

Survival of Indianmeal Moth and Navel Orangeworm (Lepidoptera: Pyralidae) at Low Temperatures

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J. Econ. Entomol. 100(4): 1482–1488 (2007)

ABSTRACT Concerns over insect resistance, regulatory action, and the needs of organic processors have generated renewed interest in developing nonchemical alternative postharvest treatments to fumigants used on dried fruits and nuts. Low-temperature storage has been identified as one alternative for the Indianmeal moth, *Plodia interpunctella* (Hübner), and navel orangeworm, *Amyelois transitella* (Walker) (Lepidoptera: Pyralidae), common postharvest pests in California dried fruits and nuts. The response of eggs, nondiapausing larvae, and pupae of both species to exposure to low temperatures (0, 5, and 10°C) was evaluated. Eggs of both species were the least tolerant of low temperatures. At 0 and 5°C, pupae were most tolerant, but at 10°C, nondiapausing larvae of both species were most tolerant, with lethal time (LT)₉₅ values of 127 and 100 d for Indianmeal moth and navel orangeworm, respectively. The response of diapausing Indianmeal moth larvae to subfreezing temperatures also was evaluated. Diapausing larvae were very cold tolerant at -10°C, with LT₉₅ values of 20 and 17 d for long-term laboratory and recently isolated cultures, respectively. Diapausing larvae were far less tolerant at lower temperatures. At -15°C, LT₉₅ values for both cultures were <23 h, and at -20°C, LT₉₅ values were <7 h. Refrigeration temperatures of 0–5°C should be useful in disinfesting product contaminated with nondiapausing insects, with storage times of 3 wk needed for adequate control. Relatively brief storage in commercial freezers, provided that the temperature throughout the product was below -15°C for at least 48 h, also shows potential as a disinfestation treatment, and it is necessary when diapausing Indianmeal moth larvae are present.

KEY WORDS cold treatment, Indianmeal moth, navel orangeworm, dried fruits, tree nuts

The central valley of California produces nearly all of the almonds, *Amygdalus communis* L.; pistachios, *Pistacia vera* L., walnuts, *Juglans regia* L., raisins, *Vitis vinifera* L., dried plums, *Prunus domestica* L., and figs, *Ficus carica* L., in the United States, resulting in an annual production of >1.4 million metric tons of commodity valued at >\$3 billion (USDA 2006). A major problem in the storage and marketing of these products is infestation by a variety of postharvest insect pests, including field pests of possible phytosanitary importance such as navel orangeworm, *Amyelois transitella* (Walker), and common stored-product pests such as Indianmeal moth, *Plodia interpunctella* (Hübner) (Simmons and Nelson 1975). Currently, California dried fruit and tree nut processors depend on fumigation with methyl bromide or phosphine to disinfest large volumes of incoming product after harvest, and to control infestations during storage. Regulatory actions against methyl bromide (UNEP 2006) as well as insect resistance to hydrogen phosphide (Ben-

halima et al. 2004), may make these fumigants costly or unavailable to the nut industry. In addition, as the organic industry expands the need for nonchemical postharvest insect control methods increases. These recent concerns over resistance, regulatory action, and the needs of the organic industry have generated a renewed interest in developing nonchemical alternative treatments.

Low temperatures have been used to control postharvest insects, either by slowing developing populations through chilled or ambient air aeration (Mason and Strait 1998, Arthur and Siebenmorgen 2005) or by disinfesting product by exposure to lethal temperatures (Gould 1994, Johnson and Valero 2003). Temperatures of ≤10°C also were shown to prevent reinfestation of clean product (Johnson et al. 1998, 2002). Dried fruit and nut processors may use cold storage at temperatures of 0–5°C to maintain product quality (Hardenburg et al. 1986). Although these temperatures are useful in preventing reinfestation of clean product, because they are below developmental thresholds for most stored-product insects, they also should provide some degree of disinfestation, given suitable exposure periods. Processors of organic almonds and walnuts use freezing treatments of 1–2 wk

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to disinfest product of field and storage pests (Asai et al. 1992, Klonsky et al. 1994), but there seems to be little documentation of the efficacy of this treatment. In this study, I determined the relative susceptibility of various life stages of Indianmeal moth and navel orangeworm to 0, 5, and 10°C at different humidity levels, and I also examined the response of diapausing Indianmeal moth larvae to -10, -15, and -20°C.

