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Research

To Acclimate or Not to Acclimate? Simultaneous Positive and Negative Effects of Acclimation on Susceptibility of *Tribolium confusum* (Coleoptera: Tenebrionidae) and *Oryzaephilus surinamensis* (Coleoptera: Silvanidae) to Low Temperatures

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Abstract

Laboratory tests on acclimated and nonacclimated life stages of *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) (adults, pupae, larvae, and eggs) and *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae) (adults, larvae, and eggs) were conducted at 0, –5, –10, and –15°C to evaluate effects of acclimation on susceptibility to cold treatment. Acclimation of all tested life stages for 7 d at 15°C affected susceptibility of both species to the cold temperatures. After 1 d exposures for ≥2 h, acclimated adults had a noticeable increase in cold tolerance compared with nonacclimated adults for both tested species. Nonacclimated pupae of *T. confusum* were equally susceptible to cold compared with acclimated pupae at short exposures to low temperatures. Exposure of nonacclimated life stages of *T. confusum*, at –10°C for 1 d gave 0% survival. Similarly, almost all (99.6%) nonacclimated individuals of *O. surinamensis* died at –10°C. At 0°C, nonacclimated larvae were more cold tolerant than acclimated larvae, but this trend was reversed when larvae were exposed to –5°C. Mixed results were obtained for larvae of *O. surinamensis* because in some of the combinations tested, nonacclimated larvae were more tolerant, even at temperatures that were lower than 0°C. In contrast to *O. surinamensis*, eggs of *T. confusum* that had not been exposed to cold were not affected by acclimation, while exposure to cold showed increased cold hardiness in acclimated eggs. Results show that individual stored-product insect species may have mixed susceptibility to cold temperatures, which must be taken into account when using cold treatment as a management strategy.

Key words: low temperature, cold treatment, nonchemical management, stored-product Coleoptera

The application of low temperatures, known also as 'cold treatment' as an analogue of 'heat treatment', can be an effective strategy for control of stored-product insects (Fields 1992, Abdelghany et al. 2010, Arthur et al. 2015, Flinn et al. 2015, Athanassiou et al. 2018a). Given that stored-product insects can tolerate temperatures of 0°C for several weeks (Fields 1992, 2001), recent studies have focused on temperatures at about –18°C to decrease the exposure interval required for complete mortality of exposed insects. For example, all life stages of the red flour beetle, *Tribolium castaneum* (Herbst) (Coleoptera: Tenebrionidae) and the larger cabinet beetle, *Trogoderma inclusum* LeConte (Coleoptera: Dermestidae), were killed in <3 d when exposed at –18°C (Arthur et al. 2015). Nevertheless, there is an interaction between temperature and exposure interval. Recently, Athanassiou et al. (2018a) found that 77% of the adults of the psocid *Liposcelis bostrychophila* Badonnel (Psocoptera: Liposcelididae) could survive for 3 d at –10°C, while survival was about 3% at –15°C. In contrast, all eggs of the Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), died at exposure of 1 d to –5°C, and mortality at the same temperature was about 87% at just 8 h of exposure. The available series of data are currently sufficient to estimate mortality at different time–temperature combinations for the species described in the previous cited studies.

Most of the studies published in the scientific literature have examined efficacy of low temperatures in nonacclimated stored-product insects (Fields 1992, 2001, Arthur et al. 2015, Athanassiou et al. 2018a,b). However, acclimation of insects is a key consideration when evaluating cold temperatures for control and disinfestation, as acclimated insects may require longer exposure intervals or lower temperatures, which could affect economic aspects of cold treatments. This could be applicable in colder climates, where gradually cooling temperatures may enable insects to acclimate more easily compared with warmer climates. Cold hardiness is defined as the capacity of an organism to survive at temperatures lower than those normally expected (Andreadis and Athanassiou 2017). Acclimation is not the only factor in cold hardiness, as there are numerous biotic and abiotic factors that affect this phenomenon (Lee 1991, Andreadis and Athanassiou 2017). Wilches et al. (2017) evaluated the cold hardiness of the khapra beetle, *Trogoderma granarium* Everts (Coleoptera: Dermestidae) and found that the most cold tolerant life stage was the larval stage. Furthermore, diapausing larvae were more cold tolerant than nondiapausing larvae. However, the authors concluded that acclimation was probably more important than diapause in conferring cold hardiness. In most cases cited in the literature, exposure to low temperatures leads to an increase in survival (Fields 1992, Andreadis and Athanassiou 2017). Andreadis and Athanassiou (2017) also stated that many stored-product insects may be opportunistic in terms of cold tolerance, as their susceptibility is often buffered by the bulk mass of stored grains and bulk flour (Flinn et al. 2015), thus acclimation may have both positive and negative effects in terms of susceptibility to low temperatures.

The confused flour beetle, *Tribolium confusum* Jacquelin du Val Coleoptera: Tenebrionidae), and the saw-toothed grain beetle, *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae), are two stored-product insects that have global distribution and a wide range of food preferences (Aitken 1975). Recently, Athanassiou et al. (2018b) evaluated different temperatures ranging between 0 and −1°C for the control of different life stages of these species, and found that for both species, most exposed individuals of different life stages died 1 d exposure at −10°C, but the susceptibility of these species varied remarkably according to the life stage. Larvae and pupae of *T. confusum* were more cold tolerant than eggs or adults, while eggs, and adults of *O. surinamensis* were more cold tolerant than larvae (Athanassiou et al. 2018b). This highlights another paradigm of alteration of cold tolerance levels among different life stages of stored-product insects, as for some life stages exposure interval is more critical than temperature per se. However, in the above study, the authors used nonacclimated individuals, and the effect of acclimation remains unexplored. Thus, the objective of this study was to compare acclimated and nonacclimated individuals of these two species with temperatures between 0 and −15°C to estimate the effect of acclimation on susceptibility to cold treatments.

Materials and Methods

Insects

Tribolium confusum and *O. surinamensis* were obtained from laboratory standard cultures maintained at the Center for Grain and Animal Health Research (CGAHR), Manhattan, Kansas at 27.5°C and 70% relative humidity (RH). *Tribolium confusum* were reared on a diet of 95 wt.% whole-wheat flour and 5 wt.% Brewer's yeast, *O. surinamensis* were reared on a diet of rolled oats. Both species had been in culture for >30 yr. All life stages of *T. confusum* were used in the test, while all life stages of *O. surinamensis* were used except pupae because of expected fragility of the pupae (i.e., physical damages caused while handling). For both species, adults were <7 d old, larvae were <14 d old, and eggs were 0–2 d old. Pupae, <5 d old, were used only for *T. confusum*.

