

Effect of Relative Humidity and Product Moisture on Response of Diapausing and Nondiapausing Indianmeal Moth (Lepidoptera: Pyralidae) Larvae to Low Pressure Treatments

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ABSTRACT Low pressure treatment in flexible polyvinyl chloride containers is a potential alternative to chemical fumigants for California tree nuts. Laboratory studies investigated the effect of relative humidity and product moisture on weight loss and mortality of diapausing and nondiapausing larvae of the Indianmeal moth, *Plodia interpunctella* (Hübner) (Lepidoptera: Pyralidae), exposed to 50 mmHg. Diapausing larvae were far more tolerant than nondiapausing larvae to low pressure; exposure times nearly twice those of nondiapausing larvae were required to obtain comparable weight loss or mortality levels in diapausing larvae. Relative humidity was found to have a large effect on both weight loss (assumed to be due to moisture loss) and mortality of both nondiapausing and diapausing larvae. Mortality and weight loss increased as humidity levels decreased. By controlling the relative humidity of the treatment chamber, product moisture also strongly affected weight loss and mortality. The results suggest that for tree nuts, product moisture levels may affect the efficacy of low pressure treatments.

KEY WORDS vacuum treatment, relative humidity, product moisture, Indianmeal moth

The central Sacramento and San Joaquin valleys of California produce nearly all of the almonds [*Prunus dulcis* (Mill.) D.A. Webb], pistachios (*Pistacia vera* L.), and walnuts (*Juglans* L.) in the United States, each year resulting in the production of >900,000 metric tons of commodity valued at US\$3 billion (USDA 2008). These three products are also among the top 10 California agricultural exports, bringing into the California economy an average of >US\$2 billion each year (CDFA 2009). Almonds are currently the leading export for California and were responsible for 17% (US\$1.88 billion) of the US\$10.9 billion 2007 California export market (CDFA 2009).

A major problem in the storage and marketing of these products is infestation by a variety of postharvest insect pests. Of concern are field pests of phytosanitary importance such as navel orangeworm, *Amyelois transitella* (Walker), and codling moth, *Cydia pomonella* (L.), as well as the cosmopolitan stored product pest Indianmeal moth, *Plodia interpunctella* (Hübner). Currently, California tree nut processors depend on fumigation with methyl bromide, phosphine, or sulfur fentanyl fluoride to disinfest large volumes of incoming product after harvest and to control infestations during storage. Regulatory actions against methyl bro-

mide (UNEP 2006), insect resistance to phosphine (Benhalima et al. 2004), and the identification of sulfur fentanyl fluoride as a potentially significant greenhouse gas (Mühle et al. 2009) 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 (CCOF 2008). These recent concerns over resistance, regulatory actions and the needs of the organic industry have generated a renewed interest in developing nonchemical alternative treatments.

One possible nonchemical alternative is the use of low atmospheric pressures (vacuum) to disinfest product. Numerous researchers have examined the potential of low pressures for insect control, including Back and Cotton (1925), Bare (1948), Calderon et al. (1966), Navarro and Calderon (1979), and Al-Azawi et al. (1983), but the need for sturdy vacuum chambers to treat product limited the utility of the method to relatively small-scale applications. Flexible polyvinyl chloride containers developed for temporary grain storage (Navarro and Donahay 1985) were found to have utility as vacuum treatment enclosures, making the treatment more economical and practical (Navarro et al. 2001). Johnson and Zettler (2009) demonstrated the potential for vacuum treatments for tree nuts, and identified diapausing larvae as being relatively tolerant to low pressures.