Materials and Methods

Test Insects. All test insects were from San Joaquin Valley Agricultural Sciences Center (SJVASC) laboratory cultures maintained on a wheat bran diet (Tebets et al. 1978). Rearing conditions for nondiapausing larvae were 27°C, 60% RH, and a photoperiod of 14:10 (L:D) h. The navel orangeworm culture was originally obtained in 1966 from the University of California, Berkeley, and the laboratory Indianmeal moth culture was originally obtained from a walnut packinghouse in Modesto, CA, in November 1967. I also used recently isolated Indianmeal moth from a culled fig warehouse in Fresno, CA. Moths were collected directly or oviposition traps baited with wheat bran diet were used to obtain new isolates from the culled fig warehouse every year of the study.

Survival of Eggs at Low Temperatures. Indianmeal moth eggs were obtained from adults 1–2 d after their emergence. Oviposition jars containing 100–150 adults of both sexes were set up and placed in environmental chambers kept at the above-listed rearing conditions. Eggs were collected 16 h later. Scales were removed from eggs by gently shaking the collection container under a fume hood. The eggs were then passed through a 32-mesh brass screen to remove clumps of eggs and moth body parts. An analytical balance was used to weigh out 2.1 mg of eggs (≈ 100 eggs) into dishes made from hollow plastic test tube caps (9 mm in height, 13 mm in diameter). The bottoms of the caps were cut off, and 100-mesh brass screen was heat sealed to the cut edges. Plastic petri dishes lined with plastic packing foam (3 mm thick) held the egg dishes. Holes punched through the foam with a cork borer securely held the egg dishes and kept them from tipping during treatment. Indianmeal moth eggs were held under rearing conditions until tested 1, 24 and 48 h after collection, when egg age was 9 ± 8 , 32 ± 8 , and 56 ± 8 h, respectively.

Navel orangeworm eggs were collected over a 24-h period from adults 2–3 d old. Paper towels were used as an oviposition substrate. Strips with ≈ 50 eggs were cut from the paper towels. The paper strips were held in plastic petri dishes during treatment. Navel orangeworm eggs were treated when $\approx 15 \pm 12$, 39 ± 12 , and 63 ± 12 h old. Specific egg ages for both species were selected because earlier work showed a difference in cold tolerance between young, middle-aged, and old eggs (Johnson et al. 1997).

Petri dishes with eggs were placed in plastic shoe boxes covered with plastic wrap. Small jars (100 ml) containing glycerol solutions were used to maintain the relative humidity within the shoe box at ≈ 20 , 50 or

80%. The glycerol solutions were adjusted to certain specific gravities, measured with a hydrometer, to obtain the desired relative humidities (Braun and Braun 1958). Data loggers (Omnicdata, Logan, UT) were used to record both temperature and relative humidity in the shoe boxes. The shoe boxes were placed in constant temperature rooms held at 0, 5, or 10°C. Because we had earlier examined Indianmeal moth egg mortality at 10°C (Johnson et al. 1997), only navel orangeworm eggs were placed at 10°C in the current study. Also, only two humidity levels (20 and 80%) were used at 10°C.

For each exposure, two Indianmeal moth egg dishes and two navel orangeworm egg strips were removed and placed in petri dishes containing wheat bran diet. The petri dishes were held under rearing conditions for 7–10 d before hatched and unhatched eggs were counted. At each temperature, four to five exposures were used. Eggs placed in shoe boxes maintained at rearing conditions for 24 h were used as controls.