Bioassays

All tests were carried out in a freezing unit (Percival I36NLXC9, Percival Scientific, Perry, Iowa) set as described by Arthur et al. (2015). Plastic cylindrical vials of 50 mm in height and 25 mm in diameter (Thornton Plastics, Salt Lake City, UT) were the experimental units for the tests. Each vial contained 10 individuals of a given species and life stage (different vials for each species and life stage), and also about 50 mg of rearing media, which was flour and oats for *T. confusum* and *O. surinamensis*, respectively. Then, the vials were placed in the freezing unit for 0 h (controls), 2 h, 4 h, 8 h, 1 d, 2 d, 3 d, and 7 d (different series of vials for each interval). Different series of experiments were carried out at each of four temperatures: 0, −5, −10, and −15°C. There was an additional series of vials that was also placed in the freezing unit but contained insects that had been previously placed for 7 d in an incubator set at 15°C and 65% RH. These were considered the 'acclimated' individuals, contrary to the 'nonacclimated' individuals that had not been previously exposed to 15°C. Two additional series of vials were prepared, one containing acclimated and one containing nonacclimated insects, were maintained in an incubator at 30°C and 65% RH, that served as controls. For each combination of the above, there were three vials, and the same procedure was repeated three times, with new series of vials; hence, there were three replicates with three subreplicates (3 × 3 = 9 vials for each case) (Athanassiou et al. 2018b). After each exposure interval, the vials were removed from the freezer, and placed in a walk-in environmental chamber set at 30°C and 65% RH. Survival of adults and larvae alongside egg hatch and adult eclosion from pupae were observed after 7 d of exposure period.

Data Analysis

Data were analyzed separately for each species and life stage (adults, pupae, larvae, and eggs for *T. confusum*; adults, larvae, and eggs for *O. surinamensis*), by a three-way ANOVA with exposure period, temperature, and condition (acclimated vs. nonacclimated) as main effects while survival was the response variable. The associated interactions of the main effects were also incorporated in the analysis. All analyses were conducted by using JMP 13 software (SAS Institute Inc. 2013). Means were separated by the Tukey–Kramer (honest significant difference) [HSD]) test at 0.05 probability (Sokal and Rohlf 1995).

Results

Tribolium confusum

For all life stages, all main effects and interactions were significant, with the exception of condition for larvae (Table 1). Regarding adults of *T. confusum*, almost all individuals survived in the control vials, for both acclimated and nonacclimated condition (Table 2). At 0°C, there was practically no effect on adults until the exposure interval was >1 d. One day later, adult survival was reduced, but was still high (77–79%), without any differences between acclimated and nonacclimated individuals. In contrast, at 3 d of exposure, survival of the acclimated adults was 59%, which was more than two times the respective level of survival of nonacclimated adults. There was no adult survival at 7 d of exposure to 0°C. At −5°C, there was no effect on adults at 2 h of exposure, while at 4 h there was a significant reduction in survival, but only in the acclimated adults. In contrast, at the 8-h exposure interval, survival was significantly higher in the acclimated adults, when compared with the nonacclimated adults. Similarly, at the 1-d exposure interval, survival in the acclimated adults was about 3.5 times higher than that of nonacclimated adults, while 1 d later survival was recorded only for the acclimated adults. No adult survival was noted at longer exposures. There was no effect on adults at 2 h of exposure at −10°C. At 4 and 8 h, there was a significant reduction in survival of the nonacclimated adults when compared to the acclimated adults. At −15°C, adult survival was significantly reduced at 2 h of exposure when compared with the controls.

No significant differences were noted between acclimated and nonacclimated pupae in the control vials (Table 3). Moreover, at 0°C, no significant differences were recorded between the two pupal categories within each exposure interval, but in general, at 4 h of exposure, more acclimated pupae survived compared with nonacclimated pupae. At 7 d of exposure at this temperature (0°C), adult emergence was noted only for acclimated pupae. At −5°C, pupae were affected even at the shortest exposure interval (2 h) when compared with the controls, as adults that emerged from acclimated pupae were two times more than those that emerged from nonacclimated pupae. Survival was further decreased at 4 h, while no adult emergence was recorded at exposures >1 d. At those intervals, there were no significant differences between the two pupal categories. At −10°C, even the shortest (2 h) exposure significantly reduced adult emergence, which was completely suppressed at exposures that were greater than 8 h. Furthermore, at this temperature, there were no significant differences between the two pupal categories. Finally, at −15°C, adult emergence was significantly reduced at 2 h, when compared with the control pupae. Paradoxically, at this exposure, adult emergence from the acclimated pupae was significantly lower than that from the nonacclimated pupae, but at the 4-h interval, adults emerged only from acclimated pupae. Only one pupa survived beyond this interval at this temperature.

Table 1. ANOVA parameters of main effects and their interactions for *T. confusum* life stages (total df = 575)

Life stage	Source	df	F	P
Adults	Condition	1	89.1	<0.01
	Exposure	7	1535.3	<0.01
	Temperature	3	1794.6	<0.01
	Condition × exposure	7	9.5	<0.01
	Condition × temperature	3	22.3	<0.01
	Exposure × temperature	21	192.6	<0.01
	Condition × exposure × temperature	21	27.5	<0.01
Pupae	Condition	1	10.9	<0.01
	Exposure	7	520.6	<0.01
	Temperature	3	294.8	<0.01
	Condition × exposure	7	2.9	<0.01
	Condition × temperature	3	11.5	<0.01
	Exposure × temperature	21	27.1	<0.01
	Condition × exposure × temperature	21	5.0	<0.01
Larvae	Condition	1	0.2	0.66
	Exposure	7	569.7	<0.01
	Temperature	3	876.7	<0.01
	Condition × exposure	7	4.7	<0.01
	Condition × temperature	3	9.4	<0.01
	Exposure × temperature	21	65.0	<0.01
	Condition × exposure × temperature	21	3.6	<0.01
Eggs	Condition	1	285.0	<0.01
	Exposure	7	871.0	<0.01
	Temperature	3	602.8	<0.01
	Condition × exposure	7	16.5	<0.01
	Condition × temperature	3	147.2	<0.01
	Exposure × temperature	21	42.6	<0.01
	Condition × exposure × temperature	21	24.2	<0.01

