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Freezing for control of stored-product psocids

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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.

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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

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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

Table 1

Initial and final survival (% mean $\pm$ SE) of *L. bostrychophila* young and old eggs. Time (h) is the hours the eggs were exposed to -18°C ; time 0 is untreated control. When significant ($P < 0.05$, t -test), differences at each time interval between young and old eggs for initial survival and between young and old eggs for final survival are denoted with different lower case letters.

Time (h)	Initial Survival		Final Survival	
	Young	Old	Young	Old
0	74.5 $\pm$ 4.6	85.3 $\pm$ 4.4	73.5 $\pm$ 4.8	82.7 $\pm$ 5.2
8	52.0 $\pm$ 3.5a	20.0 $\pm$ 3.7b	46.5 $\pm$ 4.5a	12.7 $\pm$ 3.3b
16	49.0 $\pm$ 5.1a	7.3 $\pm$ 3.7b	42.0 $\pm$ 5.3a	1.3 $\pm$ 0.8b
24	20.5 $\pm$ 5.4a	0.7 $\pm$ 0.7b	18.5 $\pm$ 5.8a	0 $\pm$ 0b
32	13.7 $\pm$ 2.1a	0 $\pm$ 0b	15.0 $\pm$ 3.3a	0 $\pm$ 0b
40	5.7 $\pm$ 2.4a	0 $\pm$ 0b	5.1 $\pm$ 2.3a	0 $\pm$ 0b
48	4.0 $\pm$ 1.5a	0 $\pm$ 0b	4.0 $\pm$ 1.5a	0 $\pm$ 0b
56	7.5 $\pm$ 3.7a	0 $\pm$ 0b	7.5 $\pm$ 3.7a	0 $\pm$ 0b
64	4.0 $\pm$ 3.0	0 $\pm$ 0	4.0 $\pm$ 2.9	0 $\pm$ 0
72	6.0 $\pm$ 1.3a	0 $\pm$ 0b	5.5 $\pm$ 1.3a	0 $\pm$ 0b
80	0.5 $\pm$ 0.5	0 $\pm$ 0	0.5 $\pm$ 0.5	0 $\pm$ 0

exposure, after which time there was no initial or final survival (Table 2).

Main effects time, age, and survival were all significant for the nymph stage at $P < 0.01$ (time: $F = 746.4$, $df = 9$, 199; age: $F = 9.4$, $df = 1$, 199; initial vs final survival: $F = 106.9$, $df = 1$, 199); time by age and time by survival interactions were significant ($P < 0.05$), but others were not ($P \geq 0.05$). Initial and final survival of control young nymphs was not different from survival of control old nymphs, but there were differences between initial and final survival of both nymph age classes at the 0.5 and 0.75-h exposure intervals, and for old nymphs at the 1-h exposure (Table 3). There was little decline in initial survival of either young or old nymphs, or between initial and final survival, at the first exposure interval of 0.25 h compared to unexposed controls. After 0.5 h of exposure, initial survival of young nymphs declined to 70.7% compared to 91% in old nymphs, while final survival for both age classes declined to 12.7 and 38%, respectively. Another big decrease in initial and final survival occurred at the next exposure interval of 0.75 h, and no initial survival was recorded after the 1.5 h exposures.

Main effects time and survival, along with the time by survival interaction, were significant for the adult stage at $P < 0.01$ (time: $F = 663.8$, $df = 9$, 199; initial vs final survival: $F = 80.1$, $df = 1$, 199; time by survival: $F = 21.6$, $df = 9$, 199), but neither main effect age nor any other interactions were significant ($P \geq 0.05$). Survival of untreated controls and at the 0.25 h interval ranged from 98 to 100%, but at the 0.5 h interval there was a difference between initial and final survival for both age classes (Table 3). Initial survival was greater than final survival for both egg age classes from the

Table 2

Initial and final survival (% mean $\pm$ SE) of *L. bostrychophila* young eggs for the additional series of tests to determine the endpoint exposure time for no initial or final survival. Time (h) is the hours the eggs were exposed to -18°C ; time 0 is untreated control. Data for controls averaged for the two sets of six replicates. Exposure times were 64–168 h, with no initial or final survival after 128 h.