Survival of Larvae and Pupae at Low Temperatures. Last (sixth or fifth) instars of navel orangeworm and nondiapausing fifth instars of Indianmeal moth were obtained from laboratory cultures maintained under rearing conditions. Indianmeal moth pupae were removed from corrugated cardboard strips placed along the inside walls of culture jars as pupation sites. Navel orangeworm pupae were obtained by placing mature fifth instars in 12- by 75-mm glass culture tubes and holding them until pupation. Because mature navel orangeworm larvae are capable of chewing through plastic tube caps, the culture tubes were inverted into larger tubes (15 by 85 mm) to prevent escape of the larvae.

We placed 25 test larvae or pupae of either species in 18- by 63-mm vials made from 32 mesh stainless steel screen and capped with cork stoppers. The vials were held in plastic desiccator jars where humidity levels of ≈ 20 , 50, or 80% were maintained with glycerol solutions. The desiccator jars were held in environmental chambers kept at 0, 5, and 10°C. Data loggers (Onset Computer, Bourne, MA) were used to record both temperature and relative humidity in the desiccator jars. Three vials of each species and stage were removed at the desired intervals. After exposure, vials were opened and placed in 0.4-liter canning jars with ≈ 10 g of rearing diet. The jars were held under rearing conditions for 1–2 wk before evaluation. Response of test insects was based on development to the next stage; survival was determined by the number of test larvae successfully pupating, and the number of pupae successfully emerging as adults.

For all temperatures, both species and stages were tested at the same time. However, initial tests at 10°C showed that the exposures chosen were inadequate to estimate 95% mortality of larvae for either species. Consequently, separate tests for larvae with prolonged exposures at 10°C were added to the study. For all tests, controls were three vials of test insects kept at 28°C in desiccator jars at the three relative humidity levels for 24 h. Five treatment exposures were used for

Table 1. Lethal times (d) for navel orangeworm (NOW) and Indianmeal moth (IMM) eggs of three different ages exposed to low temperatures

Insect	Age (h)	n	Slope (±SE)	Intercept (±SE)	LT ₅₀	95% CL		LT ₉₅	95% CL	
						Lower	Upper		Lower	Upper
0°C										
IMM	9 ± 8	13,235	0.69 ± 0.013	-0.95 ± 0.026	1.4	1.1	1.6	3.8	3.4	4.2
	32 ± 8	14,110	0.57 ± 0.010	-1.21 ± 0.024	2.1	1.7	2.5	5.0	4.3	6.1
	56 ± 8	14,117	1.10 ± 0.019	-1.55 ± 0.032	1.4	1.3	1.6	2.9	2.7	3.2
NOW	15 ± 12	6,650	2.24 ± 0.064	-0.86 ± 0.042	0.4	0.07	0.7	1.1	0.8	2.0
	39 ± 12	6,691	0.92 ± 0.023	-1.03 ± 0.037	1.1	0.9	1.3	2.9	2.6	3.3
	63 ± 12	6,749	1.20 ± 0.018	-0.99 ± 0.030	0.8	0.1	1.5	2.2	1.5	3.5
5°C										
IMM	9 ± 8	12,502	0.38 ± 0.006	-1.17 ± 0.026	3.0	1.6	4.2	7.3	5.8	10.7
	32 ± 8	13,141	0.39 ± 0.005	-1.65 ± 0.028	4.3	4.0	4.5	8.5	8.1	8.9
	56 ± 8	13,528	0.52 ± 0.008	-1.41 ± 0.027	2.7	2.1	3.3	5.9	5.0	7.3
NOW	15 ± 12	8,048	0.46 ± 0.012	-0.43 ± 0.028	0.9	0.5	1.3	4.5	3.9	5.3
	39 ± 12	7,922	0.46 ± 0.010	-0.92 ± 0.029	2.0	1.8	2.2	5.6	5.2	6.0
	63 ± 12	8,083	0.46 ± 0.008	-1.39 ± 0.031	3.0	2.8	3.3	6.6	6.3	7.1
10°C										
IMM ^a	9 ± 8	10,313	0.30 ± 0.005	-1.87 ± 0.033	6.1	5.3	6.9	11.5	10.3	13.3
	32 ± 8	10,173	0.34 ± 0.006	-2.86 ± 0.050	8.4	7.5	9.4	13.3	11.8	15.6
	56 ± 8	7,868	0.37 ± 0.007	-1.92 ± 0.042	5.2	4.4	6.0	9.6	8.5	11.3
NOW	15 ± 12	2,851	0.42 ± 0.014	-1.53 ± 0.079	3.6	3.3	3.9	7.5	7.2	7.9
	39 ± 12	2,799	0.36 ± 0.012	-1.86 ± 0.084	5.1	4.4	5.7	9.7	8.9	10.7
	63 ± 12	2,913	0.36 ± 0.012	-1.67 ± 0.079	4.6	3.9	5.3	9.2	8.4	10.2