Table 2. Mean survival (out of 10 individuals per vial ± SE) of *T. confusum* acclimated or nonacclimated adults at different temperatures and exposure intervals (*n* = 9)

Exposure/condition	Temperature				F	P
	0°C	−5°C	−10°C	−15°C		
0 h acclimated	10.0 ± 0.0a	10.0 ± 0.0a	9.9 ± 0.1a	10.0 ± 0.0a	1.0	0.41
0 h nonacclimated	10.0 ± 0.0a	10.0 ± 0.0a	10.0 ± 0.0a	9.9 ± 0.1a	1.0	0.41
2 h acclimated	9.8 ± 0.2aA	9.8 ± 0.2aA	9.4 ± 0.2aA	2.0 ± 0.2bB	317.9	<0.01
2 h nonacclimated	10.0 ± 0.0aA	10.0 ± 0.0aA	9.0 ± 0.4aA	1.3 ± 0.4cB	253.2	<0.01
4 h acclimated	9.6 ± 0.3aA	7.9 ± 0.6cdB	9.2 ± 0.3aAB	0.6 ± 0.2dC	125.9	<0.01
4 h nonacclimated	10.0 ± 0.0aA	9.7 ± 0.2abA	2.4 ± 0.5bB	0.3 ± 0.2dC	317.9	<0.01
8 h acclimated	9.8 ± 0.2aA	8.1 ± 0.5bcB	2.2 ± 0.4bC	0.2 ± 0.2dD	161.1	<0.01
8 h nonacclimated	10.0 ± 0.0aA	6.1 ± 0.4eB	0.0 ± 0.0cC	0.0 ± 0.0dC	638.8	<0.01
1 d acclimated	9.8 ± 0.2aA	6.3 ± 0.7deB	0.0 ± 0.0cC	0.0 ± 0.0dC	191.2	<0.01
1 d nonacclimated	9.8 ± 0.2aA	1.7 ± 0.3fB	0.0 ± 0.0cC	0.0 ± 0.0dC	834.0	<0.01
2 d acclimated	7.7 ± 0.8bA	1.0 ± 0.4fgB	0.0 ± 0.0cB	0.0 ± 0.0dB	72.9	<0.01
2 d nonacclimated	7.9 ± 0.6bA	0.0 ± 0.0gB	0.0 ± 0.0cB	0.0 ± 0.0dB	195.8	<0.01
3 d acclimated	5.9 ± 0.4cA	0.0 ± 0.0gB	0.0 ± 0.0cB	0.0 ± 0.0dB	280.9	<0.01
3 d nonacclimated	2.3 ± 0.9dA	0.0 ± 0.0gB	0.0 ± 0.0cB	0.0 ± 0.0dB	65.3	<0.01
7 d acclimated	0.0 ± 0.0e	0.0 ± 0.0g	0.0 ± 0.0c	0.0 ± 0.0d	–	–
7 d nonacclimated	0.0 ± 0.0e	0.0 ± 0.0g	0.0 ± 0.0c	0.0 ± 0.0d	–	–
F	161.3	196.8	429.8	620.7		
P	<0.01	<0.01	<0.01	<0.01		

Within each column, means followed by the same lowercase letter are not significantly different, in all cases df = 15, 143; HSD test at 0.05. Within each row, means followed by the same uppercase letter are not significantly different, in all cases df = 3, 35; HSD test at 0.05. Where no letters exist, no significant differences were recorded. Where dashes exist, no analysis was conducted because there was no survival at that temperature.

Table 3. Mean survival (out of 10 individuals per vial ± SE) of *T. confusum* acclimated or nonacclimated pupae at different temperatures and exposure intervals ($n = 9$)

	Temperature					
Exposure/condition	0°C	−5°C	−10°C	−15°C	*F*	*P*
0 h acclimated	9.0 ± 0.3abB	10.0 ± 0.0aA	9.7 ± 0.2aAB	9.1 ± 0.3aB	4.3	0.01
0 h nonacclimated	9.9 ± 0.1aA	10.0 ± 0.0aA	9.8 ± 0.2aA	8.9 ± 0.2aB	13.9	<0.01
2 h acclimated	8.4 ± 0.6abcA	7.4 ± 0.4bAB	6.1 ± 0.8cdB	0.9 ± 0.3cdC	35.3	<0.01
2 h nonacclimated	7.4 ± 0.4abcdA	3.6 ± 0.5cB	8.6 ± 0.2abA	4.1 ± 0.5bB	31.3	<0.01
4 h acclimated	8.8 ± 0.3abA	3.4 ± 0.7cB	7.6 ± 0.5bcA	0.7 ± 0.3cdC	65.0	<0.01
4 h nonacclimated	6.0 ± 0.5bcdefA	2.4 ± 0.4cdB	6.9 ± 0.4bcA	1.3 ± 0.5cB	39.3	<0.01
8 h acclimated	8.0 ± 0.6abcdA	2.3 ± 0.5cdC	5.1 ± 0.5dB	1.1 ± 0.3cdC	36.8	<0.01
8 h nonacclimated	6.7 ± 0.6bcdeA	1.2 ± 0.2deC	4.6 ± 0.7dB	0.0 ± 0.0dC	43.5	<0.01
1 d acclimated	5.7 ± 0.9cdefA	0.1 ± 0.1eB	0.0 ± 0.0eB	0.0 ± 0.1dB	35.2	<0.01
1 d nonacclimated	5.1 ± 0.4defA	0.2 ± 0.2eB	0.0 ± 0.0eB	0.0 ± 0.0dB	147.1	<0.01
2 d acclimated	4.1 ± 0.6efgA	0.0 ± 0.0eB	0.0 ± 0.0eB	0.0 ± 0.0dB	48.9	<0.01
2 d nonacclimated	4.0 ± 0.5efgA	0.0 ± 0.0eB	0.0 ± 0.0eB	0.0 ± 0.0dB	57.6	<0.01
3 d acclimated	3.6 ± 1.4fgA	0.0 ± 0.0eB	0.0 ± 0.0eB	0.0 ± 0.0dB	7.0	<0.01
3 d nonacclimated	1.6 ± 0.3ghA	0.0 ± 0.0eB	0.0 ± 0.0eB	0.0 ± 0.0dB	21.2	<0.01
7 d acclimated	1.7 ± 0.9ghA	0.0 ± 0.0eB	0.0 ± 0.0eB	0.0 ± 0.0dB	3.6	0.03
7 d nonacclimated	0.0 ± 0.0h	0.0 ± 0.0e	0.0 ± 0.0e	0.0 ± 0.0d	–	–
F	23.2	141.1	134.2	172.9		
P	<0.01	<0.01	<0.01	<0.01		

Within each column, means followed by the same lowercase letter are not significantly different, in all cases df = 15, 143; HSD test at 0.05. Within each row, means followed by the same uppercase letter are not significantly different, in all cases df = 3, 35; HSD test at 0.05. Where no letters exist, no significant differences were recorded. Where dashes exist, no analysis was conducted because there was no survival at that temperature.

