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Population growth and development of *Liposcelis obscurus* Broadhead (Psocodea: Liposcelididae) at constant temperatures and relative humidities

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Abstract

The effects of nine temperatures (22.5, 25, 27.5, 30, 32.5, 35, 37.5, 40, and 42.5°C) and four RHs (43, 55, 63, and 75%) on the population growth and development of the parthenogenetic *Liposcelis obscurus* Broadhead (Psocodea: Liposcelididae) were investigated in laboratory studies. Results showed that *L. obscurus* did not survive at 43% RH at all temperatures tested. At 55% RH, *L. obscurus* survived at 22.5, 25, and 27.5°C; none survived at 42.5°C and ≤63% RH. *Liposcelis obscurus* survived and the population increased 56–fold from an initial population of five adult females at 42.5°C and ≤75% RH. Population growth was highest at 40°C and 75% RH, where population increase was 215-fold. *Liposcelis obscurus* has three-to-five nymphal instars, and the percentages of third, fourth, and fifth instars were 52, 41, and 7%, respectively. Temperature-dependent developmental equations were developed for *L. obscurus* eggs, individual nymphal, combined nymphal, and combined immature stages. *Liposcelis obscurus* populations grew much faster at 30–42.5°C and 75% RH. These

data provide a better understanding of *L. obscurus* population dynamics, and can be used to develop effective management strategies for this psocid.

Keywords: psocid, stored-product, population growth, development rate, booklouse

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 (Nayak et al., 2014). Psocids are mostly found in grain food stores, 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 cause grain weight loss but also result in significant germination failure by feeding on the germ and endosperm of seeds (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 they can also 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 (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, 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 other species that are economically important include *L. corrodens* Heymons, *L. brunnea* Motschulsky, *L. obscurus* Broadhead, and *L. rufa* Broadhead (Lienhard and Smithers, 2002; Gautam et al., 2010).

The psocid species *L. obscurus* Broadhead has been found infesting storage structures in the US. *Liposcelis obscurus* is an obligate parthenogen (Mockford, 1993). The only ecological study conducted on *L. obscurus* published in scientific literature investigated the effects of temperature and food on the reproductive parameters of this species (Khalafalla, 1990). In the present study, objectives were to determine the effects of constant temperatures and relative humidities on the population growth of *L. obscurus* and to quantify the effects of temperature on the development of this species.

2. Materials and Methods

2.1. Insects

Cultures of *L. obscurus* used in this study were started using insects collected from peanut (*Arachis hypogaea*) warehouses in Oklahoma (USA). Voucher specimens of 100 *L. obscurus* 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) 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 ± 1°C in plastic boxes (42 x 29 x 24 cm high) painted black, which had saturated NaCl solution beneath perforated false floors to maintain a RH of 75 ± 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

The effects of nine temperatures (22.5, 25, 27.5, 30, 32.5, 35, 37.5, 40, and 42.5°C) and four RHs (43, 55, 63, and 75%) on the population growth of *L. obscurus* over a 30-d period were determined. The

inner sides of 108 Petri dishes (100 x 25-mm high) were coated with Fluon to prevent psocids from escaping. Into each Petri dish, 5 g of red colored diet, 1 g of cracked duster wheat, and 0.5 g wheat germ (hereafter referred to as diet) were placed. The mixture of red colored diet, cracked duster wheat, and wheat germ was used as diet because *L. obscurus* did not survive well on only cracked wheat diet. The plastic Petri dish lids were replaced. 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 diet. Petri dishes with diet were randomly put in four plastic boxes (42 x 29 x 24 cm high) containing each of the saturated solutions of K_2CO_3 (43%), NaBr (55%), $NaNO_2$ (63%), and 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. Each box had 27 Petri dishes.

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

After 4 wk of diet equilibration, five 1- to 2-wk-old adult *L. obscurus* 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, 27.5, 30, 32.5, 35, 37.5, 40, and 42.5°C, where four plastic boxes (17 x 17 x 12 cm high) containing saturated solutions of K_2CO_3, NaBr, $NaNO_2$, and 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. Methods by Gautam et al. (2010) and Aminatou et al. (2011) were then followed.

The experiment had three temporal replications, and the experimental design was a randomized complete block design (RCBD) with subsampling. 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. Data on psocid numbers were transformed using the square root transformation to stabilize variances before analysis. Untransformed means and standard errors are reported for straightforward interpretation. 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., 2002a).