^a Data from Johnson et al. (1997).

pupae at all temperatures and larvae at 0 and 5°C. Larvae at 10°C were treated at eight to 10 exposures.

Survival of Diapausing Indianmeal Moth Larvae at Subfreezing Temperatures. To determine the exposure times needed for control of diapausing Indianmeal moth larvae, I used both our long-term laboratory culture and cultures recently isolated from culled figs. For each replicate, a new culture from culled figs was isolated one to three generations before treatment. Diapausing larvae were reared in 0.48-liter plastic deli cups containing ≈125 g of wheat bran diet. Approximately 100 Indianmeal moth eggs from either culture were added to each cup. I modified the plastic lids of the cups by punching a 25-mm hole in the center and taping organdy cloth over the hole to provide ventilation. The cups were held under ambient laboratory conditions for 1 wk and then moved to a protected, unheated outside area in early November, to allow the larvae to enter diapause under natural conditions. Larvae were tested 8–12 wk later.

Because Indianmeal moth diapausing larvae are considerably more cold tolerant than other stages, I used subfreezing treatment temperatures of -10, -15, and -20°C. For the -10 and -15°C treatment temperatures, 10 exposure periods from 12 to 480 h and from 6 to 120 h, respectively, were used and seven exposure periods from 3 to 24 h were used at the -20°C treatment temperature. Treatment temperatures were applied in an upright Ultra Low Temperature Freezer (Revco Scientific, Asheville, NC). Deli cups containing diapausing larvae were placed in the freezer, and three cups for each culture were removed after each of the exposure periods. Three untreated cups from each culture kept at 28°C were used for controls. After treatment, cups were held under rearing conditions until adult emergence. Treatment survival was estimated by comparing adult emergence

from treatments with that from untreated controls. The test was replicated four times at -15 and -20°C and three times at -10°C.

Data Analysis. All survival data were analyzed using the probit regression procedure in SPSS 12.0 for Windows (SPSS Inc. 2003). When appropriate because of overlapping confidence limits, data for different relative humidity levels were pooled. For each species and nondiapausing stage, lethal time (LT)₉₅ estimates were plotted against treatment temperature and curves were fitted using SigmaPlot 9.01 for Windows (Systat Software, Inc. 2004).

Results

Survival of Eggs at Low Temperatures. Estimated exposure times needed for 50 and 95% mortality of Indianmeal moth and navel orangeworm eggs are given in Table 1. Because no differences were detected among relative humidity levels based on overlapping confidence limits, the data were pooled before further analysis.

Middle-age Indianmeal moth eggs (32 ± 8 h old) were consistently the most tolerant to all treatment temperatures. The difference was significant at 0 and 10°C, based on nonoverlapping confidence limits at the LT₅₀ mortality level. At 5°C, the response of younger eggs (9 ± 8 h old) was similar to middle-aged eggs, whereas older eggs (56 ± 8 h old) were significantly less tolerant.