The mode of action of low pressure treatments has been shown to be largely due to low oxygen tensions

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at high humidities (Navarro and Calderon 1979), and humidity may affect the response of test insects to low oxygen environments (Jay et al. 1971, Navarro 1978, Soderstrom et al. 1986, Ofuya and Reichmuth 2002). The relative humidity experienced by insects infesting stored tree nuts is largely a function of the moisture content of the product. To minimize fungal growth and oxidation, the recommended storage conditions for California tree nuts include relative humidity levels <65% and product moistures of 5–8% (López et al. 1995, Kader and Thompson 2002), although other references give optimal storage humidities of 60–70% (Codex Alimentarius 1972, 2006). Variations in farm-stored product and drier efficiency may result in product with more variable moisture contents and relative humidities, which may affect the response of target insects to vacuum treatments. This article investigates the effect of product moisture on vacuum treatment efficacy by examining mortality and weight loss of diapausing and nondiapausing Indianmeal moth larvae under low pressures at different relative humidities.

Materials and Methods

Test Insects. Indianmeal moths were from a laboratory culture at the San Joaquin Valley Agricultural Sciences Center (Parlier, CA), originally obtained from a walnut packinghouse in Modesto, CA, in November 1967 and maintained on a wheat bran diet (Tebbetts et al. 1978, Tebbets 2009). Rearing conditions for nondiapausing Indianmeal moth were $28 \pm 0.5^\circ\text{C}$, $60 \pm 8\%$ RH, and a photoperiod of 14:10 (L:D) h. Diapausing Indianmeal moth larvae were obtained by holding rearing jars recently infested with eggs under normal rearing conditions for 1 wk, then transferring the jars first to an environmental chamber held at 17°C for 1 wk and then to another environmental chamber held at 14°C for at least 4 wk. Both the 17°C and 14°C chambers were kept at a photoperiod of 10:14 (L:D) h. Under these conditions larvae from this isolate uniformly entered diapause and were recognized by their color, behavior, and increased size (Tsuji 1958).

Treatment Chambers. Treatments were done in cylindrical (45.7 cm in height by 30.5 cm in diameter) aluminum vacuum chambers (Laco Technologies, Salt Lake City, UT). Chambers were closed with clear acrylic lids and gasket seals and held in an environmental room kept at either 25 or 30°C . Chambers were connected with vacuum hose in series to a vacuum pump (model D25, Precision Scientific, Winchester, VA) and simultaneously pumped down to the target pressure of 50 mmHg (equivalent to 1.38% O_2 at normal atmospheric pressure). Pressure levels were determined with an absolute pressure capsule gauge (CG-100, Becker Pumps Co., Cuyahoga Falls, OH). Pressures within individual chambers also were recorded throughout each test with data loggers (PRTemp1000, PTC Instruments, Los Angeles, CA). Once the chamber pressures reached 50 mmHg (45–60 min), individual chambers were isolated and the treatment was considered to have started. Pressure levels were monitored and readjusted 2–3 times to 50

mm during the first 2 h of the treatment, after which time pressures remained stable at 50 ± 2 mmHg for the remainder of the test.

Treatment Protocol. To examine the effect of relative humidity and product moisture on vacuum efficacy, two different experiments were performed. In the first, humidity levels were obtained by placing on the bottom of each chamber a 200-ml beaker containing 200 ml of a glycerol solution calibrated to provide low, medium, and high humidity levels (≈ 30 , 50, and $80 \pm 8\%$ RH, respectively). These levels corresponded to vapor pressure deficits of ≈ 2.21 , 1.74, and 0.63 kPa at 25°C and 2.96, 2.33, and 0.85 kPa at 30°C , respectively. Glycerol solutions were mixed in bulk using the methods of Braun and Braun (1958), and checked using hydrometers. In the second experiment, two woven poly mesh bags containing ≈ 3800 cm^3 of inshell walnuts with either low or high relative moisture content were placed in each vacuum chamber. The desired nut moisture contents were achieved by holding the bags of walnuts in a 0.23- m^3 environmental chamber (30BL, Percival Scientific, Inc., Perry, IA) kept at 25°C in which a pan of either deionized water or 100% glycerol was placed. Nuts were held in the chambers until they reached equilibrium moisture content (at least 4 wk). Moisture contents of whole nuts were determined just before and after treatments. Nuts were ground to a fine meal using a RAS mill (Romer Labs, Union, MO), and the moisture content was determined with an infrared moisture balance (MB200, Ohaus, Pine Brook, NJ).