Time (h)	Initial Survival	Final Survival
0	80.7 $\pm$ 3.2	80.7 $\pm$ 3.2
64	12.7 $\pm$ 5.7	12.0 $\pm$ 5.8
72	5.3 $\pm$ 1.3	4.0 $\pm$ 2.4
80	6.0 $\pm$ 2.2	5.3 $\pm$ 1.9
88	4.7 $\pm$ 1.9	3.3 $\pm$ 1.6
96	1.3 $\pm$ 0.8	0.7 $\pm$ 0.7
104	2.7 $\pm$ 1.9	2.7 $\pm$ 1.9
112	3.3 $\pm$ 1.6	3.3 $\pm$ 1.6
120	1.3 $\pm$ 0.8	1.3 $\pm$ 0.8
128	0 $\pm$ 0	0 $\pm$ 0

0.50–1.5-h intervals, and there was no initial survival of adults after 2 h of exposure (Table 3).

3.2. *Liposcelis paeta*

All main effects time, age, and survival were significant for eggs at $P < 0.01$ (time: $F = 408.1$, $df = 6$, 139; age: $F = 38.21$, $df = 1$, 139; initial vs final survival: $F = 16.4$, $df = 1$, 139), as was the age by time interaction ($F = 4.2$, $df = 6$, 139). The other two interactions were not significant ($P \geq 0.05$). Survival in unexposed controls ranged from 64.7 to 87.3%, but was 41% or less at the first exposure interval of 4 h (Table 4). Final survival after 4 h of exposure was greater in young versus old eggs, and initial survival of young eggs was greater than in old eggs at the 8 and 12 h exposures (Table 4). No other differences were observed. There were no differences for either egg age class between initial and final survival according to the t -tests, even though the generalized ANOVA had indicated a significant effect for initial versus final survival. All eggs were dead after 24 h of exposure.

Main effects exposure time and survival were significant for nymphs at $P < 0.01$ (time: $F = 345.1$, $df = 6$, 199; initial vs final survival: $F = 3.6$, $df = 1$, 199), but neither main effect age nor any interactions were significant. Survival of unexposed nymphs was 94–100%, but was only 33.3–47.3% after 0.25 h of exposure (Table 5). The only significant differences were greater initial survival of young and old nymphs compared to final survival at the 0.5 h interval. All nymphs were dead after 1.5 h of exposure. Similarly, main effects exposure time and survival were significant for adults at $P < 0.01$ (time: $F = 942.9$, $df = 6$, 199; initial vs final survival: $F = 8.6$, $df = 1$, 199), but main effect adult age was not significant nor were any interactions. Survival of unexposed controls was above 99% but survival was below 40% after the first exposure interval of 0.25 h (Table 5). Initial and final survival was greater in young versus old adults at the 0.5 h interval, which was the only time there was a difference between the adult ages or between initial versus final survival of young or old adults (Table 5). All adults were dead after one hour of exposure.

3.3. *Liposcelis decolor*

All main effects time, age, and survival were significant for eggs at $P < 0.01$ (time: $F = 206.8$, $df = 9$, 199; age: $F = 35.2$, $df = 1$, 139; initial vs final survival: $F = 53.0$, $df = 1$, 139), as were the age by time and the egg by survival interactions (age by time: $F = 5.12$, $df = 9$, 139, egg by survival: $F = 16.0$, $df = 1$, 199). The time by survival and three-way interactions were not significant ($P \geq 0.05$). The exposure intervals for eggs were the same as those used for nymphs and adults because eggs were more susceptible to the cold temperature compared to the previous species. Declines in initial and final survival of young and old eggs were more gradual compared to the previous species, and along with the shortened exposure intervals, indicate a greater susceptibility of *L. decolor* eggs compared to *L. bostrychophila* and *L. paeta*. Initial survival was greater in young versus old eggs at the 0.5–6 h intervals, and initial survival was greater than final survival for young eggs at these same intervals (Table 6). All eggs were dead after 12 h of exposure.

Main effects exposure time, survival, and the exposure by age and the age by survival interactions were all significant for nymphs at $P < 0.05$ (time: $F = 350.0$, $df = 9$, 99, initial vs final survival: $F = 64.0$, $df = 1$, 199, exposure by age: $F = 2.3$, $df = 9$, 199; age by survival: $F = 4.0$, $df = 1$, 1100). Main effect age was not significant, nor were any other interactions ($P \geq 0.05$). Initial and final survival of unexposed nymphs ranged from 83.3 to 98.6%, but after 0.25 h of exposure survival was 19.3–56.7% (Table 7). Even though the overall analysis showed that age was not significant ($P = 0.056$), the

Table 3

Initial survival and final survival (% mean $\pm$ SE) of *L. bostrychophila* young and old nymphs, and young and old adults. Time (h) is the hours the nymphs or adults were exposed to -18°C ; time 0 is untreated control. When significant ($P < 0.05$, t -test), differences at each time interval between young and old eggs for initial survival and between young and old eggs for final survival are denoted with different lower case letters; differences between initial and final survival of young life stages, and between initial and final survival of old life stages, are denoted with different upper case letters.