Larval survival in the control vials was not affected by acclimation, and most larvae survived (Table 4). Moreover, both acclimated and nonacclimated larvae were not affected at 0°C, even at 1 d of exposure. At 2 d of exposure, larval survival was reduced to 77%, but only for the acclimated larvae, while almost all nonacclimated larvae survived. Similarly, at the 3-d exposure interval, fewer acclimated larvae survived when compared with the nonacclimated larvae, but survival of nonacclimated larvae was reduced when compared with the previous exposure intervals. At this temperature, larval survival was recorded even at the 7-d exposure intervals, for both larval categories. Two hours of exposure at −5°C did not affect *T. confusum* larvae, but longer periods did reduce survival. Although, in most of the cases, there were no significant differences between the two larval categories, survival percentages were generally higher for the nonacclimated larvae. However, more acclimated larvae survived at 1 d of exposure, while 1 d later (i.e., 2 d of exposure) at −5°C, the only larvae that had survived were from the acclimated ones. No larval survival was recorded further than these exposures. At −10°C, larval survival was not affected at 2 h of exposure, but at 4 h for both larval categories, about one-third of the exposed individuals were still alive. No survival was recorded at exposures >8 h. Similarly, at −15°C, there were no differences between the two larval categories, while survival was notably affected at 2 h of exposure, and no survival was recorded at exposures >4 h.

There were no differences between acclimated and nonacclimated eggs in the control vials, except at −15°C (Table 5). Moreover, at 0°C, eggs were not affected until the 4-h exposure interval, with the exception of nonacclimated eggs. At 2 d of exposure at this temperature, egg hatch was reduced, but only for nonacclimated eggs. At longer intervals, egg hatch was much higher in the acclimated eggs than in the nonacclimated eggs. For example, at 3 d of exposure, egg hatch of acclimated and nonacclimated eggs was 66 and 3%, respectively, while at 7 d of exposure, the respective percentages were 23 and 0%. At −5°C, eggs were affected at 2 h of exposure, but again, this was only for nonacclimated eggs. There were no significant differences between the two egg categories for the other exposure intervals where egg hatch was recorded, i.e., 4 and 8 h. These differences in egg hatch were not apparent at −10°C, where egg hatch at 8 h was higher than that at −5°C. At −15°C, egg hatch was recorded only at 2 h of exposure and was much higher in acclimated eggs.

Oryzaephilus surinamensis

For the three life stages examined, all main effects and interactions were significant (Table 6). There were no differences between acclimated and nonacclimated adults in the control vials (Table 7). Also, adults were not significantly affected even at 3 d of exposure at 0°C, while at the 7-d interval, adult survival was 57%. Similarly, at −5°C, adults were affected only at 1 d of exposure, and only for nonacclimated adults. In contrast, acclimated adults remained unaffected until 3 d of exposure, where survival of acclimated and nonacclimated adults was 79 and 6%, respectively. There was no adult survival at 7 d of exposure. At −10°C, at only 2 h of exposure, less than half of the nonacclimated adults survived, in comparison to the acclimated adults. Furthermore, there was no adult survival for the nonacclimated adults at intervals that were longer than 2 h. Conversely, acclimated adults survived at the 4 and 8 h intervals (percentages of 82 and 10%, respectively). Finally, at −15°C, all adults were dead in both adult categories by 2 h.

There were no significant differences between the two larval categories in the controls at 0, −10, and −15°C (Table 8). At 0°C, acclimated larvae were not affected even at 2 d of exposure. In contrast, survival of nonacclimated larvae at 2 h, 4 h, 1 d, 3 d, and 7 d of exposure was significantly lower than that of the untreated (control) larvae. Significantly fewer acclimated larvae survived at 4 h, 8 h, and 1 d of exposure at −5°C than nonacclimated larvae. No significant differences between nonacclimated and acclimated larvae were recorded at 2 d of exposure at −5°C. At this temperature, no survival was recorded beyond 2 d of exposure. At −10°C, at 2 h, survival of acclimated and nonacclimated larvae was 80 and 7%, respectively, whereas the respective values at 8 h were 4 and 0%, with no survival beyond this exposure. At −15°C, even 2 h of exposure killed all larvae.

Table 4. Mean survival (out of 10 individuals per vial ± SE) of *T. confusum* acclimated or nonacclimated larvae at different temperatures and exposure intervals (*n* = 9)

Exposure/condition	Temperature				*F*	*P*
	0°C	−5°C	−10°C	−15°C		
0 h acclimated	9.7 ± 0.2a	9.9 ± 0.1a	10.0 ± 0.0a	9.9 ± 0.1a	1.0	0.42
0 h nonacclimated	10.0 ± 0.0a	10.0 ± 0.0a	9.8 ± 0.2a	9.8 ± 0.2a	1.5	0.23
2 h acclimated	9.6 ± 0.4aA	10.0 ± 0.0aA	9.0 ± 0.3abA	3.8 ± 0.7bB	46.7	<0.01
2 h nonacclimated	10.0 ± 0.0aA	9.6 ± 0.2aAB	8.0 ± 0.5bB	2.1 ± 0.7bcC	77.7	<0.01
4 h acclimated	10.0 ± 0.0aA	6.9 ± 0.4bcB	3.6 ± 0.5cC	1.8 ± 1.1bcC	35.2	<0.01
4 h nonacclimated	9.9 ± 0.1aA	8.2 ± 0.5abA	3.2 ± 0.5cB	1.8 ± 1.1bcB	34.2	<0.01
8 h acclimated	10.0 ± 0.0aA	6.8 ± 0.8bcB	2.8 ± 1.0cC	0.0 ± 0.0cD	46.0	<0.01
8 h nonacclimated	9.9 ± 0.1aA	7.3 ± 0.5bcB	2.3 ± 0.4cC	0.0 ± 0.0cD	190.9	<0.01
1 d acclimated	9.8 ± 0.2aA	5.7 ± 1.0cB	0.0 ± 0.0dC	0.0 ± 0.0cC	94.0	<0.01
1 d nonacclimated	10.0 ± 0.0aA	2.1 ± 0.5dB	0.0 ± 0.0dC	0.0 ± 0.0cC	437.1	<0.01
2 d acclimated	7.7 ± 0.4bA	0.2 ± 0.2deB	0.0 ± 0.0dB	0.0 ± 0.0cB	267.0	<0.01
2 d nonacclimated	9.3 ± 0.4aA	0.0 ± 0.0eB	0.0 ± 0.0dB	0.0 ± 0.0cB	627.2	<0.01
3 d acclimated	4.7 ± 0.6cA	0.0 ± 0.0eB	0.0 ± 0.0dB	0.0 ± 0.0cB	56.0	<0.01
3 d nonacclimated	8.9 ± 0.3abA	0.0 ± 0.0eB	0.0 ± 0.0dB	0.0 ± 0.0cB	734.2	<0.01
7 d acclimated	1.3 ± 0.4dA	0.0 ± 0.0eB	0.0 ± 0.0dB	0.0 ± 0.0cB	9.1	<0.01
7 d nonacclimated	2.1 ± 0.4dA	0.0 ± 0.0eB	0.0 ± 0.0dB	0.0 ± 0.0cB	36.1	<0.01
F	93.7	116.8	118.0	54.3		
P	<0.01	<0.01	<0.01	<0.01		

