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Freezing for control of stored-product psocids

Frank H. Arthur [a, *], Kris L. Hartzer [a], James E. Throne [b], Paul W. Flinn [a, 1]

[a] USDA, Agricultural Research Service, Center for Grain and Animal Health Research, 1515 College Avenue, Manhattan, KS 66502, USA
[b] USDA, Agricultural Research Service, San Joaquin Valley Agricultural Sciences Center, 9611 South Riverbend Avenue, Parlier, CA, USA

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ABSTRACT

A series of studies was conducted by exposing young and old eggs, nymphs, and adults of the psocids *Liposcelis bostrychophila* (Badonnel), *L. paeta* (Pearman), *L. decolor* (Pearman), and *L. entomophila* (Enderlein) to −18 °C for various time intervals. Survival was assessed as initial and final, at different times depending on the life stage. Young eggs of *L. bostrychophila* were the most tolerant life stage of any of the species, with scattered survival out to 120 h of exposure to −18 °C. Eggs were the most tolerant life stage for each species, requiring 24, 12, and 2 h of exposure for complete kill of *L. paeta*, *L. decolor*, and *L. entomophila*, respectively. Nymphs and adults of all species were far more susceptible than eggs, with no final survival after two hours of exposure. Results show the extreme variation between different psocid life stages and species to cold temperatures, and provide guidelines for using cold as a control strategy for psocids. Our results show that 24 h at −18 °C is sufficient to kill all life stages of the psocid species tested, except for young *L. bostrychophila* eggs which will require at least 128 h of exposure at −18 °C for complete mortality.

1. Introduction

Psocids are a group of insects that are considered to be emerging pests in stored products throughout much of the world, with reports of heavy infestations in a variety of stored-product environments, including processing and food storage facilities (see review by Nayak et al., 2014). Partial explanations for this sudden increase of psocids include natural tolerance to fumigants, particularly the egg stage (Nayak et al., 2003a); development of resistance to phosphine (Nayak and Collins, 2008); natural ecology including high reproductive rates and short developmental times at high temperatures (Tang et al., 2008; Wang et al., 2009); and ability to survive on a variety of food products (Opit and Throne, 2008; Athanassiou et al., 2010).

The four major species of psocids found worldwide are *Liposcelis bostrychophila* (Badonnel) (Psocoptera: Liposcelididae), *L. decolor* (Pearman), *L. entomophila* (Enderlein), and *L. paeta* (Pearman) (Nayak et al., 2014), and, in general, they are more tolerant to contact insecticides compared to stored-product beetles (Athanassiou et al., 2009). However, the susceptibility of psocids to various insecticides varies considerably depending on the individual insecticide, species, and exposure substrate. Nayak et al. (2014) summarized the results of previous research, which emphasized the challenges of controlling psocids in stored products.

As described above, there is extensive research on insecticides for control of psocids, but less research on using extreme temperatures as a disinfestation strategy, especially for packaged food products. Cold temperatures have recently been investigated for control of *Tribolium castaneum* (Herbst), the red flour beetle, and *Trogoderma inclusum* (L.), the larger cabinet beetle (Flinn et al., 2015; Arthur et al., 2015). However, there are no recent studies published in the scientific literature evaluating susceptibility of psocids to cold temperature. Therefore, the objective of this study was to determine susceptibility of psocids to −18 °C, a common industry standard for commercial freezers in the US (Johnson and Valero, 2003; Flinn et al., 2015).

2. Materials and methods

2.1. Experimental methods

This study was conducted at the USDA-ARS Center for Grain and Animal Health Research (CGAHR), Manhattan, KS, using *L. bostrychophila*, *L. decolor*, *L. entomophila*, and *L. paeta*. All four species were originally collected during 2005–2007 from wheat

* Corresponding author.
E-mail address: frank.arthur@ars.usda.gov (F.H. Arthur).
[1] Retired.

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stored in outside bins at the CGAHR or from the CGAHR grain elevator, and voucher specimens from the original colonies were deposited in the Kansas State University Museum of Entomological and Prairie Arthropod Research (#202, 205, 182, and 207, respectively). The psocids had been subsequently colonized and reared on a diet of 97% cracked wheat kernels, 2% brewer's yeast, and 1% wheat germ inside a walk-in chamber set at 30 °C, 75% r.h., and a 16:8 light: dark cycle. The freezer used in the study was a Percival upright model I36NLXC9 purchased in 2011 (Percival Scientific, Perry, IA, USA), modified as described by Arthur et al. (2015) to mitigate spiking of temperatures during the defrost cycle. The ages of the different life stages of each species used in the study varied depending on duration of the stages for each species at the rearing temperature (based on Supplemental Tables in Nayak et al., 2014). Ages of young and old eggs, respectively, were 0–4 and 5–8 days for *L. bostrychophila* and *L. paeta* and 0–3 and 4–6 days for *L. decolor* and *L. entomophila*. The ages in days of young nymphs were 0–5, 0 to 7, 0 to 5, and 0 to 8 for *L. bostrychophila*, *L. paeta, L. decolor,* and *L. entomophila*, respectively. The ages in days of old nymphs were 6–12, 8–14, 6–14, and 9–18 for *L. bostrychophila*, *L. paeta, L. decolor,* and *L. entomophila*, respectively. Young and old adults were 0–7 and 14–21 days after adult emergence for all species. Eggs were collected by placing adults on flour for 3 or 4 days, and then sieving the flour through a 70-mesh sieve to obtain eggs.

