Response of Postharvest Tree Nut Lepidopteran Pests to Vacuum Treatments

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ABSTRACT Industry concerns over insect resistance, regulatory action, and the needs of organic processors have renewed interest in nonchemical alternative postharvest treatments to fumigants used for California tree nuts. The development of inexpensive polyvinyl chloride containers capable of holding low pressures has increased the practicality of vacuum treatments for durable commodities such as tree nuts. To develop vacuum treatment protocols, we determined the relative tolerance to vacuum (50 mmHg) at 25 and 30°C of different life stages of three postharvest pests of tree nuts: codling moth, *Cydia pomonella* (L.), navel orangeworm, *Amyelois transitella* (Walker), and Indianmeal moth, *Plodia interpunctella* (Hübner). At both temperatures, nondiapausing codling moth larvae were the least tolerant stage tested. LT_{95} values for diapausing Indianmeal moth larvae were similar to Indianmeal moth eggs at both temperatures. Indianmeal moth diapausing larvae and eggs were the most tolerant at 25°C, whereas navel orangeworm eggs were most tolerant at 30°C. Field tests using GrainPro Cocoons (GrainPro, Inc., Concord, MA) to treat shelled almonds, *Prunus dulcis* (Mill.) D.A.Webb, in bins at vacuum levels of 18–43 mmHg at average winter temperatures (6–10°C) showed that diapausing codling moth larvae were the most tolerant under these conditions and that exposures of 7–13 d provided incomplete control. Summer field tests treating in-shell almonds in bags at average temperatures of 25–30°C provided complete control with 48 h exposure to average vacuum levels of 50 mmHg, and navel orangeworm eggs were the most tolerant stage.

KEY WORDS vacuum treatment, tree nuts, codling moth, navel orangeworm, Indianmeal moth

The central valley of California produces nearly all of the almonds, *Prunus dulcis* (Mill.) D.A.Webb; pistachios, L *Pistacia vera.*; and walnuts (*Juglans* spp.) in the United States, resulting in an average annual production of >800,000 metric tons of commodity valued at ≈$2.4 billion (USDA 2007). These three products are also among the top 10 California agricultural exports, bringing into the California economy an average of >$1.5 billion each year (CDFA 2007). Almonds are currently the leading export for California and were responsible for nearly 20% ($1.8 billion) of the unprecedented $9.3 billion 2005 California export market (CDFA 2007).

A major problem in the storage and marketing of these products is infestation by a variety of postharvest insect pests. Of particular concern are field pests of possible phytosanitary importance such as navel orangeworm, *Amyelois transitella* (Walker), which infests all three nut crops, and codling moth, *Cydia pomonella* (L.), which is a major pest of walnuts. To avoid invasion by these pests, importing countries may require phytosanitary inspections or treatments before allowing California nut products entrance to their markets. Also of concern is the cosmopolitan stored product pest Indianmeal moth, *Plodia interpunctella* (Hübner). Conversations with quality control managers within the tree nut industry in California indicate that Indianmeal moth is the most common reason for customer returns, which reflect the results found within south central U.S. grocery stores (Platt et al. 1998).

Currently, California 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 (Benhalima 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 to fumigants, regulatory action, and the pest management 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

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product. The lethal effects of vacuum were noted as early as the 17th century (Back and Cotton 1925). 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 (known as Volcani Cubes or GrainPro Cocoons; GrainPro, Inc., Concord, MA) developed for temporary grain storage (Navarro and Donahaye 1985) also were found to have utility as vacuum treatment enclosures, making the treatment more economical and practical (Navarro et al. 2001). This study investigates the potential of vacuum for disinfesting postharvest tree nut pests by first determining the relative tolerance to low pressures of different life stages of target pests, particularly diapausing larval stages, which are often tolerant of other treatments such as fumigation (Bell 1977a,b; Bond 1984; Cox et al. 1984) and cold storage (Johnson 2007). Field tests were then conducted with almonds treated under vacuum in five MT GrainPro Cocoons.

Materials and Methods

Test Insects. All test insects were from laboratory cultures at the San Joaquin Valley Agricultural Sciences Center (SJVASC), Parlier, CA. The navel orangeworm culture was originally obtained from the University of California, Berkeley, in 1966, and the Indianmeal moth culture was originally obtained from a walnut packinghouse in Modesto, CA, in November 1967. The codling moth culture was obtained from an apple orchard near North Fork, CA, in September 1984.