For both experiments, data loggers (Hobo H8 RH/TEMP, Onset Computer, Bourne, MA) were placed inside each chamber to record relative humidity levels. Three treatment chambers, one for each exposure, were used at each relative humidity level or product moisture. Exposures used for nondiapausing larvae were 12, 16, and 20 h at 25°C and 6, 8, and 10 h at 30°C . Exposures used for diapausing larvae were 18, 30, and 42 h at 25°C and 10, 15, and 20 h at 30°C . A single unevacuated chamber was used at each relative humidity level or product moisture as an untreated control, and test larvae were removed from these chambers at each of the above-mentioned exposure times.

Cages used to contain test larvae within chambers were 18- by 63-mm tubes made from 32 mesh stainless steel screen. Five tubes containing 15 fifth-instar diapausing or nondiapausing Indianmeal moth larvae were used for each treatment exposure. Tubes containing test larvae were closed with neoprene stoppers. Nondiapausing larvae were placed in tubes just before treatment. Because diapausing larvae are known to respond to fumigation treatments differently after being disturbed (Tebbetts et al. 1986), diapausing larvae were placed in tubes and held at 14°C and a photoperiod of 10:14 (L:D) h ≈ 2 –3 wk before treatment. Tubes containing test larvae were weighed just before and immediately after treatment on an analytical balance (AB104-S, Mettler Toledo Inc., Columbus, OH) to determine moisture loss. Because neoprene absorbs moisture, stoppers were removed before weight measurement. Larvae were removed

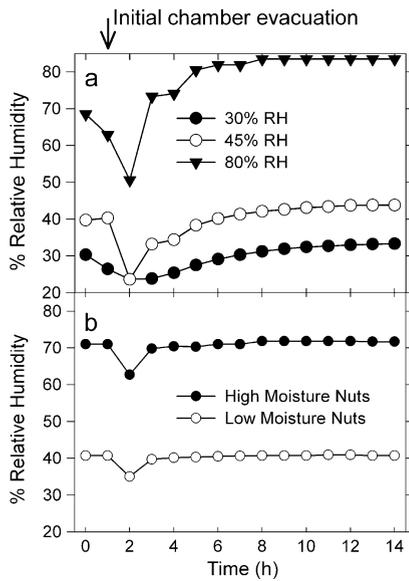


Fig. 1. Typical relative humidity levels recorded during vacuum treatments at 25°C when humidity levels were controlled by glycerol solutions (a) or product moisture (b).

from tubes and evaluated for mortality ≈24 h after treatment to allow for recovery of test larvae from any treatment-induced stupor. (Preliminary tests showed that little or no additional mortality occurred in the first 24 h after treatment, and that nearly all larval mortality occurred during treatments.) Larvae were considered to be alive if they responded to a gentle probe. All treatments were replicated three times.

Data Analysis. Weight loss values for the first experiment comparing the effect of relative humidity were analyzed using the analysis of variance (ANOVA) procedure from SPSS 17.0 for Windows (SPSS Inc. 2008). Weight loss values for the second experiment comparing the effect of product moisture were analyzed using the SPSS *t*-test procedure. Mortality values

for both experiments were compared using the SPSS Mann-Whitney *U* test.