Within each column, means followed by the same lowercase letter are not significantly different, in all cases df = 15, 143; HSD test at 0.05. Within each row, means followed by the same uppercase letter are not significantly different, in all cases df = 3, 35; HSD test at 0.05. Where no letters exist, no significant differences were recorded.

Table 5. Mean survival (out of 10 individuals per vial ± SE) of *T. confusum* acclimated or nonacclimated eggs at different temperatures and exposure intervals (*n* = 9)

Exposure/condition	Temperature				*F*	*P*
	0°C	−5°C	−10°C	−15°C		
0 h acclimated	9.2 ± 0.5a	8.9 ± 0.3a	8.6 ± 0.4a	8.1 ± 0.3b	1.6	0.22
0 h nonacclimated	8.4 ± 0.3ab	8.7 ± 0.4a	8.6 ± 0.3a	9.2 ± 0.4a	1.0	0.43
2 h acclimated	8.7 ± 0.3aA	9.6 ± 0.2aA	6.8 ± 0.5bB	6.3 ± 0.5cB	13.6	<0.01
2 h nonacclimated	7.3 ± 0.3abA	5.0 ± 0.7bB	8.0 ± 0.4abA	0.2 ± 0.2dC	64.3	<0.01
4 h acclimated	8.7 ± 0.3aA	1.7 ± 0.5cC	4.7 ± 0.3cB	0.0 ± 0.0dD	138.9	<0.01
4 h nonacclimated	6.6 ± 0.4bA	2.8 ± 0.5cB	2.6 ± 0.4dB	0.0 ± 0.0dC	47.8	<0.01
8 h acclimated	8.9 ± 0.5aA	0.0 ± 0.0dB	0.4 ± 0.2eB	0.0 ± 0.0dB	322.2	<0.01
8 h nonacclimated	4.0 ± 0.6cA	0.1 ± 0.1dB	1.3 ± 0.4deB	0.0 ± 0.0dB	24.4	<0.01
1 d acclimated	8.1 ± 0.5abA	0.0 ± 0.0dB	0.0 ± 0.0eB	0.0 ± 0.0dB	250.8	<0.01
1 d nonacclimated	2.1 ± 0.5cdeA	0.0 ± 0.0dB	0.0 ± 0.0eB	0.0 ± 0.0dB	21.6	<0.01
2 d acclimated	7.7 ± 0.3abA	0.0 ± 0.0dB	0.0 ± 0.0eB	0.0 ± 0.0dB	705.3	<0.01
2 d nonacclimated	0.4 ± 0.2defA	0.0 ± 0.0dB	0.0 ± 0.0eB	0.0 ± 0.0dB	6.4	<0.01
3 d acclimated	6.6 ± 0.5bA	0.0 ± 0.0dB	0.0 ± 0.0eB	0.0 ± 0.0dB	153.0	<0.01
3 d nonacclimated	0.3 ± 0.2ef	0.0 ± 0.0d	0.0 ± 0.0e	0.0 ± 0.0d	2.0	0.13
7 d acclimated	2.3 ± 0.4cdA	0.0 ± 0.0dB	0.0 ± 0.0eB	0.0 ± 0.0dB	32.7	<0.01
7 d nonacclimated	0.0 ± 0.0f	0.0 ± 0.0d	0.0 ± 0.0e	0.0 ± 0.0d	–	–
F	76.0	168.9	158.2	331.4		
P	<0.01	<0.01	<0.01	<0.01		

Within each column, means followed by the same lowercase letter are not significantly different; in all cases df = 15, 143; HSD test at 0.05. Within each row, means followed by the same uppercase letter are not significantly different, in all cases df = 3, 35; HSD test at 0.05. Where no letters exist, no significant differences were recorded. Where dashes exist, no analysis was conducted because there was no survival at that temperature.

In all control vials, egg hatch of the nonacclimated eggs was higher than that of the acclimated eggs (Table 9). Although initially eggs were not affected at 0°C, as exposure time increased egg hatch decreased. Moreover, even though egg hatch at <1 d was higher in the nonacclimated eggs, at longer intervals of 1 and 2 d, egg hatch was similar in acclimated and nonacclimated eggs. Furthermore, at 3 d, the values reversed, since only acclimated eggs survived. However, at −5°C, nonacclimated eggs survived until 1 d of exposure, while all acclimated eggs died at only 4 h of exposure. Similar trends were also recorded at −10°C. At −15°C, even 2 h of exposure killed all acclimated eggs, but some nonacclimated eggs (2%) survived for 4 h.

Discussion

In a previous study, Athanassiou et al. (2018b) evaluated the susceptibility of the same strains of *T. confusum* and *O. surinamensis* to the same temperatures (i.e., 0, −5, −10, and −15°C), by using the same experimental protocol but using nonacclimated life stages. In that study, *T. confusum* was generally more susceptible to cold than *O. surinamensis*. For *T. confusum*, larvae and pupae were more tolerant than adults and eggs, while for *O. surinamensis*, adults and eggs were more tolerant than larvae (Athanassiou et al. 2018b). The results of our study for nonacclimated individuals agree with previous results (Athanassiou et al. 2018b).