2.3. Effects of temperature on development

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 in Opit and Throne (2008). The Petri dishes were placed in two black Rubbermaid plastic boxes (30 x 23 x 9 cm high) that contained saturated NaCl solution (75% RH) beneath a perforated false floor. Boxes were placed in an incubator maintained at 40 ± 1°C. After 2 d, adult females were taken off, and the diet in each Petri dish was examined for eggs by using a dissecting microscope at 25x magnification. Procedures used for setting up the experiment to monitor development of eggs were analogous to those used by Opit and Throne (2018). Thirty centrifuge caps (associated with vial caps and Petri dishes) were randomly placed in each of nine Rubbermaid plastic boxes (37 x 22 x 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. obscurus* eggs do not hatch at temperatures above 42.5°C. The experiment had two temporal replications. To estimate the incubation period of eggs and to mark insects after egg hatch to determine when one developmental stage ended and the next began, procedures analogous to those used by Opit and Throne (2018) were used.

2.4. Data analysis

In the determination of the effects of temperature on the duration of development of *L. obscurus,* 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 an RCBD. Regression (TableCurve 2D; Systat Software, 2002b) 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 10.0 (Systat Software, 2006). 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 a 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. obscurus* was determined by fitting a linear equation to development rate (reciprocal of development time) and temperature data using TableCurve 2D (Systat Software Inc., 2002b). The upper developmental thresholds for *L. obscurus* developmental stages were found by determining the temperature at which the rate of development begins to decrease (Zilahi-Balogh and Pfeiffer, 1998). The upper developmental thresholds were obtained by fitting the appropriate equation to all the development rate and temperature data and by using the "EVALUATION" procedure in TableCurve 2D (Systat Software Inc., 2002b) to determine the upper developmental thresholds.

3. Results

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

The nine temperatures and four RHs tested affected *L. obscurus* population growth (Fig. 1). No live *L. obscurus* were found at 43% RH for all temperatures; at 55% RH and 30–42.5C; and 63% RH at 42.5°C. Numbers of *L. obscurus* at 35 and 37.5°C and 75% RH were very similar—approximately a 143-fold increase in population, in 30 d, for each temperature. Population growth was highest at 40.°C and 75% RH, where population increase was 215-fold (Figure 1). At 42.5°C and 75% RH, *L. obscurus* populations increased 56–fold from an initial population of five adult females.

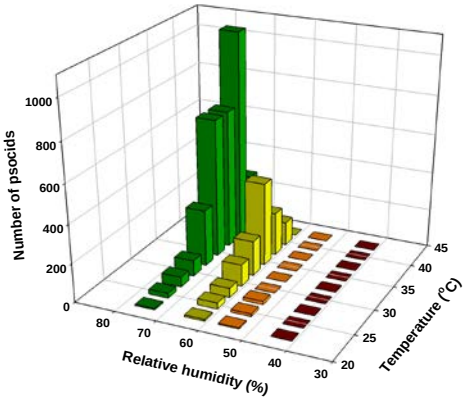

Fig. 1. Effects of temperature and relative humidity on *Liposcelis obscurus* population growth.

3.2. Effects of temperature on *L. obscurus* 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. 2A). The optimal incubation temperature is 40.0°C, and development is completed in 4.1 d.

3.2.2. Nymphal and Combined Nymphal Stages

Duration of the nymphal and combined nymphal stages varied with temperature (Fig. 2B–E). Quadratic equations described the relationship between temperature and development time well for individual nymphal and combined nymphal stages. Temperature had a significant effect on development time for N1 (first instar), N2 (second instar), and N3 (third instar) (Fig. 2B–D); where development time decreased with increasing temperature. Based on analysis of data for all nymphs that developed to adults, combined nymphal development time averaged 28.6 d at 25ºC and declined to 11.6 d at 40ºC. However, developmental time increased slightly at 42.5ºC and development is completed in 11.8 d, respectively. Based on the quadratic equation for the combined nymphal stages, the predicted optimal developmental temperature is 41.1ºC and development is completed in 11.7 d.

3.2.3. Combined Immature Stages

The 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, and a quadratic equation fit the data well (Fig. 2F). Total developmental time from egg to adults averaged 42.7 d at 25°C and declined to 15.8 d at 40°C. However, developmental time increased slightly at 42.5°C and development is completed in 16 d. The upper developmental threshold was estimated as 43.9°C. The lower developmental threshold was estimated as 13.2ºC using a linear equation that best described the development rate and temperature relationship. Based on this study, *L. obscurus* has three to five nymphal instars, and the percentages of third, fourth, and fifth instars were 52, 41, and 7%, respectively.