Indianmeal moth and navel orangeworm were maintained on a wheat bran diet (Tebbets et al. 1978); codling moth were reared on a lima bean-based agar diet (Tebbets et al. 1986). Rearing conditions for navel orangeworm and nondiapausing Indianmeal moth were 28°C, 60% RH, and a photoperiod of 14:10 (L:D) h. Rearing conditions for nondiapausing codling moth were 27°C, 60% RH, and a photoperiod of 16:8 (L:D) h. Diapausing Indianmeal moth larvae were obtained by holding rearing jars recently infested with eggs under normal rearing conditions for 1 wk. The jars were then transferred 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 and 14°C chambers were kept at a photoperiod of 10:14 (L:D) h. Under these conditions, larvae from our Indianmeal moth isolate uniformly entered diapause and were recognized by their color, behavior, and increased size (Tsuji 1958). Diapausing codling moth larvae were reared at 18°C, 60% RH, and a photoperiod of 8:16 (L:D) h (Tebbets et al. 1986). Codling moth still in the larval stage 50 d after being placed in diapause conditions were assumed to be in diapause.

Eggs, nondiapausing larvae, and diapausing larvae of codling moth, and eggs and nondiapausing larvae of navel orangeworm were chosen for study. Although there is some evidence that navel orangeworm has some form of diapause (Gal 1978, Legner 1983, Johnson et al. 2002), it is poorly understood; consequently, well-defined diapausing larvae were unavailable for study. Preliminary tests with codling moth and navel orangeworm indicated that pupae were far less tolerant to low pressures than larvae and were not included in our study (J.A.J., unpublished data). Adults of these two species also were excluded, because they are unlikely to be found in treated product. Because the response of most stages of the Indianmeal moth to low pressures has already been examined (Mbata and Phillips 2001), we selected diapausing larvae, which had not been previously tested, and eggs, which had been determined to be the most tolerant life stage for this species.

Laboratory Dose Response. Treatments were done in cylindrical, 45.7-cm-tall by 30.5-cm-diameter stainless steel 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. Pressure levels were determined with an absolute pressure capsule gauge (CG-100, Becker Pumps Co., Cuyahoga Falls, OH). 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 two to three times to 50 mm during the first 2 h of the treatment, after which time pressures remained stable for the remainder of the test.

Test larvae were treated in 18- by 63-mm tubes made from 32 mesh stainless steel screen. Five tubes containing 10–15 fifth instar diapausing or nondiapausing larvae were used for each treatment exposure. Strips of corrugated cardboard were added to tubes with nondiapausing codling moth larvae to give them harborage and reduce cannibalism. Because codling moth was likely to chew into and seek refuge within cork stoppers, tubes containing nondiapausing codling moth were closed with neoprene stoppers. Tubes containing all other test larvae were closed with cork stoppers. Nondiapausing larvae were placed in tubes just before treatment. Because diapausing larvae were shown to be more susceptible to methyl bromide fumigation after being disturbed (Tebbets et al. 1986), diapausing larvae of both species were placed in tubes and held at 14°C and 10:14 (L:D) h ≈1–3 wk before treatment.

Codling moth, Indianmeal moth and navel orangeworm eggs were held under rearing conditions (28°C) and treated when 6–30, 6–54, and 30–54 h old, respectively. Test eggs were treated in 9.0-cm plastic petri dishes. Strips of ovipositional substrate containing codling moth or navel orangeworm eggs were attached to the bottom of a petri dish with double stick tape, whereas Indianmeal moth eggs were placed di-

rectly on removable double stick tape attached to card stock which was fixed to the bottom of the petri dish. One dish containing ≈100 eggs of each species was used for each treatment exposure.

Preliminary studies showed that eggs and diapausing larvae were more tolerant than nondiapausing larvae, so the latter were treated separately with a different series of exposures. Five exposures were used for all life stages and temperatures. Exposures for nondiapausing larvae were from 6 to 30 h with 6-h intervals at 25°C and from 6 to 14 h with 2-h intervals at 30°C. Exposures for all other life stages were from 12 to 72 h with 12-h intervals at 25°C and 8–24 h with 4-h intervals at 30°C. Preliminary studies also indicated that the presence of product could affect efficacy of vacuum treatments, so treatment chambers contained ≈3.5 kg (≈7.5 liter) of in-shell walnuts at ≈3.3–4.4% kernel moisture. Tubes, petri dishes, or both with test insects were placed on top of the walnuts during treatment, with one chamber used for each exposure. Control insects were held in a chamber not connected to the vacuum pump and removed after the longest vacuum treatment exposure was completed.