Results

Typical relative humidity levels recorded during vacuum treatments when humidities were controlled with glycerol solutions or product moisture levels are shown in Fig. 1. In all cases, relative humidity levels dropped during chamber evacuation, but returned to equilibrium levels shortly after the vacuum pump was switched off. Relative humidity levels in chambers containing low and high moisture walnuts were ≈40 and 70%, respectively. These levels corresponded to vapor pressure deficits of 1.9 and 0.95 kPa at 25°C, and 2.54 and 1.27 kPa at 30°C, respectively. Average whole nut moisture levels ± SEM remained consistent, even after vacuum treatments and were 9.0 ± 0.12, 8.9 ± 0.16, 5.6 ± 0.13, and 5.8 ± 0.12% for high moisture pretreatment, high moisture posttreatment, low moisture pretreatment, and low moisture post-treatment, respectively.

The percentage weight loss recorded for both non-diapausing and diapausing Indianmeal moth larvae exposed to 50 mmHg at varying relative humidity levels is given in Table 1. Weight loss, which is assumed to be primarily due to water loss, was much greater in all vacuum treatments (12.7–63.0%) than in the untreated controls (1.0–10.8%). Untreated diapausing larvae also lost less weight (1.0–2.7%) than untreated nondiapausing larvae (4.4–10.8%), even though diapausing larvae were held for longer periods.

For vacuum treated larvae at all exposures, weight loss was significantly different ($F = 75.8-576.6$, $df = 2$, $P \leq 0.05$) among different humidity levels. Weight loss was always greatest at the lowest (30%) humidity level and least at the highest (80%) humidity level, and this was true for both nondiapausing and diapausing larvae at both temperatures. This trend was not always seen in untreated larvae; in half of the exposures, no significant differences were noted; and differences, when detected, were usually between the humidity

Table 1. Percentage of weight loss of nondiapausing (NDIMM) and diapausing (DIMM) Indianmeal moth larvae exposed to low pressure (50 mmHg) or normal pressure (760 mmHg) at different temperatures, exposure times, and humidity levels

Stage	Exposure (h)	Treatment (50 mmHg)			Control (760 mmHg)		
		30% RH	45% RH	80% RH	30% RH	45% RH	80% RH
25°C							
NDIMM	12	51.4 ± 0.47a	44.6 ± 0.91b	24.7 ± 0.51c	7.7 ± 0.49a	7.5 ± 0.62a	6.8 ± 0.46a
	16	58.8 ± 0.56a	51.7 ± 0.47b	30.0 ± 0.83c	8.1 ± 0.59ab	8.5 ± 0.80a	6.5 ± 0.52b
	20	63.0 ± 0.58a	57.0 ± 2.46b	36.3 ± 0.82c	9.4 ± 0.48a	8.5 ± 0.32ab	7.7 ± 0.24b
DIMM	18	33.7 ± 0.83a	30.7 ± 0.74b	17.3 ± 1.33c	1.5 ± 0.08a	1.5 ± 0.12a	1.1 ± 0.09b
	30	48.2 ± 0.71a	42.8 ± 1.17b	23.2 ± 0.73c	2.0 ± 0.17a	1.8 ± 0.10a	1.7 ± 0.15a
	42	54.7 ± 0.60a	50.4 ± 0.90b	31.0 ± 1.52c	2.6 ± 0.17a	2.7 ± 0.48a	2.2 ± 0.18a
30°C							
NDIMM	6	45.7 ± 0.68a	35.7 ± 0.96b	20.6 ± 0.84c	5.3 ± 0.41a	4.8 ± 0.35a	4.4 ± 3.9a
	8	53.6 ± 1.07a	44.2 ± 1.24b	25.0 ± 1.07c	10.8 ± 4.1a	5.5 ± 0.28a	7.6 ± 1.57a
	10	59.2 ± 0.86a	50.2 ± 1.17b	29.4 ± 1.03c	7.3 ± 0.47ab	10.1 ± 2.20a	6.1 ± 0.40b
DIMM	10	34.7 ± 0.78a	25.3 ± 0.65b	12.7 ± 0.43c	1.3 ± 0.07a	1.0 ± 0.08b	1.0 ± 0.07b
	15	44.9 ± 0.52a	34.7 ± 0.92b	17.8 ± 0.54c	1.5 ± 0.09a	1.3 ± 0.07ab	1.3 ± 0.08b
	20	51.4 ± 0.51a	43.6 ± 0.75b	22.6 ± 0.58c	1.9 ± 0.05a	1.8 ± 0.08a	1.6 ± 0.07b

For each treatment, values in the same row followed by a different letter are significantly different ($P \leq 0.05$; ANOVA, SPSS 17.0).