3.3. 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 65–73%.

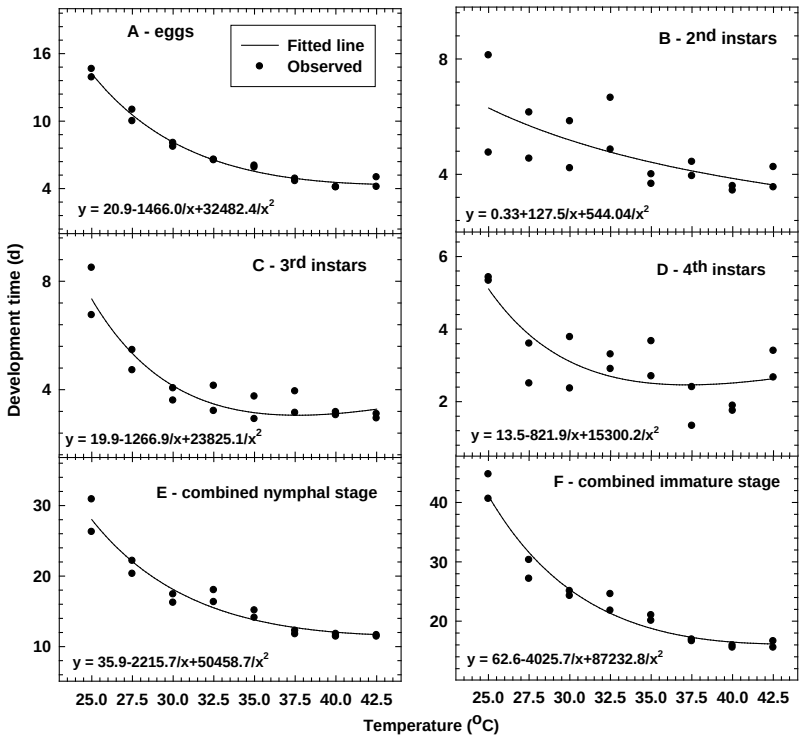

Fig. 2. Development of female *Liposcelis obscurus* at constant temperatures and 75% RH: (A) eggs, (B) second, (C) third, and (D) fourth instars, and (E) combined nymphal and (F) combined immature stages.

Discussion

Results from this study show that *L. obscurus* did not survive at 43% RH at any of the temperatures tested; at 55% RH and 30–42.5°C; and at 63% RH and 42.5°C. The optimal temperature and RH for population growth of is *L. obscurus* are 40°C and 75% RH. *Lepinotus reticulatus*, *L. brunnea*, *L. rufa*, *L. pearmani*, and *L. fusciceps* have also been reported not to survive at 43% RH (Opit and Throne, 2008b; Opit and Throne, 2009; Gautam et al., 2010; Aminatou et al., 2011; Gautam et al., 2015). Although *L. obscurus* survived and barely multiplied at 55% RH and 22.5–27.5°C over 30 d, data indicate it will not thrive at this low RH. At 63% RH, a low temperature of 22.5°C results in limited increase in population, and a higher temperature of 42.5°C kills all psocids. At 75% RH, a low temperature of 22.5°C results in limited increase in *L. obscurus* population. Rees and Walker (1990) observed that *L. bostrychophila, L. entomophila,* and *L. paeta* did not survive at low RHs (<60%). Knulle and Spadora (1969) stated that below the equilibrium RHs of psocids, death occurs. According to Devine (1982), high atmospheric water vapor of ≥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. obscurus* 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). *L. 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%.

The highest population growth for *L. obscurus* occurred at 40ºC and 75% RH. 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*. Optimum temperatures for these species were 30C° for *L. fusciceps;* 32.5°C for *L. reticulatus*, *L. pearmani, and L. brunnea;* and 35°C for *L. rufa* (Opit and Throne, 2008b; Opit and Throne, 2009; Gautam et al., 2010; Aminatou et al., 2011; Gautam et al. 2015). Optimal RH for *L. brunnea* (63%) explains why it mainly occurs in the relatively drier parts of US compared with other species (Gautam et al., 2010). However, its distribution may be limited by high temperatures of 35.0°C or higher (Opit and Throne 2009). Rees and Walker (1990) showed that the optimum conditions for *L. bostrychophila*, *L. entomophila,* and *L. paeta* are 30.0°C and 80% RH, 30°C and 70% RH, and 33°C and 70% RH, respectively. *L. rufa* barely survives at 40.0°C (Gautam et al., 2010). Therefore, higher temperatures may limit *L. rufa* distribution although it reproduces relatively well at lower RHs and temperatures (55% RH and 22.5–30.0ºC) compared to *L. obscurus*. The optimum conditions for *L. obscurus* (40.0ºC and 75% RH) imply that it is expected to have a broader distribution than *L. rufa,* and be more abundant in hot and humid areas. Based on this study, *L. obscurus* is capable of surviving and multiplying at moderately high rates at 42.5°C.