	Time (h)	Initial Survival		Final Survival	
		Young	Old	Young	Old
Nymphs	0	98.7 $\pm$ 1.3	99.7 $\pm$ 0.8	97.0 $\pm$ 1.3	97.0 $\pm$ 2.0
	0.25	98.7 $\pm$ 0.8	98.0 $\pm$ 0.9	98.8 $\pm$ 0.8	96.0 $\pm$ 1.5
	0.5	70.7 $\pm$ 5.6bA	91.0 $\pm$ 4.9aA	12.7 $\pm$ 4.9bB	38.0 $\pm$ 10.0aB
	0.75	38.0 $\pm$ 8.7A	38.0 $\pm$ 8.5A	0 $\pm$ 0bB	7.3 $\pm$ 2.2aB
	1	1.3 $\pm$ 1.3b	7.3 $\pm$ 2.8aA	0 $\pm$ 0	0.7 $\pm$ 0.7B
	1.5	0.7 $\pm$ 0.7	1.3 $\pm$ 1.3	0 $\pm$ 0	0 $\pm$ 0
Adults	0	99.0 $\pm$ 0.7	100 $\pm$ 0	99.3 $\pm$ 0.7	99.0 $\pm$ 0.7
	0.25	100 $\pm$ 0	100 $\pm$ 0	100 $\pm$ 0	98.0 $\pm$ 1.4
	0.5	96.0 $\pm$ 2.1A	98.0 $\pm$ 1.4A	58.0 $\pm$ 11.4bB	84.0 $\pm$ 2.9aB
	0.75	60.7 $\pm$ 9.7A	55.3 $\pm$ 7.1A	11.0 $\pm$ 2.8B	12.7 $\pm$ 2.8B
	1	25.3 $\pm$ 6.7A	24.0 $\pm$ 7.9A	3.3 $\pm$ 1.6B	6.0 $\pm$ 3.3B
	1.5	5.3 $\pm$ 2.5A	6.7 $\pm$ 3.7A	0 $\pm$ 0B	0 $\pm$ 0B
	2.0	0 $\pm$ 0	1.3 $\pm$ 1.3	0 $\pm$ 0	0 $\pm$ 0

Table 4

Initial and final survival (% mean $\pm$ SE) of *L. paeta* young and old eggs. Time (h) is the hours the eggs were exposed to -18°C ; time 0 is untreated control. When significant ($P < 0.05$, t -test), differences at each time interval between young and old eggs for initial survival and between young and old eggs for final survival are denoted with different lower case letters.

Time (h)	Initial Survival		Final Survival	
	Young	Old	Young	Old
0	87.3 $\pm$ 3.9	77.3 $\pm$ 4.7	82.0 $\pm$ 4.0a	64.7 $\pm$ 4.1b
4	41.3 $\pm$ 3.7	32.0 $\pm$ 3.4	34.7 $\pm$ 3.2a	20.7 $\pm$ 2.4b
8	22.7 $\pm$ 2.7a	11.3 $\pm$ 3.2b	14.7 $\pm$ 3.5	6.0 $\pm$ 1.3
12	14.0 $\pm$ 5.0a	3.3 $\pm$ 1.6b	6.0 $\pm$ 3.0	0.0 $\pm$ 2.0
16	0.7 $\pm$ 0.7	0 $\pm$ 0	0.7 $\pm$ 0.7	0 $\pm$ 0
20	0.7 $\pm$ 0.7	0 $\pm$ 0	0.7 $\pm$ 0.7	0 $\pm$ 0
24	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0

Table 5

Initial survival and final survival (% mean $\pm$ SE) of *L. paeta* young and old nymphs, and young and old adults. Time (h) is the hours the nymphs or adults were exposed to -18°C ; time 0 is untreated control. When significant ($P < 0.05$, t -test), differences at each time interval between young and old eggs for initial survival and between young and old eggs for final survival are denoted with different lower case letters; differences between initial and final survival of young life stages, and between initial and final survival of old life stages, are denoted with different upper case letters.