2.2. Exposure intervals

The experimental unit was 25 individuals of a particular species and life stage in a 7-dram plastic vial (50 mm in height by 25 mm diameter). Preliminary tests were done to define approximate exposure intervals at the target temperature that would prevent egg hatch or cause direct mortality of nymphs and adults. Immediate mortality of nymphs and adults of all species was assessed after 0 (untreated control), 15, 30, or 45 min, and after 1, 1.5, 2, 4, 6, and 8 h of exposure. Thus, for each replicate, there were separate vials for each age of the life stages at each particular exposure interval (40 per replicate, 6 replicates, 240 total vials per species). The pre-testing had indicated eggs of all species except *L. entomophila* were more tolerant than nymphs or adults. The exposure intervals used for both ages of *L. bostrychophila* eggs were 0, 8, 16, 24, 32, 40, 48, 56, 64, 72, and 80 h for a total of 132 separate vials. There was still some initial survival and eventual adult emergence of young *L. bostrychophila* eggs after 80 h, so a second series of six replicates was done at 64, 72, 80, 96, and 104 h with just young eggs, along with an untreated control (36 vials for treatments, 6 for untreated controls). Again, there was survival after 104 h, so a third and final series of six replicates was done from 112–168 h in 8-h increments along with untreated controls (42 vials for treatments, 6 for untreated controls). The exposure intervals used for *L. paeta* eggs were 0, 4, 8, 12, 16, 20, and 24 h for a total of 84 vials. The exposure intervals used for *L. decolor* and *L. entomophila* eggs were the same as those used for nymphs and adults, for a total of 120 vials.

2.3. Mortality assessment

The untreated controls were held in a chamber set at 30 °C and 60% r.h., as described in detail in Arthur et al. (2015). Adult mortality was defined as no discernible movement when touched or prodded with a probe, and was assessed as follows. Upon completion of an exposure interval the vials were removed from the freezer, transferred to the chamber, and allowed to warm for approximately 48 h. An initial mortality assessment was made at that time. After this initial assessment, approximately 25–30 mg of the standard diet was added to each vial. The vials were returned to the chamber, and mortality assessments for adults were done again at 7 days, which was considered as the delayed mortality assessment, and after that time the vials were discarded. Direct mortality for nymphs was defined as direct death, and indirect mortality was defined as failure to advance to the adult stage. The initial mortality assessment was made after the 2-day holding period in the chamber, and the final delayed survival assessments were made at 14 and 7 days after removal from the freezer for young and old nymphs, respectively. At that time, the number of adults that emerged from those nymphs was recorded (final survival). Initial egg mortality was defined as the number of nymphs present one week after the vials were removed from the freezer, but time period for this assessment occasionally varied to 14 days because egg hatch was often delayed as the exposure interval increased. Eventual adult emergence was also recorded as the final survival assessment. However, for all life stages, especially the untreated controls at time 0, some individuals may have escaped from the exposure arenas while those arenas were in the 30 °C chamber. Hence, the presence of individuals in the exposure arena was equated with survival.

2.4. Statistical analysis

Survival for each species and life stage was analyzed using the Mixed Models Procedure of the Statistical Analysis System (SAS Institute, version 9.2, Cary, NC, USA), with replicate as the random effect, to determine significance of main effects exposure time (time), young versus old of each life stage (age), and initial versus final survival (survival). A *t*-test (PROC *t*-test, SAS Institute) was then performed at each exposure interval to determine significance between the ages (young and old) of the particular life stage with respect to survival at a particular exposure interval and also differences between initial versus final survival for each stage and age ($P < 0.05$). There were occasions when one of the two comparison means was 0. When this occurred, a one-sample *t*-test was done on the other positive mean to determine if it was >0 (one-tailed *t*-test, PROC *t*-test, SAS Institute). Data were also examined by determining the first exposure interval that produced 100% initial mortality or 100% final mortality of each species, life stage, and life stage age. For young *L. bostrychophila* eggs, only the data for the first trial up to 80 h was analyzed as described in the previous sentences, as the purpose of the extra trials was simply to find the end point where there was no initial or final survival.

3. Results

3.1. Liposcelis bostrychophila

The main effects time, age, and the time by age interaction for eggs were significant at $P < 0.01$ (time: $F = 239.1$, df = 10, 271; age: $F = 147.3$, df = 1, 271; interaction: $F = 23.5$, df = 10, 271); main effect initial versus final survival and all other interactions were non-significant ($P \geq 0.05$). Initial and final survival of *L. bostrychophila* eggs for the time 0 untreated controls was at least 70%, but initial and final survival were less at the first exposure assessment of 8 h (Table 1). There was a rapid decline in initial and final survival of young and old eggs between 8 and 32 h, followed by a gradually lower level of survival of young eggs approaching but not reaching 0 at 80 h; however, initial and final survival of old eggs was 0 at 32 h (Table 1). Generally both initial and final survival of young eggs was higher at each exposure interval compared to old eggs (Table 1). The data for the second and third trials with young *L. bostrychophila* eggs shows some slight discrepancies for the time points where there was some overlap with the first trial, but the end result was that initial and final survival occurred out to 120 h of

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