The response of middle-aged (39 ± 12 h) and older (63 ± 12 h) navel orangeworm eggs to 0 and 10°C was similar, and it was significantly more tolerant, based on nonoverlapping confidence limits at the LT₅₀ mortality level, than younger (15 ± 12 h) eggs. At 5°C, older eggs were most tolerant, and middle-aged eggs were more tolerant than younger eggs. At all temperatures,

Table 2. Lethal times (d) for navel orangeworm (NOW) and Indianmeal moth (IMM) larvae and pupae exposed to low temperatures

Insect	Stage	n	Slope (±SE)	Intercept (±SE)	LT ₅₀	95% CL		LT ₉₅	95% CL		
						Lower	Upper		Lower	Upper	
0°C											
IMM	Larva	1,322	0.33 ± 0.017	-1.02 ± 0.093	3.1	2.3	3.8	8.1	7.2	9.3	
	Pupa	1,341	0.28 ± 0.013	-1.28 ± 0.090	4.6	3.8	5.2	10.4	9.4	11.9	
NOW	Larva	1,337	0.64 ± 0.037	-1.07 ± 0.104	1.7	-0.3	3.5	4.3	2.7	9.8	
	Pupa	1,353	0.35 ± 0.017	-1.08 ± 0.090	3.1	2.3	3.7	7.7	6.9	8.9	
5°C											
IMM	Larva	1,316	0.17 ± 0.009	-0.91 ± 0.081	5.3	2.8	7.3	14.8	12.1	19.9	
	Pupa	1,138	0.15 ± 0.008	-0.77 ± 0.076	5.1	3.8	6.2	16.0	14.3	18.3	
NOW	Larva	1,330	0.27 ± 0.016	-1.33 ± 0.105	4.8	3.9	5.7	10.9	9.6	12.7	
	Pupa	1,157	0.14 ± 0.007	-0.96 ± 0.074	6.8	5.4	8.1	18.4	16.3	21.4	
10°C											
IMM	Larva	1,912	0.03 ± 0.001	-1.60 ± 0.082	62.6	57.7	67.2	127.1	118.9	137.7	
	Pupa	1,338	0.14 ± 0.008	-1.09 ± 0.100	7.5	6.0	8.9	18.9	17.0	21.3	
NOW	Larva	2,440	0.03 ± 0.001	-1.09 ± 0.066	39.7	34.2	44.3	99.6	90.9	111.8	
	Pupa	1,352	0.11 ± 0.006	-0.93 ± 0.088	8.4	6.0	10.4	23.3	20.6	27.2	

the most tolerant Indianmeal moth egg age was significantly more tolerant than the most tolerant navel orangeworm egg age.

Survival of Larvae and Pupae at Low Temperatures. Estimated exposure times needed for 50 and 95% mortality of Indianmeal moth and navel orangeworm larvae and pupae exposed to low temperatures are given in Table 2. As with the eggs, there was no consistent difference between relative humidity levels, consequently data were pooled before further analysis.

Larvae and pupae of both moth species were more tolerant than eggs. Indianmeal moth pupae were significantly more tolerant to 0°C than the other stages. Response of Indianmeal moth larvae, navel orangeworm larvae and navel orangeworm pupae to 0°C was similar, as was the response of larvae and pupae of both species to 5°C, based on overlapping confidence limits for LT₅₀ mortality level. At 10°C, the response of Indianmeal moth and navel orangeworm pupae was similar, but larvae of both species were considerably more tolerant. The estimated LT₉₅ for navel orangeworm larvae (99.6 d) was >4 times that of navel orangeworm pupae (23.3 d), whereas the LT₉₅ for Indianmeal moth larvae (127.1 d) was nearly 7 times that for Indianmeal moth pupae (18.9 d). Indianmeal moth larvae were also significantly more tolerant than navel orangeworm larvae at 10°C.