Table 2. Percentage of mortality of nondiapausing (NDIMM) and diapausing (DIMM) Indianmeal moth larvae exposed to low pressure (50 mmHg) or normal pressure (760 mmHg) at different temperatures, exposure times, and humidity levels

Stage	Exposure (h)	Treatment (50 mmHg)			Control (760 mmHg)		
		30% RH	45% RH	80% RH	30% RH	45% RH	80% RH
25°C							
NDIMM	12	98.7 ± 0.71a	88.5 ± 2.27b	1.3 ± 0.71c	0.9 ± 0.63a	1.3 ± 0.71a	1.3 ± 0.71a
	16	100.0 ± 0.0a	98.2 ± 0.79a	3.1 ± 1.44c	0.4 ± 0.44a	0.4 ± 0.42a	2.3 ± 1.07a
	20	100.0 ± 0.0a	99.6 ± 0.44a	15.3 ± 2.06c	0.4 ± 0.44a	2.2 ± 1.41a	4.5 ± 1.55b
DIMM	18	27.9 ± 5.34a	10.2 ± 4.41b	0.9 ± 0.61b	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	30	98.1 ± 2.20a	75.1 ± 5.94b	0.0 ± 0.0c	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
	42	99.1 ± 0.89a	92.1 ± 2.28b	24.2 ± 7.60c	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
30°C							
NDIMM	6	95.6 ± 1.55a	44.3 ± 4.81b	4.5 ± 1.7c	2.2 ± 1.06a	0.9 ± 0.61a	1.3 ± 0.71a
	8	100.0 ± 0.0a	92.9 ± 1.89b	8.0 ± 2.54c	1.7 ± 0.99a	5.5 ± 0.28a	0.0 ± 0.0a
	10	99.6 ± 0.44a	99.6 ± 0.44a	11.9 ± 3.00b	0.0 ± 0.0a	0.2 ± 1.07a	2.2 ± 1.06a
DIMM	10	54.9 ± 6.63a	6.2 ± 2.10b	0.4 ± 0.44b	0.0 ± 0.0a	0.0 ± 0.0a	0.9 ± 0.61a
	15	94.2 ± 1.70a	43.0 ± 5.16b	0.9 ± 0.61c	0.4 ± 0.44a	0.0 ± 0.0a	0.0 ± 0.0a
	20	100.0 ± 0.0a	95.1 ± 1.37b	4.0 ± 2.23c	0.0 ± 0.0a	0.0 ± 0.0a	0.1 ± 0.10a

For each treatment, values in the same row followed by a different letter are significantly different ($P \leq 0.05$; Mann-Whitney U test, SPSS 17.0).

extremes. Diapausing larvae were more resistant than nondiapausing larvae to moisture loss under vacuum; to obtain comparable moisture loss levels, diapausing larvae were treated at exposures that were at least twice those used for nondiapausing larvae.

Mortality results of test larvae exposed to 50 mmHg at three different relative humidity levels are presented in Table 2. Mortality of untreated larvae was negligible (0–5.5%), and no significant differences ($P \geq 0.5$) were detected among humidity levels. In the vacuum treatments, striking differences were seen between humidity levels for both nondiapausing and diapausing larvae. For example, at 30°C and the longest exposure times (10 and 20 h for nondiapausing and diapausing larvae, respectively), mortality for both larval stages at 30% RH was >99%, whereas mortality at 80% RH was <12%. Mortality levels were much lower for diapausing larvae than nondiapausing larvae at comparable exposure times.