	Time (h)	Initial Survival		Final Survival	
		Young	Old	Young	Old
Nymphs	0	94.7 $\pm$ 5.3	100 $\pm$ 0	99.3 $\pm$ 0.7	99.3 $\pm$ 0.7
	0.25	47.3 $\pm$ 9.3	39.3 $\pm$ 8.9	38.0 $\pm$ 13.1	33.3 $\pm$ 6.9
	0.5	14.7 $\pm$ 4.9A	10.7 $\pm$ 3.8A	2.0 $\pm$ 2.0B	1.3 $\pm$ 0.8B
	0.75	0.7 $\pm$ 0.7	0.3 $\pm$ 0.3	0 $\pm$ 0	0 $\pm$ 0
	1.0	0 $\pm$ 0	1.3 $\pm$ 1.3	0 $\pm$ 0	0 $\pm$ 0
Adults	0	100 $\pm$ 0	99.3 $\pm$ 0.7	100 $\pm$ 0	100 $\pm$ 0
	0.25	37.3 $\pm$ 5.9	36.0 $\pm$ 5.6	37.3 $\pm$ 6.0	35.3 $\pm$ 6.3
	0.5	19.3 $\pm$ 3.5a	4.0 $\pm$ 2.1b	12.7 $\pm$ 2.6a	2.7 $\pm$ 0.8b
	0.75	3.3 $\pm$ 3.3	0 $\pm$ 0	3.3 $\pm$ 3.3	0 $\pm$ 0

t -test clearly indicated that initial survival of young nymphs was greater than for old nymphs for most of the exposure intervals, including the unexposed controls. Final survival of young nymphs was greater than for old nymphs at the 0.25, 0.5, and 0.75 h intervals. There were only two instances where there were differences between initial and final survival for either young or old nymphs. All nymphs were dead after two hours of exposure.

All main effects exposure time, age, and survival, plus the time

Table 6

Initial and final survival (% mean $\pm$ SE) of *L. decolor* young and old eggs. Time (h) is the hours the eggs were exposed to -18°C ; time 0 is untreated control. When significant ($P < 0.05$, t -test), differences at each time interval between young and old eggs for initial survival and between young and old eggs for final survival are denoted with different lower case letters; differences between initial and final survival of young eggs, and between initial and final survival of old eggs, are denoted with different upper case letters.

Time (h)	Initial Survival		Final Survival	
	Young	Old	Young	Old
0	75.3 $\pm$ 5.3	70.7 $\pm$ 5.7	62.0 $\pm$ 5.2	65.3 $\pm$ 7.7
0.25	69.3 $\pm$ 5.5	66.7 $\pm$ 2.5	56.0 $\pm$ 5.4	57.37 $\pm$ 5.3
0.50	72.0 $\pm$ 3.6aA	47.3 $\pm$ 3.0b	54.7 $\pm$ 4.2aB	44.0 $\pm$ 1.5b
0.75	59.3 $\pm$ 1.6aA	30.7 $\pm$ 4.5b	44.7 $\pm$ 3.5aB	25.3 $\pm$ 5.1b
1	46.0 $\pm$ 5.8aA	31.3 $\pm$ 3.3b	25.3 $\pm$ 4.1B	24.7 $\pm$ 5.6
1.5	32.7 $\pm$ 4.7aA	14.7 $\pm$ 4.3b	12.0 $\pm$ 2.1B	12.7 $\pm$ 3.2
2	23.3 $\pm$ 4.9aA	16.0 $\pm$ 1.8b	8.7 $\pm$ 1.9B	11.3 $\pm$ 2.8
4	9.3 $\pm$ 2.2aA	2.7 $\pm$ 0.8b	1.3 $\pm$ 0.8B	2.0 $\pm$ 0.9
6	2.7 $\pm$ 0.9aA	0 $\pm$ 0b	0 $\pm$ 0B	0 $\pm$ 0
8	2.7 $\pm$ 1.3	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0

by age and the time by survival interactions, were significant for adults at $P < 0.01$ (time: $F = 413.0$, $df = 9$, 198; age: $F = 40.9$, $df = 1$, 198; survival: $F = 27.0$, $df = 1$, 198; time by age: $F = 12.5$, $df = 9$, 198; time by survival: $F = 16.6$, $df = 9$, 198). The other two interactions were not significant ($P \geq 0.05$). Survival of unexposed controls ranged from 82.0 to 95.3 (Table 7). More young adults initially survived at the 0.25–0.75-h exposure intervals compared to old adults, final survival for both age classes was less than initial survival at 0.25 and 0.5 h, and there was no survival after one hour of exposure (Table 7).

3.4. *Liposcelis entomophila*

The main effects exposure time and survival, plus the time by age and the time by survival interactions, were all significant for eggs at $P < 0.01$ (time: $F = 230.0$, $df = 9$, 199; initial vs final survival: $F = 29.7$, $df = 1$, 199; time by age: $F = 5.5.0$, $df = 9$, 199; time by survival: $F = 16.6$, $df = 9$, 198), and the main effect age was significant at $P < 0.02$ ($F = 6.0$, $df = 1$, 199). The other two interactions were not significant ($P \geq 0.05$). However, low egg hatch combined with possible nymph mortality before the 1-week initial assessment was such that survival of unexposed controls was less than 50% (Table 8). Survival did decline with increasing exposure time, but there were only three instances where there were significant

Table 7

Initial and final survival (% mean $\pm$ SE) of *L. decolor* young and old nymphs, and young and old adults. Time (h) is the hours the nymphs or adults were exposed to -18°C ; time 0 is untreated control. When significant ($P < 0.05$, *t*-test), differences at each time interval between young and old eggs for initial survival and between young and old eggs for final survival are denoted with different lower case letters; differences between initial and final survival of young life stages, and between initial and final survival of old life stages, are denoted with different upper case letters.