Interestingly, acclimation of all tested life stages for both species changed their susceptibility to cold. We selected +15°C as a realistic temperature that was close to the lowest thermal threshold for the development of both species. For example, the lowest temperatures where *T. confusum* and *O. surinamensis* complete their biological cycles are 19 and 20°C, respectively (Rees 2004). For the same reason, we exposed the insects to +15°C for 1 wk, to avoid elevated mortality, which might have occurred in some life stages at longer acclimation exposure intervals. For example, regarding *T. confusum* and *O. surinamensis*, there is no egg hatch at 15°C, and probably longer exposures to these temperature levels will lead to an increase in egg mortality (Lin et al. 1954, Howe 1956a,b).

The effect of previous acclimation has been correlated with increased cold tolerance in several stored-product insect species (Fields 1992, Fields and Timlick 2010, Abdelghany et al. 2015, Wilches et al. 2017). The survival of diapausing and nondiapausing larvae of *P. interpunctella* that had been acclimated for 4 wk at −10°C was significantly higher than nonacclimated larvae at −5 and −10°C (Fields and Timlick 2010). Abdelghany et al. (2015) reported that cold-acclimated diapausing larvae of the warehouse beetle, *Trogoderma variabile* (Ballion; Coleoptera: Dermesitidae), were more tolerant than nonacclimated diapausing larvae. Nevertheless, the current tests showed that previous acclimation to 15°C had both positive and negative effects on cold tolerance. This trend was not consistent and was expressed in three levels. First, exposure to different temperatures resulted in different susceptibility levels in acclimated individuals for both species. Secondly, these differential effects were life stage-mediated, as often one life stage of a given species responded in a different way to acclimation than another life stage. Finally, in some cases, within the same temperature and life stage, the difference in susceptibility between acclimated and nonacclimated individuals

Table 6. ANOVA parameters of main effects and their interactions for *O. surinamensis* life stages (total df = 575)

Life stage	Source	df	*F*	*P*
Adults	Condition	1	175.9	<0.01
	Exposure	7	732.1	<0.01
	Temperature	3	2981.9	<0.01
	Condition × exposure	7	17.0	<0.01
	Condition × temperature	3	65.4	<0.01
	Exposure × temperature	21	153.2	<0.01
	Condition × exposure × temperature	21	36.1	<0.01
Larvae	Condition	1	22.8	<0.01
	Exposure	7	545.0	<0.01
	Temperature	3	731.9	<0.01
	Condition × exposure	7	15.0	<0.01
	Condition × temperature	3	220.6	<0.01
	Exposure × temperature	21	62.1	<0.01
	Condition × exposure × temperature	21	22.8	<0.01
Eggs	Condition	1	431.1	<0.01
	Exposure	7	407.8	<0.01
	Temperature	3	51.7	<0.01
	Condition × exposure	7	90.0	<0.01
	Condition × Temperature	3	3.1	0.02
	Exposure × temperature	21	17.8	<0.01
	Condition × exposure × temperature	21	7.9	<0.01

Table 7. Mean survival (out of 10 individuals per vial ± SE) of *O. surinamensis* acclimated or nonacclimated adults at different temperatures and exposure intervals (*n* = 9)

Exposure/condition	Temperature				*F*	*P*
	0°C	−5°C	−10°C	−15°C		
0 h acclimated	10.0 ± 0.0a	10.0 ± 0.0a	9.8 ± 0.2a	10.0 ± 0.0	2.3	0.10
0 h nonacclimated	10.0 ± 0.0a	10.0 ± 0.0a	9.9 ± 0.1a	10.0 ± 0.0	1.0	0.41
2 h acclimated	9.0 ± 0.3aB	10.0 ± 0.0aA	9.6 ± 0.2aAB	0.0 ± 0.0C	537.7	<0.01
2 h nonacclimated	9.6 ± 0.3aA	10.0 ± 0.0aA	4.1 ± 0.7cB	0.0 ± 0.0C	167.4	<0.01
4 h acclimated	9.4 ± 0.2aA	9.8 ± 0.2aA	8.2 ± 0.4bB	0.0 ± 0.0C	355.1	<0.01
4 h nonacclimated	9.8 ± 0.2aA	9.9 ± 0.1aA	0.0 ± 0.0dB	0.0 ± 0.0B	2088.7	<0.01
8 h acclimated	9.2 ± 0.3aB	9.9 ± 0.1aA	1.0 ± 0.2dC	0.0 ± 0.0D	940.5	<0.01
8 h nonacclimated	9.6 ± 0.2aA	9.0 ± 0.3abA	0.0 ± 0.0dB	0.0 ± 0.0B	809.8	<0.01
1 d acclimated	8.9 ± 0.5aB	9.9 ± 0.1aA	0.0 ± 0.0dC	0.0 ± 0.0C	539.4	<0.01
1 d nonacclimated	8.6 ± 0.5aA	8.0 ± 0.5bA	0.0 ± 0.0dB	0.0 ± 0.0B	172.5	<0.01
2 d acclimated	8.8 ± 0.4aB	9.9 ± 0.1aA	0.0 ± 0.0dC	0.0 ± 0.0C	676.8	<0.01
2 d nonacclimated	8.8 ± 0.5aA	5.4 ± 0.4cB	0.0 ± 0.0dC	0.0 ± 0.0C	209.0	<0.01
3 d acclimated	8.2 ± 0.4aA	7.9 ± 0.4bA	0.0 ± 0.0dB	0.0 ± 0.0B	235.8	<0.01
3 d nonacclimated	8.1 ± 0.8aA	0.6 ± 0.2dB	0.0 ± 0.0dB	0.0 ± 0.0B	85.5	<0.01
7 d acclimated	5.7 ± 0.6bA	0.0 ± 0.0dB	0.0 ± 0.0dB	0.0 ± 0.0B	105.1	<0.01
7 d nonacclimated	5.7 ± 0.7bA	0.0 ± 0.0dB	0.0 ± 0.0dB	0.0 ± 0.0B	60.8	<0.01
F	9.3	289.9	371.7	–		
P	<0.01	<0.01	<0.01	–		

Within each column, means followed by the same lowercase letter are not significantly different, in all cases df = 15, 143; HSD test at 0.05. Within each row, means followed by the same uppercase letters are not significantly different, in all cases df = 3, 35; HSD test at 0.05. Where no letters exist, no significant differences were recorded. Where dashes exist, no analysis was conducted.