The percentage weight loss recorded for both nondiapausing and diapausing Indianmeal moth larvae exposed to 50 mmHg at varying product moisture is

given in Table 3. Again, most of the weight loss is assumed to be due to moisture loss. Because low moisture product produced an equilibrium relative humidity ($\approx 40\%$ RH) that was lower than that found for high moisture product ($\approx 70\%$ RH), it is expected that moisture loss should follow a pattern similar to the first experiment. Untreated larvae lost much less moisture than did vacuum treated larvae, and untreated diapausing larvae lost less than untreated nondiapausing larvae, even at longer exposure times. For untreated larvae at 25°C, there was significant difference in weight loss between product moisture levels only for diapausing larvae at the longest exposure ($t = 27.5$, $df = 28$, $P \leq 0.05$). At 30°C for untreated larvae, significant differences in weight loss between product moisture levels were seen for all exposures ($t = 2.87$ – 4.80 , $df = 28$, $P \leq 0.05$), with the highest loss always in the low moisture nuts. In all vacuum treatments, insects treated with low moisture nuts lost significantly more moisture than those with high moisture nuts ($t = 8.87$ – 32.77 , $df = 28$, $P \leq 0.05$). Diapausing larvae lost less moisture than did nondiapausing lar-

Table 3. Percentage of weight loss of nondiapausing (NDIMM) and diapausing (DIMM) Indianmeal moth larvae exposed to low pressure (50 mmHg) or normal pressure (760 mmHg) at different temperatures, exposure times, and low (5.7%) and high (9.0%) product moisture levels

Stage	Exposure (h)	Treatment (50 mmHg)		Control (760 mmHg)	
		Low moisture	High moisture	Low moisture	High moisture
25°C					
NDIMM	12	45.5 ± 0.73a	23.3 ± 0.81b	8.0 ± 0.42a	6.8 ± 0.51a
	16	54.0 ± 0.88a	31.0 ± 0.94b	7.9 ± 0.22a	7.8 ± 0.39a
	20	59.1 ± 0.54a	35.6 ± 0.94b	10.1 ± 0.73a	8.6 ± 0.50a
DIMM	18	32.5 ± 2.11a	13.1 ± 0.55b	1.0 ± 0.05a	1.4 ± 0.49a
	30	42.4 ± 0.53a	23.3 ± 0.70b	1.5 ± 0.09a	1.4 ± 0.15a
	42	50.5 ± 0.62a	29.0 ± 0.98b	1.9 ± 0.09a	1.4 ± 0.08b
30°C					
NDIMM	6	41.7 ± 1.38a	20.3 ± 0.83b	5.0 ± 0.28a	3.4 ± 0.19b
	8	50.4 ± 0.95a	25.3 ± 1.14b	5.5 ± 0.23a	4.5 ± 0.26b
	10	56.8 ± 1.35a	30.6 ± 1.41b	7.1 ± 0.42a	4.8 ± 0.22b
DIMM	10	32.4 ± 0.52a	14.4 ± 0.40b	1.3 ± 0.05a	1.0 ± 0.10b
	15	44.5 ± 0.48a	21.5 ± 0.66b	1.7 ± 0.06a	1.2 ± 0.10b
	20	52.4 ± 0.34a	26.6 ± 0.71b	2.1 ± 0.16a	1.5 ± 0.03b

For each treatment, values in the same row followed by a different letter are significantly different ($P \leq 0.05$; t -test, SPSS 17.0).