	Time (h)	Initial Survival		Final Survival	
		Young	Old	Young	Old
Nymphs	0	98.6 $\pm$ 0.8a	83.3 $\pm$ 9.1b	97.0 $\pm$ 1.3	94.7 $\pm$ 2.0
	0.25	56.7 $\pm$ 8.3a	33.3 $\pm$ 0.9bA	44.0 $\pm$ 5.4a	19.3 $\pm$ 5.2bB
	0.5	47.3 $\pm$ 7.5aA	1.3 $\pm$ 4.9b	32.0 $\pm$ 3.4aB	4.7 $\pm$ 2.2b
	0.75	13.3 $\pm$ 5.7a	0 $\pm$ 0b	5.3 $\pm$ 2.0a	0 $\pm$ 0b
	1	3.3 $\pm$ 2.6	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
	1.5	0.7 $\pm$ 0.7	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0
Adults	0	95.3 $\pm$ 0.7	92.7 $\pm$ 3.7	92.0 $\pm$ 1.8	82.0 $\pm$ 4.8
	0.25	66.7 $\pm$ 6.7aA	42.7 $\pm$ 5.6bA	36.0 $\pm$ 11.0aB	16.7 $\pm$ 3.7bB
	0.5	61.7 $\pm$ 7.1aA	20.0 $\pm$ 4.0bA	18.0 $\pm$ 4.2aB	4.0 $\pm$ 2.1bB
	0.75	8.7 $\pm$ 2.9aA	0.7 $\pm$ 0.7b	1.3 $\pm$ 1.3B	0 $\pm$ 0
	1	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0

Table 8

Initial and final survival (% mean $\pm$ SE) of *L. entomophila* young and old eggs. Time (h) is the hours the eggs were exposed to -18°C ; time 0 is untreated control. When significant ($P < 0.05$, *t*-test), differences at each time interval between young and old eggs for initial survival and between young and old eggs for final survival are denoted with different lower case letters; differences between initial and final survival of young eggs, and between initial and final survival of old eggs, are denoted with different upper case letters.

Time (h)	Initial Survival		Final Survival	
	Young	Old	Young	Old
0	49.3 $\pm$ 3.7	41.3 $\pm$ 4.4	44.3 $\pm$ 5.2	39.3 $\pm$ 4.1
0.25	43.3 $\pm$ 4.8a	28.0 $\pm$ 4.3b	32.7 $\pm$ 5.4	24.7 $\pm$ 2.4
0.50	30.0 $\pm$ 4.0aA	10.0 $\pm$ 3.0b	10.7 $\pm$ 4.2B	8.7 $\pm$ 1.3
0.75	10.7 $\pm$ 2.5a	4.0 $\pm$ 1.5b	12.7 $\pm$ 3.5	10.0 $\pm$ 0.9
1	2.7 $\pm$ 0.8a	0 $\pm$ 0b	4.0 $\pm$ 1.0	1.3 $\pm$ 0.9
1.5	0.7 $\pm$ 0.7	0 $\pm$ 0	0 $\pm$ 0	0 $\pm$ 0

differences between survival of young and old eggs or in initial versus final survival (Table 8). There was no egg survival after the 1.5-h exposure interval. Nymphs and adults of *L. entomophila* were extremely susceptible to exposure at -18°C , as there was little survival after 0.25 h of exposure, and no survival after 0.5 h of exposure (Table 9). No overall analyses were performed, only the *t*-test comparisons for unexposed controls.

3.5. Time to 100% initial and 100% final mortality

Complete 100% initial or final mortality was not achieved for young eggs of *L. bostrychophila* until 128 h of exposure to -18°C , while 32 and 24 h were required for 100% initial and final mortality,

Table 9

Initial and final survival (% mean $\pm$ SE) of *L. entomophila* young and old nymphs, and young and old adults. Time (h) is the hours the nymphs or adults were exposed to -18°C ; time 0 is untreated control. When significant ($P < 0.05$, *t*-test), differences at each time interval between young life stages for initial and final survival, and between old life stages for initial and final survival, are denoted with different lower case letters; differences between initial and final survival of young life stages, and between initial and final survival of old life stages, are denoted with different upper case letters.