When LT₉₅ values were plotted against treatment temperature for all life stages and species (Fig. 1), the difference in the response of larvae from the response of pupae and eggs is clearer. For pupae and eggs, the response curve fit a simple linear regression, with r² > 0.95. For larvae of both species, the response curve was perfectly described by a three-parameter exponential growth curve.

Survival of Diapausing Indianmeal Moth Larvae at Subfreezing Temperatures. The response of diapausing Indianmeal moth larvae to subfreezing temperatures is summarized in Table 3. Based on overlapping confidence limits at the LT₅₀ mortality level, there was no significant difference between the laboratory culture and the recently isolated cultures. Diapausing larvae from both cultures withstood prolonged exposure to -10°C, with estimated LT₉₅ values of 482.8 and

410.4 h (20 and 17 d) for the laboratory and recently isolated cultures, respectively. Decreasing the treatment temperature reduced survival dramatically. For the laboratory and recently isolated cultures, respectively, LT₉₅ values were 14.4 and 22.6 h at -15°C and 6.2 and 6.9 h at -20°C.

Discussion

Earlier studies have noted that eggs of Indianmeal moth may differ in their response to low temperatures. Cline (1970) found that Indianmeal moth eggs <4.5 h old were most susceptible to 2.4°C, requiring only 5 d of exposure for complete mortality, whereas eggs 17.5–72.0 h old required at least 8 d. Lewthwaite et al. (1998), in studying the use of cold storage after heat treatments for control of Indianmeal moth, concluded that 3-d-old eggs (48–72 h old) were most tolerant and that 1-d-old eggs (0–24 h old) were most susceptible to temperatures of 0.5–12°C. In contrast, I found

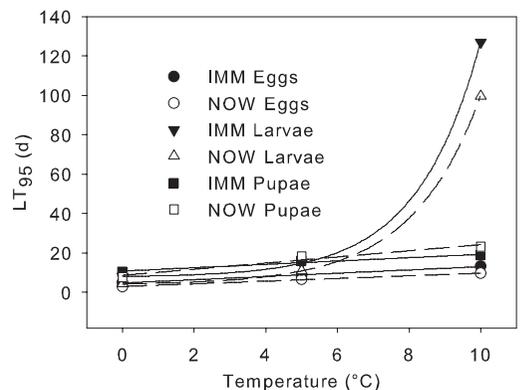


Fig. 1. Comparison of estimated LT₉₅ values for eggs, larvae, and pupae of Indianmeal moth and navel orangeworm. Lines represent regression equations where y is LT₉₅ (d) and x is temperature (°C). Indianmeal moth (IMM) eggs: $y = 4.78 + 0.83x$. Navel orangeworm (NOW) eggs: $y = 3.00 + 0.68x$. IMM pupae: $y = 10.85 + 0.85x$. NOW pupae: $y = 8.67 + 1.56x$. IMM larvae: $y = 7.67 + 0.43e^{0.56x}$. NOW larvae: $y = 3.77 + 0.53e^{0.52x}$.

Table 3. Lethal times (h) for diapausing Indianmeal moth from a SJVASC laboratory culture and a recently isolated culture

Culture	n	Slope (±SE)	Intercept (±SE)	LT ₅₀	95% CL		LT ₉₅	95% CL	
					Lower	Upper		Lower	Upper
-20°C									
Lab	4,605	5.60 ± 0.216	-2.78 ± 0.138	3.1	2.1	3.9	6.2	4.9	10.3
Wild	4,210	5.17 ± 0.188	-2.69 ± 0.125	3.3	2.7	3.9	6.9	5.8	9.2
-15°C									
Lab	7,904	4.21 ± 0.143	-3.24 ± 0.139	5.9	4.6	6.9	14.4	12.2	18.8
Wild	6,088	2.51 ± 0.091	-1.76 ± 0.104	5.0	2.4	7.3	22.6	16.7	38.9
-10°C									
Lab	7,389	1.68 ± 0.039	-2.88 ± 0.073	51.0	33.8	72.3	482.8	259.4	1,669.4
Wild	5,940	1.95 ± 0.046	-3.46 ± 0.086	59.0	36.7	90.9	410.4	212.3	1,907.2