Table 4. Percent mortality of non-diapausing (NDIMM) and diapausing (DIMM) Indianmeal moth larvae exposed to low pressure (50 mmHg) or normal pressure (760 mmHg) at 25°C at different temperatures, exposure times and low (5.7%) and high (9.0%) product moisture levels

Stage	Exposure (h)	Treatment (50 mmHg)		Control (760 mmHg)	
		Low moisture	High moisture	Low moisture	High moisture
25°C					
NDIMM	12	92.0 ± 1.81a	1.1 ± 0.79b	1.0 ± 0.95a	3.4 ± 1.63a
	16	98.7 ± 0.71a	4.9 ± 1.62b	1.8 ± 1.02a	2.7 ± 1.82a
	20	100.0 ± 0.0a	31.0 ± 6.33b	1.4 ± 0.75a	2.9 ± 1.17a
DIMM	18	18.0 ± 3.48a	1.3 ± 0.96b	1.3 ± 0.71a	0.0 ± 0.0a
	30	70.1 ± 7.04a	0.7 ± 0.48b	0.0 ± 0.0a	0.4 ± 0.44a
	42	96.2 ± 1.17a	16.5 ± 6.79b	0.4 ± 0.44a	0.5 ± 0.48a
30°C					
NDIMM	6	85.3 ± 5.29a	5.0 ± 7.72b	0.5 ± 0.56a	0.9 ± 0.61a
	8	99.5 ± 0.44a	4.1 ± 1.43b	0.0 ± 0.0a	0.9 ± 0.63a
	10	100.0 ± 0.0a	29.2 ± 7.03b	1.3 ± 0.96a	0.9 ± 0.61a
DIMM	10	41.9 ± 5.28a	0.3 ± 0.33b	0.0 ± 0.0a	4.8 ± 0.47a
	15	97.3 ± 1.9a	4.4 ± 1.25b	0.4 ± 0.44a	2.7 ± 2.23a
	20	99.6 ± 0.44a	9.8 ± 3.04b	1.2 ± 0.93a	0.0 ± 0.0a

For each treatment, values in the same row followed by a different letter are significantly different ($P \leq 0.05$; Mann-Whitney U test, SPSS 17.0).

vae; as with the first experiment, exposures were at least doubled for diapausing larvae to obtain moisture loss values comparable to nondiapausing larvae.

Mortality of nondiapausing and diapausing Indianmeal moth larvae exposed to 50 mmHg at varying product moisture followed a trend similar to the first experiment (Table 4). Among both diapausing and nondiapausing untreated larvae at both temperatures, no significant differences were noted in mortality between the different product moisture levels, and mortality remained low ($\leq 4.8\%$). However, in all cases significantly higher mortality ($P \leq 0.05$) was noted in vacuum-treated larvae at lower product moisture levels. Again, considerably longer exposure times for diapausing larvae were needed to achieve mortality levels comparable of nondiapausing larvae.

Discussion

The mode of action of low pressure treatments is believed to be largely due to low oxygen tensions (Navarro and Calderon 1979), although Galun and Fraenkel (1961) noted that lack of oxygen could not be the only cause for mortality of *Aedes aegypti* (L.) adults and *Musca vicina* Macquart pupae at low pressures. Insects in low oxygen environments undergo metabolic arrest, leading to decreased ATP production, increased membrane permeability and eventual cell damage or death (Hochachka 1986). When oxygen levels are below the anaerobic compensation point, anaerobic metabolism is begun, resulting in the accumulation of potentially lethal metabolic end products (Mitcham et al. 2006).

Dehydration has also been identified as a possible contributing factor in mortality under low pressure environments, particularly at low humidities. Numerous studies have noted a reduction in weight due to moisture loss in vacuum-treated insects and that high humidities can reduce efficacy of vacuum treatments (El Nahal 1953, Bhambhani 1956, Chen et al. 2006). Jay et al. (1971) noted that mortality of stored product

insects increased with decreasing relative humidity under low oxygen treatments at atmospheric pressures, and attributed this effect to dehydration. Gaseous exchange in most insects is controlled by the spiracles, with low oxygen and high CO_2 concentrations in the trachea causing spiracles to open and high oxygen concentrations causing spiracles to close (Beckel and Schneiderman 1957, Burkett and Schneiderman 1974). The purpose of spiracular control is seen to be the reduction of respiratory water loss (Lighton et al. 2004). It is generally felt that the loss of moisture under low oxygen conditions is due to reduced oxygen concentrations causing spiracles to remain open, thereby increasing water loss (Navarro 1978, Jay and Cuff 1981).