Larvae were removed from tubes and evaluated for mortality ≈24 h after treatment. Larvae were considered to be alive if they responded to a light probe. Approximately 3 g of wheat bran diet was added to petri dishes containing eggs immediately after treatment to provide food for newly hatched larvae and prevent cannibalization. Dishes were held for at least 10 d after treatment before examination under a dissecting microscope to count hatched and unhatched eggs. All treatments were replicated three to five times.

Field Trials. Two series of field trials were conducted using five M/T V-HF Cocoons (GrainPro, Inc., Concord, MA), airtight rectangular structures made of UV-resistant polyvinyl chloride. The structures consist of top and bottom pieces joined together with a tongue-and-groove zipper similar to Ziploc bag closures. When filled to capacity, the cocoons were 1.5 m high by 2.95 m long by 1.7 m wide. Vacuum pressures were obtained with a 3-hp rotary, oil-lubricated vacuum pump (U-4–70, Becker Pumps Co.) connected to the Cocoon with a 4.5-m, 44-mm diameter metal reinforced polyvinyl chloride vacuum hose with quick disconnect. A bellows type pressure control switch (Bulletin 836T, Rockwell Automation, Milwaukee, WI) on the pump was available to maintain the pressure level within the treatment Cocoon. Pressures within the Cocoon were monitored with an absolute pressure capsule gauge. In all tests, pressure levels in the Cocoons were below 100 mmHg 10–30 min after evacuation began.

The first series of trials was done using shelled almonds in 1.2- by 1.2- by 1.2-m wooden bins. Two bins of almonds were placed in each of two Cocoons. The bins in one Cocoon were completely filled with almonds (≈1 MT) and served as treatments. The bins in the second Cocoon were partially filled (≈800 kg) and were used as untreated controls. Test insects and stages used were Indianmeal moth and navel orangeworm eggs, diapausing Indianmeal moth, and codling moth larvae, and nondiapausing navel orangeworm larvae. Although codling moth is a pest of walnuts and not of almonds, they were included in these field tests because almonds were the only product available. Except for Indianmeal moth eggs, all test insects were placed in 240-ml plastic cups with snap-on lids. A 22-mm-diameter hole was cut into each of the lids and organdy cloth was taped over the hole to permit ventilation. Cups were filled with almond kernels and ≈50 test larvae of a single species or a strip of paper with 50–100 navel orangeworm eggs were added. Diapausing codling moths were added as cocooned larvae within corrugated cardboard strips. Diapausing larvae of Indianmeal moth and codling moth were added to the cups and held under diapause conditions ≈1 wk before treatment. Indianmeal moth eggs (≈75–100) were treated in 18-ml glass vials with screen-centered caps. Four cups (one each for navel orangeworm larvae, navel orangeworm eggs, diapausing Indianmeal moth larvae, and diapausing codling moth larvae,) a vial with Indianmeal moth eggs, and a temperature and humidity data logger (Onset Computer, Bourne, MA) were placed together in plastic mesh bags and buried ≈30 cm below the surface of the almonds. Two bags were buried in each of the bins in both treatment and control Cocoons.

Corrugated cardboard was taped around the edges and corners of the bins, and Styrofoam sheeting was placed around the bins to fill out and protect the Cocoon. Both treatment and control Cocoons were sealed, but only the treatment Cocoon was attached to the vacuum pump. Because the tests were conducted during November and December, when maximum outside temperatures in the central valley of California averaged ≈17 and 12°C, respectively, lengthy exposures were necessary. Three tests were done with exposures of 168, 216, and 312 h (7, 9, and 13 d). The vacuum pump was allowed to run continuously during the treatments, and the resulting pressure levels, measured by the capsule gauge, were recorded periodically throughout the tests. In all three tests, the average pressure was well below the target of 50 mmHg (see Table 3). After each treatment, the Cocoons were opened and test insects were removed and held at rearing conditions for evaluation. Larvae were evaluated for mortality 1–7 d after treatment. Eggs were placed in Petri dishes containing ≈3 g of wheat bran diet and held at least 10 d before being examined under a dissecting microscope to count the numbers of hatched and unhatched eggs.

