, *L. brunnea* distribution may be limited by high temperatures of 35.0 °C or higher (Opit and Throne, 2009). The optimum conditions for *L. obscura* of 40.0 °C and 75% RH imply that it is expected to have a broader distribution than most psocids of genus *Liposcelis*, and be more abundant in hot and humid areas. What is notable is that *L. obscura* is such a small insect and yet it is capable of surviving and proliferating at 42.5 °C and 75% RH, with a 56-fold population increase in 30 d.

Rees and Walker (1990) observed that *L. bostrychophila*, *L. entomophila*, and *L. paeta* did not survive at low RHs (<60%). Knulle and Spadafora (1969) stated that below the equilibrium RHs of psocids, death occurs. According to Devine (1982), high atmospheric water vapor of  $\geq 60\%$  RH is necessary for psocids to maintain body water levels by absorption. However, below this level, more moisture is lost than gained, which results in dehydration and death. At 30.0 °C and 55% RH, *L. obscura* did not survive, but *L. brunnea*, *L. rufa*, and *L. fusciceps* populations grew, although growth was slow (Opit and Throne, 2009; Gautam et al., 2010, 2016). *Liposcelis brunnea*, *L. rufa*, and *L. fusciceps* are probably well adapted in a manner that enables them to absorb atmospheric water vapor even when RH is as low as 55%. *Liposcelis obscura*, is similar to *L. reticulatus*, *L. brunnea*, *L. rufa*, *L. pearmani*, and *L. fusciceps* in that it does not survive at 43% RH (Opit and Throne, 2008b, 2009; Gautam et al., 2010, 2016; Aminatou et al., 2011).

Temperature affected *L. obscura* egg viability, and the percentage of viable eggs averaged 91.5% across all temperatures. Temperature also affected egg viability of species such as *L. bostrychophila*, *L. tricolor*, *L. badia*, *L. decolor*, *L. paeta*, and *L. yunnaniensis*. These species were geographical strains from China (Wang et al., 2000, 2009; Dong et al., 2007; Jiang et al., 2008; Tang et al., 2008; Hassan et al., 2011). Temperature did not affect the egg viability of *L. reticulatus* (87%), *L. brunnea* (80%), *L. rufa* (90%), *L. pearmani* (86%), and *L. fusciceps* (91%). These species were geographical strains from the US (Opit and Throne, 2008b, 2009; Gautam et al., 2010, 2016; Aminatou et al., 2011).

*Liposcelis obscura* has three to five nymphal instars. The percentages of individuals with three, four, and five instars were 52, 41, and 7%, respectively. *L. brunnea* females were also found to have three to five nymphal instars, with a higher percentage having four nymphal instars (78%) compared with *L. obscura*, which has a higher percentage of insects with three nymphal instars. However, Khalafalla (1990), reports that the *L. obscura* strains found in Egypt have exactly four instars. Opit and Throne (2008b) report that *L. reticulatus* (a parthenogenetic species) also has four nymphal instars. Males and females of bisexual *Liposcelis* species are found to have two-to-four or two-to-five nymphal instars, respectively (Gautam et al., 2010, 2016; Aminatou et al., 2011). Due to the additional number of instars female psocids have, the developmental period of females is longer than that of males. According to Mockford (1993), psocids usually have four to six nymphal stages.

Results of this study imply *L. obscura* should be more abundant and a major pest in hot and humid areas of the world. To our knowledge *L. obscura* has only been reported twice. It was found infesting a peanut warehouse in Oklahoma, USA, and in stored rice in Egypt. Possible reasons for why *L. obscura* has not been frequently reported world-wide may be due to lack of research or misidentification of this species.

This study demonstrates how temperature and RH affect *L. obscura* population growth and development. *L. obscura* is not expected to be a serious pest where temperatures are 27.5 °C or less. Given that *L. obscura* had a relatively higher population growth over a 30-d period compared with other *Liposcelis* species at higher temperatures of 35–42.5 °C and 75% RH, we expect it to be (or become) a predominant pest in hot, humid tropical, and subtropical geographic areas. Finally, the temperature dependent equations developed for this species could be used to understand *L. obscura* population dynamics and to develop effective management strategies. Further research investigating heat-shock proteins of *L. obscura* will be beneficial.

#### Declaration of competing interest

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