	Time (h)	Initial Survival		Final Survival	
		Young	Old	Young	Old
Nymphs	0	94.7 $\pm$ 2.0A	87.3 $\pm$ 5.2	75.3 $\pm$ 5.2B	74.7 $\pm$ 5.1
	0.25	4.0 $\pm$ 3.7	0 $\pm$ 0	0.7 $\pm$ 0.7	0 $\pm$ 0
Adults	0	91.3 $\pm$ 1.2a	74.7 $\pm$ 4.4b	87.3 $\pm$ 1.6a	63.3 $\pm$ 4.8b
	0.25	3.3 $\pm$ 1.9	0.7 $\pm$ 0.7	8.0 $\pm$ 3.4	2.0 $\pm$ 1.4

respectively, for old eggs (Fig. 1). In contrast, only 1–4 h of exposure were required for 100% mortality of nymphs and adults. Young and old eggs of *L. paeta* required 24 and 16 h of exposure for 100% initial and final mortality, respectively, but all nymphs and adults were dead after only 0.75–1.5 h of exposure (Fig. 1). For *L. decolor*, 12 h and 6 h of exposure was required for 100% initial mortality of young and old eggs, respectively, 6 h of exposure was needed for final mortality of both egg ages, and 0.75–2 h of exposure was needed for nymphs and adults, while complete mortality of all life stages of *L. entomophila* was achieved at 0.25–2 h of exposure (Fig. 1).

4. Discussion

Liposcelis bostrychophila eggs were more tolerant to -18°C than all stages of other stored-product insects previously studied. In our previous study, the eggs and both young and old larvae of *T. castaneum* were the most tolerant life stages, with 8 h of exposure required for initial mortality (Arthur et al., 2015). Old larvae were the most tolerant life stage of *T. inclusum*, requiring 64 h of exposure for 100% initial mortality. Two hours exposure to -12.5°C killed almost all eggs, larvae, pupae, and adults of the Mediterranean flour moth, *Ephestia kuehniella* (Zeller) (Andreadis et al., 2012). Exposures of 4–28, 2–4, and 4–8 h to -16°C killed larvae and adults of *Trogoderma granarium* (Everts), *Tribolium confusum* Jacqueline DuVal, and *Oryzaephilus surinaminensis* (L.) (Eliopoulos et al., 2011). Loganathan et al. (2011) recommended 3 h at -15°C to control all stages of *Callosobruchus maculatus* (F.). Fields (1992) reviewed over 30 published studies on use of cold for control of stored-product insects, but few of those studies investigated temperatures as low as -18°C . For those that did (Fields, 1992), 100% mortality took 1 h for adults of *Rhyzopertha dominica* (Fauvel); 5 h for adults of *Sitophilus granarius* (L.) and *Sitophilus oryzae* (L.); 1 h for *Plodia interpunctella* (Hübner); and 3 days for adults, nymphs, and larvae of the mite *Acarus siro* (L.). Nymphs and adults of the mold mite, *Tyrophagus putrescentiae* (Shranck), were all dead after 30 min exposure to -18°C , but a few eggs survived to 250 h (Eaton and Kells, 2011). Thus, none of the insects tested in the studies cited above were more tolerant to cold temperatures than *L. bostrychophila*.

Our results indicate that the egg stage of psocids is the life stage most tolerant to cold temperatures, with young eggs of *L. bostrychophila* as the most tolerant stage and species. Nymphs and adults of all four species were far more susceptible than eggs. With the exception of *L. bostrychophila*, 15–30 min of exposure to -18°C was sufficient to kill a large percentage of adults, similar to results for *T. castaneum* in our previous study (Arthur et al., 2015).

Fig. 1. Hours of exposure to -18°C required for 100% initial and 100% final mortality of young and old eggs, nymphs, and adults of *L. bostrychophila* (A), *L. paeta* (B), *L. decolor* (C), and *L. entomophila* (D). Young and old life stages designated as Y and O, respectively.

Young eggs were often more tolerant than the old eggs, particularly for *L. bostrychophila*, as survival of young eggs occurred out to 120 h of exposure to -18°C .