Diapausing stages are normally characterized as having reduced respiration and oxygen demands and are more tolerant to low oxygen environments than nondiapausing stages (Kukul et al. 1991, Adler 2001). As such, diapausing stages should also be more tolerant of the low oxygen environment found in vacuum treatments. Johnson and Zettler (2009) showed that the response of diapausing Indianmeal moth larvae to 50 mmHg and 50–60% RH was similar to Indianmeal moth eggs, the stage identified as being the most tolerant in previous studies (Mbatia and Phillips 2001, Mbatia et al. 2004). In winter field studies, Johnson and Zettler (2009) found that diapausing Indianmeal moth and codling moth, *Cydia pomonella* (L.), larvae were very tolerant to low pressure treatments at temperatures (5–12°C) that killed the egg stages.

Diapausing (cold-acclimated) larvae are the normal overwintering stage of the Indianmeal moth (Carrillo et al. 2006) and are among the most cold-tolerant of stored product insects (Fields 1992). Life stages that are tolerant to cold are often tolerant to desiccation as well; many of the adaptations to allow survival at low temperatures also may have evolved to survive the desiccation associated with long-term exposure to cold, dry air (Ring and Danks 1994). This characteristic may give diapausing larvae tolerance to low pres-

tures, at least when compared with nondiapausing larvae.

The egg stage of stored product insects is often identified as the most tolerant to low pressures (Bare 1948; Al-Azawi et al. 1983; Mbata and Phillips 2001; Mbata et al. 2004; Finkelman et al. 2003, 2004, 2006). However, some studies have shown eggs to be less tolerant than other stages to low oxygen at atmospheric pressures. Tunç and Navarro (1983) found that eggs of the red flour beetle, *Tribolium castaneum* (Herbst), were less tolerant of low oxygen (4% O₂ in N₂) at atmospheric pressure than adult beetles, and Donahaye et al. (1996) noted that red flour beetle eggs were the least tolerant stage of 1% O₂ in N₂. Indianmeal moth eggs were considerably less tolerant of 1.8% O₂ in N₂ at atmospheric pressure than mature larvae (Tunç 1983). Mbata and Phillips (2001), however, showed that eggs of both red flour beetle and Indianmeal moth were the most tolerant stage to low pressure. If low oxygen was the only mechanism responsible for mortality under low pressure environments, then it would be expected that relative tolerances of life stages be similar between low pressure and low oxygen treatments.

Stored product insects in general survive low moisture storage environments by efficient conservation of water and resistance to desiccation (Levinson and Levinson 1994). Indianmeal moth eggs have been shown to be relative unaffected by environmental humidity levels (Morrison and Crawford 1970). Lacking spiracles, insect eggs do not lose moisture through respiration in the same manner as larvae and pupae. It is possible that the mechanism for mortality of Indianmeal moth eggs under low pressures is unrelated to moisture loss, whereas moisture loss plays a more important role in mortality of larval stages. Adaptations by diapausing larvae to avoid desiccation may result in its increased tolerance to low pressure environments.

In the current study, product moisture had a very large effect on the weight loss and mortality of both larval stages to low pressures, presumably through changes to the humidity level in the treatment chamber. The product moistures and resulting humidities used in this study were within the range of those recommended for safe storage of walnuts (López et al. 1995, Kader and Thompson 2002). Because relatively minor changes in walnut moisture content can result in fairly large changes in storage humidities, product moisture should be considered in making treatment recommendations, extending exposure times when product moistures result in humidity levels of above 70%.

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