middle-aged Indianmeal moth eggs (32 ± 8 h old) to be more tolerant than either younger eggs (9 ± 8 h old) or older eggs (56 ± 8 h old). This agrees with Johnson and Wofford (1991) where middle-aged Indianmeal moth eggs (27 and 51 ± 6 h old) were most tolerant to -15 and -19°C .

I found the response of the most tolerant age of Indianmeal moth eggs (LT₉₅ values of 5 d at 0°C and 8.5 d at 5°C) to be similar to that reported by Cline (1970) (96% mortality after 7 d exposure to 2.4°C). Lewthwaite et al. (1998) calculated far longer exposure times for 99% mortality of all ages of Indianmeal moth eggs (19.8 d at 0.5°C and >22 d at 4 – 12°C). In contrast, Adler (1960) reported 100% mortality of Indianmeal moth eggs after only 5 h of exposure to 4°C . These variable results may be due to differences in treatment application, test insects, and analytical methods.

Le Torc'h (1977) found Indianmeal moth larvae to be relatively tolerant of low temperatures, with exposures of at least 40 d to 2°C necessary for 95% mortality. White et al. (1994) reported similar results with nondiapausing Indianmeal moth, showing that $\approx 95\%$ mortality occurred after a 50-d exposure to 0°C . I found Indianmeal moth larvae to be far more susceptible to temperatures $\leq 5^\circ\text{C}$, with LT₉₅ estimates of 8 d at 0°C and 15 d at 5°C . Although this discrepancy may be due in part to differences in test insects, I suspect that it is largely the result of my using successful pupation to determine posttreatment survival in the larval stage. Larvae that were noted to be living 24 h after treatment often were unable to pupate.

Stratil and Reichmuth (1984) examined the response of neonate Indianmeal moth larvae to temperatures of 2 – 10°C . Complete mortality occurred after a 10–12 d exposure to 2 – 4°C , and after a 21–22 d exposure to 6 – 8°C . At 10°C , a 56 d exposure was needed to obtain 97% mortality. Our results were comparable with those of Stratil and Reichmuth (1984), with relatively short exposures needed for 95% mortality at 0 and 5°C and extended exposures needed for 95% mortality at 10°C .

Tebbetts et al. (1978) evaluated the response of different life stages of navel orangeworm to 3.5°C , noting that 1–3-d-old pupae were the most tolerant (LT₉₅ = 21.4 d). Eggs were found to be the most susceptible to cold, with 0–1-d-old eggs (LT₉₅ = 5.1 d) being less tolerant than 3–4-d-old eggs (LT₉₅ = 8.8 d).

This agrees with our observation that the youngest age of navel orangeworm eggs was the least tolerant of all life stages at all treatment temperatures and that navel orangeworm pupae were most cold tolerant at 0 and 5°C .

My results show that refrigeration temperatures of 0 – 5°C , often used by dried fruit and nut processors to maintain product quality, should be useful in disinfesting product contaminated with nondiapausing insects. I found the upper 95% CL of the LT₉₅ for the most tolerant nondiapausing stage (navel orangeworm pupae) to be ≈ 21 d at 5°C , suggesting that 3 wk should provide adequate control. However, 10°C , which has been suggested for preventing reinfestation of clean product (Johnson et al. 1998, 2002), would not be a practical disinfestation temperature because of the long treatment times required for larvae (≈ 20 wk for Indianmeal moth larvae).