Table 8. Mean survival (out of 10 individuals per vial ± SE) of *O. surinamensis* acclimated or nonacclimated larvae at different temperatures and exposure intervals (n = 9)

	Temperature					
Exposure/condition	0°C	–5°C	–10°C	–15°C	*F*	*P*
0 h acclimated	8.1 ± 0.3abcdB	4.0 ± 0.7cdC	8.1 ± 0.6aB	9.9 ± 0.1aA	29.6	<0.01
0 h nonacclimated	8.7 ± 0.3abB	6.8 ± 0.5aC	8.1 ± 0.3aB	10.0 ± 0.0aA	15.8	<0.01
2 h acclimated	9.1 ± 0.4aA	0.4 ± 0.2eB	8.0 ± 0.5aA	0.0 ± 0.0bB	231.4	<0.01
2 h nonacclimated	5.4 ± 0.6efA	6.2 ± 0.5abA	0.7 ± 0.4bcB	0.0 ± 0.0bB	57.1	<0.01
4 h acclimated	8.4 ± 0.6abcA	0.2 ± 0.2eBC	1.3 ± 0.3bB	0.0 ± 0.0bC	136.8	<0.01
4 h nonacclimated	6.3 ± 0.5deA	5.0 ± 0.5bcA	0.0 ± 0.0cB	0.0 ± 0.0bB	83.4	<0.01
8 h acclimated	8.8 ± 0.4aA	0.1 ± 0.1eB	0.4 ± 0.2bcB	0.0 ± 0.0bB	319.6	<0.01
8 h nonacclimated	6.7 ± 1.1bcdeA	4.2 ± 0.5cdB	0.0 ± 0.0cC	0.0 ± 0.0bC	106.0	<0.01
1 d acclimated	7.9 ± 0.5abcdA	1.0 ± 0.4eB	0.0 ± 0.0cB	0.0 ± 0.0bB	144.5	<0.01
1 d nonacclimated	4.2 ± 0.4fA	3.2 ± 0.4dB	0.0 ± 0.0cC	0.0 ± 0.0bC	72.1	<0.01
2 d acclimated	6.4 ± 0.5cdeA	0.0 ± 0.0eB	0.0 ± 0.0cB	0.0 ± 0.0bB	164.1	<0.01
2 d nonacclimated	2.0 ± 0.5gA	0.1 ± 0.1eB	0.0 ± 0.0cB	0.0 ± 0.0bB	16.5	<0.01
3 d acclimated	5.0 ± 0.4efA	0.0 ± 0.0eB	0.0 ± 0.0cB	0.0 ± 0.0bB	150.0	<0.01
3 d nonacclimated	0.3 ± 0.2g	0.0 ± 0.0e	0.0 ± 0.0c	0.0 ± 0.0b	2.0	0.13
7 d acclimated	2.0 ± 0.4gA	0.0 ± 0.0eB	0.0 ± 0.0cB	0.0 ± 0.0bB	20.6	<0.01
7 d nonacclimated	0.0 ± 0.0g	0.0 ± 0.0e	0.0 ± 0.0c	0.0 ± 0.0b	–	–
F	54.6	53.0	168.5	14,953.0		
P	<0.01	<0.01	<0.05	<0.01		

Within each column, means followed by the same lowercase letter are not significantly different, in all cases df = 15, 143; HSD test at 0.05. Within each row, means followed by the same uppercase letters are not significantly different, in all cases df = 3, 35; HSD test at 0.05. Where no letters exist, no significant differences were recorded. Where dashes exist, no analysis was conducted.

Table 9. Mean survival (out of 10 individuals per vial ± SE) of *O. surinamensis* acclimated or nonacclimated eggs at different temperatures and exposure intervals (n = 9)

	Temperature					
Exposure/condition	0°C	–5°C	–10°C	–15°C	*F*	*P*
0 h acclimated	1.9 ± 0.4cdB	5.3 ± 0.6bA	4.9 ± 0.8bA	5.2 ± 0.5cA	7.7	<0.01
0 h nonacclimated	7.2 ± 0.6aAB	6.8 ± 0.6aB	8.1 ± 0.5aAB	9.1 ± 0.3aA	4.3	0.01
2 h acclimated	2.4 ± 0.3A	1.8 ± 0.5cA	0.6 ± 0.3dB	0.0 ± 0.0dB	12.8	<0.01
2 h nonacclimated	7.1 ± 0.5aA	5.1 ± 0.3bB	5.4 ± 0.3bB	8.0 ± 0.5bA	11.8	<0.01
4 h acclimated	1.8 ± 0.3cdeA	0.0 ± 0.0dB	0.1 ± 0.1dB	0.0 ± 0.0dB	25.9	<0.01
4 h nonacclimated	6.0 ± 0.4A	4.0 ± 0.5bB	2.6 ± 0.7cB	0.2 ± 0.2dC	25.2	<0.01
8 h acclimated	2.6 ± 0.3bcA	0.0 ± 0.0dB	0.0 ± 0.0dB	0.0 ± 0.0dB	57.2	<0.01
8 h nonacclimated	4.0 ± 0.5bA	1.2 ± 0.2cdB	1.6 ± 0.2cdB	0.0 ± 0.0dC	34.0	<0.01
1 d acclimated	2.1 ± 0.4cA	0.0 ± 0.0dB	0.0 ± 0.0dB	0.0 ± 0.0dB	36.1	<0.01
1 d nonacclimated	2.4 ± 0.5bcA	0.1 ± 0.1dB	0.4 ± 0.2dB	0.0 ± 0.0dB	16.2	<0.01
2 d acclimated	1.2 ± 0.4cdeA	0.0 ± 0.0dB	0.0 ± 0.0dB	0.0 ± 0.0dB	11.3	<0.01
2 d nonacclimated	1.1 ± 0.4cdeA	0.0 ± 0.0dB	0.0 ± 0.0dB	0.0 ± 0.0dB	6.9	<0.01
3 d acclimated	0.8 ± 0.3cdeA	0.0 ± 0.0dB	0.0 ± 0.0dB	0.0 ± 0.0dB	5.8	<0.01
3 d nonacclimated	0.0 ± 0.0e	0.0 ± 0.0d	0.0 ± 0.0d	0.0 ± 0.0d	–	–
7 d acclimated	0.0 ± 0.0e	0.0 ± 0.0d	0.0 ± 0.0d	0.0 ± 0.0d	–	–
7 d nonacclimated	0.1 ± 0.1de	0.0 ± 0.0d	0.0 ± 0.0d	0.0 ± 0.0d	1.0	0.41
F	40.4	67.0	62.2	238.0		
P	<0.01	<0.01	<0.01	<0.01		

Within each column, means followed by the same lowercase letter are not significantly different, in all cases df = 15, 143; HSD test at 0.05. Within each row, means followed by the same uppercase letters are not significantly different, in all cases df = 3, 35; HSD test at 0.05. Where no letters exist, no significant differences were recorded. Where dashes exist, no analysis was conducted.

changed with the increase of the exposure interval. All the above indicate that acclimation and cold tolerance is a very complex procedure, and may be specific within species, life stage, and experimental conditions (Andreadis and Athanassiou 2017).