A second series of trials was done using in-shell almonds in 22.7-kg woven polypropylene bags (polybags). In the treatment Cocoon, 82 polybags (≈1,860 kg of almonds) were stacked to fill the Cocoon. Because of a limited supply of product, only 30 polybags (680 kg) of almonds were used in the control Cocoon, which was not sealed. Both Cocoons were under portable canopies to provide shade.

Test insects and stages used were Indianmeal moth and navel orangeworm eggs, and diapausing Indianmeal moth and codling moth larvae. All test insects

Table 1. Lethal times (hours) for Indianmeal moth, codling moth, and navel orangeworm life stages exposed to 50 mmHg at 25°C

Stage	*n*	Slope ± SE	LT_{50}	95% CI		LT_{95}	95% CI	
				Lower	Upper		Lower	Upper
Indianmeal moth								
Eggs	3,415	5.27 ± 0.213	16.9 c	15.3	18.5	34.8 de	31.2	40.0
Diapausing larvae	2,171	7.88 ± 0.483	21.9 e	20.7	23.1	35.5 e	33.5	38.2
Codling moth								
Eggs	3,662	6.57 ± 0.443	15.0 b	12.7	17.0	26.7 b	23.8	31.1
Diapausing larvae	2,127	6.30 ± 0.306	15.8 b	14.6	17.0	28.9 bc	26.4	32.4
Nondiapausing larvae	1,774	6.90 ± 0.849	10.4 a	7.9	11.8	18.1 a	16.4	21.8
Navel orangeworm								
Eggs	2,831	11.37 ± 0.994	23.2 f	21.4	24.5	32.4 d	30.0	37.5
Nondiapausing larvae	1,781	9.07 ± 0.425	19.2 d	17.8	20.4	29.1 c	26.7	33.1

Values in the same column with different letters are significantly different ($P < 0.05$; lethal dose ratio test).

were treated in the stainless steel screen tubes described above. Test tubes were placed along with ≈0.5 kg of in-shell almonds into plastic mesh bags. Into each mesh bag were placed three tubes with strips of paper containing 50–100 navel orangeworm eggs, three tubes with ≈100 Indianmeal moth eggs attached with double-stick tape to strips of card stock, and five tubes each with 10–15 diapausing Indianmeal moth or cocooned diapausing codling moth larvae in corrugated cardboard strips. Diapausing larvae were placed in the tubes 1–4 wk before treatment. A single mesh bag with test insects were placed in each of two polybags in the treatment cocoon, whereas one mesh bag was placed in a polybag in the control Cocoon. Temperature and relative humidity data loggers were placed within one of the mesh bags in both the control and treatment Cocoon. The polybags were then closed with large binder clips, the treatment Cocoon was sealed and the treatment was begun. Pressures were again monitored periodically using a capsule gauge.

Because California central valley average temperatures in June and July are ≈33 and 36°C, respectively, treatment exposures were much shorter than in the first series of tests. Exposures of 48, 30, and 24 h were used. After treatment, test insects were removed and evaluated as described above. In the 48- and 30-h treatments, we attempted to use the pressure controller to maintain the pressure in the treatment Cocoon at ≈50 mmHg, but because the differential was very large, pressures ranged from 35 to 120 mmHg, and made it difficult to calculate an average pressure. In the 24-h treatment, a small leak resulted in pressures of only 75 mmHg, with the pump running continuously.

Data Analysis. All laboratory mortality data were analyzed using the probit procedure in PoloPlus 2.0 (Robertson et al. 2003) after a log transformation of exposures. Lethal exposure times for 50 and 95% mortality (LT_{50} and LT_{95}) were estimated for each species and stage. Estimated exposure times were compared among all life stages and species at each temperature by using the lethal-dose ratio test in PoloPlus 2.0 (Robertson et al. 2003, 2007). For field trials, mortality for each species and life stage was calculated based on the total number of test insects used.