As expected, I found diapausing Indianmeal moth larvae to be the most cold tolerant life stage. The LT₉₅ estimates for diapausing larvae exposed to -10°C (20 and 17 d for laboratory and recently isolated cultures, respectively) was considerably longer than LT₉₅ estimates for eggs (5 d), nondiapausing larvae (8 d) and pupae (10.4 d) exposed to 0°C . White et al. (1994) found acclimated diapausing Indianmeal moth larvae to be very tolerant to 0°C , with an exposure of 63 d resulting in only 50% mortality. In the same study, $\approx 90\%$ mortality occurred in acclimated diapausing larvae after a 14 d exposure to -10°C . Carrillo et al. (2006) noted that 100% mortality occurred after just 12 h exposure to -10°C in laboratory-reared (nondiapausing) fifth instars of Indianmeal moth, whereas field-collected, cold-acclimated (diapausing) fifth instars required exposure of 312 h (13 d) for 100% mortality.

Diapausing larvae from both cultures were far less tolerant to -15 and -20°C , with LT₉₅ estimates of <24 h. The temperature at which spontaneous freezing occurs, known as the supercooling point (SCP), is often used to predict the response of specific insects to cold (Bale 1987). The SCP for cold-acclimated (diapausing) fifth instars of Indianmeal moth has been determined to be between -14 and -24°C (White et al. 1994; Naeemullah et al. 1999; Carrillo et al. 2005, 2006). In earlier studies (Johnson et al. 1992), I found the mean SCP for diapausing fifth instars from our laboratory cultures to be -22.6°C , comparable with

those recorded by Carrillo et al. (2005, 2006). I feel that the relatively rapid mortality at -15 and -20°C is because treatment temperatures were approaching the SCP, although I cannot be sure that death was due to freezing or chilling injury. Casual observation of larvae after lethal exposure to -15 and -20°C showed them to be frozen, but whether freezing occurred pre- or postmortem is unknown. Carrillo et al. (2005) showed that the lower lethal temperature (the estimated temperature where 50% of the population died when exposed to a constant time of 1 min) for cold-acclimated fifth instars (diapausing) was -20.65°C , and they suggested that because this temperature was above the SCP the observed mortality was due to cumulative chilling injury rather than freezing.

On a practical level, the short exposures at -20°C needed for 95% mortality of the most cold tolerant stage of Indianmeal moth is of most interest because this temperature is very close to those used in commercial cold storage (-18°C [0°F]). This temperature should be sufficient to control both moth species because I found navel orangeworm to be less cold tolerant than Indianmeal moth, and there is no known, well-defined diapause stage for navel orangeworm. Consequently, relatively brief storage in commercial freezers, provided the temperature throughout the product was below -15°C for at least 48 h (upper 95% CL of LT_{95} at -15°C for recently isolated culture = 38.9 h), shows potential as a disinfestation treatment for both Indianmeal moth and navel orangeworm.

Normal postharvest handling of organic walnuts includes 1–2 wk of freezing treatment (Klonsky et al. 1994). Depending upon the size and configuration of the product bins or packages and their associated cooling rates, the recommended exposure period may be unnecessarily long. In earlier work on a freezing treatment for cowpea weevil, *Callosobruchus maculatus* (F.), we found that the center of bins of garbanzo beans, *Cicer arietinum* L., placed in commercial freezers took 14–19 d to reach -18°C (Johnson and Valero 2003). We estimated that a 24-h exposure to -18°C was sufficient to control all stages of cowpea weevils; however, because the slow cooling rate resulted in long exposures to subfreezing temperatures, high mortality (>98%) was found in cowpea weevil eggs (the most tolerant stage) in the center of the bins after only 7 d of exposure when the center temperature was between -5 and -10°C .

Acknowledgments

I thank Karen Valero for assistance in conducting this research and David Brandl (USDA-ARS, Parlier, CA) for technical advice. I also thank Peter Follett (USDA-ARS, Hilo, HI) and Joel Siegel (USDA-ARS, Parlier, CA) for reviewing the manuscript.

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Received 26 January 2007; accepted 19 May 2007.