Our results show that eggs of psocids are the life stage most tolerant to cold temperature, which is similar to studies with the fumigant phosphine, which also show the egg stage of psocids as being more tolerant than nymphs and adults (Nayak et al., 2003a; Nayak and Collins, 2008). A recent study by Athanassiou et al. (2012) evaluated response of different life stages of psocids to the fumigant sulfuryl flouride. A dose of 4 and 8 g/m^3 was sufficient to kill adults of *Lepinotus reticulatus* (Enderlein) and *L. paeta*, while 24 g/m^3 was required for complete kill of *L. decolor*. Nymphs of all species were killed at rates between 4 and 8 g/m^3 , while concentrations of 24 and 72 g/m^3 were required for complete kill of eggs of *Lep. reticulatus* and *L. paeta*, respectively, and survival of *L. paeta* eggs occurred even at 96 g/m^3 . In studies with modified atmospheres, Bonjour et al. (2011) reported that an application rate of

ozone at 70 ppm (parts per million by volume) killed all adult female *L. bostrychophila* and *L. paeta* after only 1 d of exposure. However, this concentration was not effective against eggs of either species at any of the exposure intervals from 1 to 4 days.

The apparent greater tolerance of *L. bostrychophila* adults to -18°C was shown by the high survival after the first exposure intervals of 0.25 and 0.5 h compared to the other three species. Studies involving exposure of adult psocids to contact insecticides have reported mixed results regarding the order of species susceptibility. Collins et al. (2000) reported that the organophosphate insecticides fenitrothion, chlorpyrifos-methyl, and pirimiphos-methyl gave only limited control of *L. bostrychophila* and *L. paeta*, and little residual control of *L. entomophila*. Azinphos-methyl was more efficacious, with control of *L. bostrychophila* for up to 36 weeks and 24 weeks on steel and concrete surfaces, respectively. Control of *L. paeta* up to 28 weeks was reported but only on the steel surface, and there was little control of *L. entomophila*. A follow-

up study with carbaryl, deltamethrin, and permethrin also indicated that *L. bostrychophila* was more susceptible than either *L. paeta* or *L. entomophila*, with *L. entomophila* identified as the most tolerant species (Nayak et al., 2002). In our test involving exposure to -18°C , *L. entomophila* was the most susceptible species. In exposure studies with the organophosphate chlorpyrifos-methyl, *L. bostrychophila* was the most tolerant species (Nayak et al., 2003b), and was also the most tolerant psocid species exposed on a concrete surface treated with the insecticidal pyrolle chlorfenapyr (Athanasios et al., 2014). This cited study used the same strains of psocids as used in the current study.

Optimal moisture conditions for laboratory rearing of different psocid species are generally stated as ranging from 70 to 80% relative humidity (r.h.) (Nayak and Collins, 2001). The holding chamber used for this study was set at 60% r.h., which could have affected development of all tested psocid species from egg hatch to the first survival assessment, which for untreated controls was 7 days after eggs were placed in the vials. The low survival of unexposed eggs of *L. entomophila* could have resulted from sub-optimal r.h. levels or from handling of the eggs. Survival of unexposed nymphs and adults of all species was generally above 90%, but the low humidity at -18°C , combined with the 60% r.h. in the holding chamber, could have been a contributing factor to both the initial and final survival for each species at each exposure interval.

The results of this study show that cold temperatures may be able to control psocids, but these results emphasize the relative tolerance of eggs compared to nymphs and adults. Also, psocid species varied in their response to cold temperatures. One day at -18°C is sufficient to kill all life stages of the psocid species tested, except for young *L. bostrychophila* eggs which will require 128 h at -18°C for complete mortality. The insulating effects of stacked bags of commodities, and the time required for cold temperatures to penetrate through the stacked bags, must be considered when using cold temperatures as a disinfestation strategy for food storage facilities (Flinn et al., 2015).

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References

Andreadis, S.S., Eliopoulos, P.A., Savopoulou-Soultani, M., 2012. Cold hardiness of immature and adult stages of the Mediterranean flour moth, *Ephestia kuehniella*. J. Stored Prod. Res. 48, 132–136.