Adults of both species reacted the same way to acclimation, as the previous exposure to +15°C for 1 wk had no effect on their survival but notably affected their susceptibility to cold. Thus, at exposures ≥2 h for both tested species, acclimated adults were more cold tolerance than nonacclimated adults. For example, only acclimated *T. confusum* and *O. surinamensis* adults were able to survive for 2 d at –5°C and for 8 h at –10°C, respectively, which is indicative of the positive effect of acclimation. Fields et al. (1998) documented that the levels of the sugar trehalose and the amino acid proline were high in acclimated adults of the rusty grain beetle, *Cryptolestes ferrugineus*

(Stephens) (Coleoptera: Laemophloeidae) and the granary weevil, *Sitophilus granarius* (L.) (Coleoptera: Curculionidae), which may affect their cold tolerance. Both trehalose and proline can solidify membranes under cold pressure (Crowe et al. 1983, Rudolph and Goins 1991).

Nonacclimated pupae of *T. confusum* were equally susceptible to cold compared with acclimated pupae at short exposures to low temperatures, and in some combinations, survival of nonacclimated pupae was higher. However, with the increase of the exposure interval, survival of acclimated pupae decreased. Pupal survival was assessed through adult emergence, which was estimated at 7 d after the termination of each treatment. However, adult emergence was checked periodically even after this interval to detect possible delayed effects (delayed adult emergence). These observations showed no delayed emergence for any of the combinations tested. However, additional experimental work is required to estimate possible changes in the life table characteristics of the adults that emerged from the treated pupae and the effect of acclimation on these adults. In a previous study, larvae of *T. castaneum* that hatched from 0 to 2-d-old eggs previously exposed to −18°C, did not complete development to the adult stage, indicating a delayed mortality effect (Arthur et al. 2015).

Larvae of *T. confusum* showed an interesting alteration in susceptibility with the increase of the exposure interval at 0°C. Thus, at this temperature, the increase of exposure revealed that nonacclimated larvae were more tolerant to cold than acclimated ones. This trend was reversed when larvae were exposed to −5°C, suggesting that there is a 'critical threshold' that triggers cold hardening in acclimated larvae, which is expressed at subzero temperatures. Larvae of *O. surinamensis* also gave mixed results in their cold tolerance, as in some of the combinations tested, nonacclimated larvae were more tolerant, even at temperatures that were lower than 0°C. Robinson (1926) and Evans et al. (1983) found that acclimation did not lead to an increased cold tolerance of *S. granarius*, while similar results have also been reported for agricultural pests (Popham et al. 1991, Hemmati et al. 2014). Further experimentation is needed to clarify this issue.

Eggs showed a different response than the other life stages. Acclimation reduced egg survival for *O. surinamenis*, suggesting that eggs of this species are susceptible even to temperatures as low as +15°C. In this context, the decreased egg survival that was observed after short exposure of the acclimated eggs to cold can be considered more as a direct consequence of the negative effect of previous acclimation, and not as an increased cold tolerance of nonacclimated eggs at these exposures. The temperature increase reversed the difference between acclimated and nonacclimated eggs, and showed that acclimated eggs were more tolerant to cold, regardless of the temperature level. In contrast with *O. surinamensis*, eggs of *T. confusum* that had not been exposed to cold were not affected by acclimation, while exposure to cold showed increased cold tolerance hardening in acclimated eggs.

In the current tests, exposure of nonacclimated and acclimated *T. confusum* at −10°C for 1 d produced complete control of all life stages. Similarly, almost all (99.6%) nonacclimated and acclimated individuals of *O. surinamensis* died at −10°C at 1 d. Consequently, this temperature–exposure combination can be proposed for practical utilization of cold treatments against both species. Differences between acclimated and nonacclimated individuals were most apparent at 2 and 4 h exposures and higher temperatures. For example, at −10°C and 4 h of exposure, survival of acclimated *T. confusum* adults was about 4× greater compared with nonacclimated adults, while at −15°C and 2 h exposure, survival was only about 1.5× greater compared with nonacclimated adults. Similar results were obtained for pupae and eggs of *T. confusum*, in that decreasing the temperature, and shortening the exposure interval from 4 to 2 h, resulted in greater survival of acclimated compared with nonacclimated pupae and eggs. However, for *O. surinamensis* adults, there was no survival at −15°C at any exposure interval, but at 2 and 4-h exposure intervals, survival of acclimated adults was 2 to 8× greater compared with nonacclimated adults. For eggs, survival of acclimated *O. surinamensis* exposed for 2 h was about 4 to 8× greater at 0, −5, and −10°C, compared with nonacclimated eggs. Thus, there was an interaction between temperature and eggs, but at the shortest time of 2 h at −10°C, survival of acclimated adults, pupae, and eggs of *T. castaneum*, and adults and eggs of *O. surinamensis*, was generally 2 to 8× greater compared with nonacclimated life stages.

From a practical point of view, previous exposure to low temperatures may have a certain acclimation effect, which can increase, instead of decrease, survival after applications that lead to temperature decrease, such as chilling/aeration in grain silos. Moreover, in areas with mild winter, the exposure to low temperatures may result in increased cold hardiness, and a further decrease of the temperature may strengthen this phenomenon. In other words, cooling the grain may be beneficial, but at the same time may lead to development of insects that can tolerate low temperatures. Hence, the time of cooling/chilling applications should be carefully selected, as overwintering individuals of the target species may be cold tolerant, which may lead to an unexpected population growth after the application, when conditions prevailing are suitable. However, if adequate temperature and exposure time can be achieved, both acclimated and nonacclimated conditions can be used to control these two species.

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