L. obscurus has three to five nymphal instars, and the percentages of third, fourth, and fifth 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. obscurus* which has a higher percentage of insects with three nymphal instars. However, Khalafalla (1990), reports that the *L. obscurus* 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 and two to five nymphal instars, respectively (Gautam et al., 2010; Aminatou et al., 2011; Gautam et al., 2015). Due to the additional number of instars female psocids have, the developmental period of females is longer than that of males. The evolution of a variable number of *Liposcelis* instars may be to prolong their survival in adverse conditions (Aminatou et al., 2011). According to Mockford (1993), psocids usually have four to six nymphal stages.

The optimal temperature for *L. obscurus* development from egg to adult was 40ºC and development was completed in 15.8 d. The optimal temperature for development of female *L. badia*, *L. bostrychophila*, *L. reticulatus*, *L. pearmani*, and *L. tricolor* was 32.5ºC and development were completed between 17 and 31 d (Wang et al., 2000; Dong et al., 2007; Jiang et al., 2008; Opit and Throne, 2008; Aminatou et al., 2011). For *L. brunnea*, *L. entomophila*, *L. decolor,* and *L. fusciceps*, the optimal temperature for development was 35.0°C and development was completed in 23.6, 21.7, 16.1, and 19.0 d, respectively; also, *L. paeta* and *L. rufa*'s development were completed in 11.5 and 21.6 d, respectively, at 37.5°C (Tang et al., 2008; Wang et al., 2008; Opit and Throne, 2009; Gautam et al., 2010; Aminatou et al., 2011; Gautam et al., 2015). At the optimal temperature of 40.0°C, development of *L. obscurus* from eggs to adult takes a slightly shorter time compared to other psocids that have been studied.

This study demonstrates how temperature and RH affect *L. obscurus* population growth and development. *L. obscurus* is not expected to be a serious pest in grain storages where temperatures are 27.5°C or less. Given that *L. obscurus* had a relatively higher population growth over a 30-d period compared to 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 and humid areas. Finally, the temperature dependent equations developed for this species could be used to understand *L. obscurus* population dynamics and to develop effective management strategies.

4. Conclusions

Based on this study, *L. obscurus* is predicted to be more abundant and a pest in hot and humid areas of the world. That being said, to the best of our knowledge *L. obscurus* 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. obscurus* has not been frequently reported may be due to lack of research or misidentification of this species.

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Circadian Rhythm of *Liposcelis entomophila* and *Liposcelis paeta* in Paddy Warehouse

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Abstract

Booklice is a small but serious stored grain pest, and understanding the circadian rhythm of booklice help to control. In this study, circadian activity of booklice were monitored with sticky traps in the grain bulk surfaces of two warehouses stored paddy rice in two different provinces in China. The results showed that the species of booklice were different and were *Liposcelis entomophila*, and *Liposcelisp paeta* for Nanning's and Zhanjiang's warehouses respectively. In term of *L.entomophila*, its activity intensity gradually decreased from 0 am to 12 pm and reached the lowest level of daily activity at 12pm. After this, there was a steady and straight upward trend, and the peak of its activity intensity is reached at 8 pm. Its circadian activity trend can be represented as: $y = -0.971x^3 + 21.88x^2 - 139.5x + 353.4$(x: time; y: quantity of booklice). Over the same period, the activity intensity of *L.paeta* varied greatly. It gradually increased, reached a peak at 8 am, dropped dramatically at 12 pm and then climbed the second peak at 6 pm.

Keyword: sticky trap, monitor, *L.entomophila*, *L.paeta*, circadian rhythm

1. Introduction

In control of stored grain pests, insect population dynamics monitoring and density inspection are important. The species, density, distribution, and damage status data of grain stored pests in the grain bulk can be timely detected, predicting the development trend of insects, avoiding unnecessary prevention cost and the economic losses, and providing scientific strategy for insect control (Bai Xuguang, 2002).