- Arthur, F.H., Hartzler, K.L., Throne, J.E., Flinn, P.W., 2015. Susceptibility of *Tribolium castaneum* (Coleoptera: Tenebrionidae) and *Trogoderma inclusum* (Coleoptera: Dermestidae) to cold temperatures. J. Stored Prod. Res. 64, 45–53.
- Athanasios, C.G., Arthur, F.H., Throne, J.E., 2009. Efficacy of grain protectants against four psocid species on maize, rice and wheat. Pest Manag. Sci. 65, 1140–1146.
- Athanasios, C.G., Opat, G.P., Throne, J.E., 2010. Influence of commodity type, percentage of cracked kernels, and wheat class on population growth of stored-product psocids (Psocoptera: Liposcelidae). J. Econ. Entomol. 103, 985–990.
- Athanasios, C.G., Phillips, T.W., Aikins, M.J., Hasan, M.M., Throne, J.E., 2012. Effectiveness of sulfur dioxide for control of different life stages of stored-product psocids (Psocoptera). J. Econ. Entomol. 105, 282–287.
- Athanasios, C.G., Kavallieratos, N.G., Arthur, F.H., Throne, J.E., 2014. Residual efficacy of chlorfenapyr for control of stored-product psocids (Psocoptera). J. Econ. Entomol. 107, 854–859.
- Bonjour, E.L., Opat, G.P., Hardin, J., Jones, C.L., Payton, M.E., Beeby, R.L., 2011. Efficacy of ozone fumigation against the major grain pests in stored wheat. J. Econ. Entomol. 104, 308–316.
- Collins, P.J., Nayak, M.K., Kopittke, R., 2000. Residual efficacy of four organophosphates on concrete and galvanized steel surfaces against three liposcelid psocid species (Psocoptera: Liposcelidae) infesting stored products. J. Econ. Entomol. 93, 1357–1363.
- Eaton, M., Kells, S.A., 2011. Freeze mortality characteristics of the mold mite *Tyrophagus putrescentiae*, a significant pest of stored products. J. Econ. Entomol. 104, 1423–1429.
- Eliopoulos, P.A., Prasodimou, G.Z., Pouliou, A.V., 2011. Time–mortality relationships of larvae and adults of grain beetles exposed to extreme cold. Crop Prot. 30, 1097–1102.
- Fields, P.G., 1992. The control of stored-product insects and mites with extreme temperatures. J. Stored Prod. Res. 28, 89–118.
- Flinn, P.W., Arthur, F.H., Throne, J.E., Friesen, K.S., Hartzler, K.H., 2015. Cold temperature disinfestation of bagged flour. J. Stored Prod. Res. 63, 42–46.
- Johnson, J.A., Valero, K.A., 2003. Use of commercial freezers to control cowpea weevil, *Callosobruchus maculatus* (Coleoptera: Bruchidae) in organic garbanzo beans. J. Econ. Entomol. 96, 1952–1957.
- Loganathan, M., Jayas, D.S., Fields, P.G., White, N.D.G., 2011. Low and high temperatures for the control of cowpea beetle, *Callosobruchus maculatus* (F.) (Coleoptera: Bruchidae) in chickpeas. J. Stored Prod. Res. 47, 244–248.
- Nayak, M.K., Collins, P.J., 2001. An improved method for mass rearing of three liposcelid psocids (Psocoptera: Liposcelidae) infesting stored commodities. J. Stored Prod. Res. 37, 323–328.
- Nayak, M.K., Collins, P.J., 2008. Influence of concentration, temperature and humidity on the toxicity of phosphine to the strongly phosphine-resistant psocid *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelidae). Pest Manag. Sci. 64, 971–976.
- Nayak, M.K., Collins, P.J., Kopittke, R.A., 2002. Comparative residual toxicities of carbaryl, deltamethrin and permethrin as structural treatments against three liposcelid psocid species (Psocoptera: Liposcelidae) infesting stored commodities. J. Stored Prod. Res. 38, 247–258.
- Nayak, M.K., Collins, P.J., Pavic, H., Kopittke, R.A., 2003a. Inhibition of egg development by phosphine in the cosmopolitan pest of stored products *Liposcelis bostrychophila* (Psocoptera: Liposcelidae). Pest Manag. Sci. 59, 1191–1196.
- Nayak, M.K., Collins, P.J., Kopittke, R.A., 2003b. Residual toxicities and persistence of organophosphorus insecticides mixed with carbaryl as structural treatments against three liposcelid psocid species (Psocoptera: Liposcelidae) infesting stored grain. J. Stored Prod. Res. 39, 343–353.
- Nayak, M.K., Collins, P.J., Throne, J.E., Wang, J., 2014. Biology and management of psocids infesting stored products. Ann. Rev. Entomol. 59, 279–297.
- Opat, G.P., Throne, J.E., 2008. Effects of diet on population growth of the psocids *Lepinotus reticulatus* and *Liposcelis entomophila*. J. Econ. Entomol. 101, 616–622.
- Tang, P.A., Wang, J.J., He, Y., Jiang, H.B., Wang, Z.Y., 2008. Development, survival, and reproduction of the psocid *Liposcelis decolor* (Psocoptera: Liposcelidae) at constant temperatures. Ann. Entomol. Soc. Am. 101, 1017–1025.
- Wang, J.J., Ren, Y., Wei, X.Q., Dou, W., 2009. Development, survival, and reproduction of the psocid *Liposcelis paeta* (Psocoptera: Liposcelidae) as a function of temperature. J. Econ. Entomol. 102, 1705–1713.