Results

Laboratory Dose Response. Results from the probit analysis at 25°C including lethal dose ratio tests for LT_{50} and LT_{95} are given in Table 1. For the purposes of predicting which stages may be most tolerant to vacuum treatments, the results for LT_{95} may be most useful. LT_{95} values for diapausing Indianmeal moth larvae and Indianmeal moth eggs (35.5 and 34.8 h, respectively) were similar and were significantly higher than most other stages. The LT_{95} for navel orangeworm eggs (32.4 h) was similar to Indianmeal moth eggs but significantly less than diapausing Indianmeal moth larvae ($P < 0.05$). Nondiapausing navel orangeworm larvae were also tolerant to vacuum, with an LT_{95} of 29.1 h, similar to that of diapausing codling moth larvae (28.9 h). The LT_{95} for codling moth eggs (26.7 h) was similar to that of diapausing codling moth larvae, but significantly less ($P < 0.05$) than nondiapausing navel orangeworm larvae. The stage most susceptible to vacuum was nondiapausing codling moth larvae, with the estimated LT_{95} (18.1 h) significantly lower than all other stages ($P < 0.05$).

As expected, estimated LT_{95} values for all life stages were lower at 30°C (Table 2). At the higher temperature, navel orangeworm eggs proved to be the most tolerant stage, with an LT_{95} (22.7 h) value significantly higher than all other stages (lethal-dose ratio test; $P < 0.05$). Codling moth eggs were the next most tolerant stage at the LT_{95} response level (19.8 h). LT_{95} values for Indianmeal moth eggs (17.9 h) and diapausing larvae (17.0 h) were similar but were significantly less ($P < 0.05$) than codling moth eggs. LT_{95} values for diapausing codling moth larvae and nondiapausing navel orangeworm larvae (14.7 and 15.4 h, respectively) were similar, and the LT_{95} value for nondiapausing codling moth larvae (12.4 h) was again significantly lower ($P < 0.05$) than all other stages.

Field Trials. In the first series of field trials (Table 3), temperatures within the Cocoons were quite low, averaging 10.5, 8.9, and 6.3°C for the 168-, 216-, and 312-h treatments, respectively. Because ambient temperatures were so low, we extended the treatment exposures to compensate. This also resulted in relatively low treatment pressures (43.1, 38.5, and 17.7

Table 2. Lethal times (hours) for Indianmeal moth, codling moth, and navel orangeworm life stages exposed to 50 mmHg at 30°C

Stage	*n*	Slope ± SE	LT_{50}	95% CI		LT_{95}	95% CI	
				Lower	Upper		Lower	Upper
Indianmeal moth								
Eggs	2,089	7.40 ± 0.36	10.7 d	10.0	11.4	17.9 c	16.7	19.5
Diapausing larvae	1,322	10.56 ± 0.71	11.8 e	11.2	12.4	17.0 c	16.0	18.4
Codling moth								
Eggs	2,140	8.09 ± 0.74	12.4 e	10.5	13.6	19.8 d	18.2	22.6
Diapausing larvae	1,257	7.53 ± 0.50	8.9 b	7.4	10.0	14.7 b	12.7	19.3
Nondiapausing larvae	1,454	7.23 ± 0.41	7.3 a	6.6	7.9	12.4 a	11.2	14.5
Navel orangeworm								
Eggs	1,894	11.38 ± 0.69	16.3 f	14.8	17.4	22.7 e	21.0	26.0
Nondiapausing larvae	1,770	8.40 ± 0.37	9.8 c	8.9	10.7	15.4 b	13.5	19.7

Values in the same column with different letters are significantly different ($P < 0.05$; lethal dose ratio test).

mmHg) because the pump was run continuously during the treatment.

The effect of the low ambient temperatures can be seen in the high control mortality levels in the egg stages found in the 216- and 312-h exposures. Consequently, the response of eggs to the vacuum treatments could not be determined. The low temperatures had little effect on the larval stages, as shown by the relatively low control mortalities for these stages. Diapausing larvae of both species were more tolerant than nondiapausing navel orangeworm larvae; no survival of navel orangeworm larvae occurred in any of the three tests. Mortality of diapausing Indianmeal moth larvae in the 168- and 216-h treatments (96.8 and 88.3%, respectively) was considerably higher than that for diapausing codling moth (75.1 and 1.5%, respectively). In the 312-h treatment, a single diapausing codling moth larva survived; 100% mortality was observed for all other species and life stages.

Temperatures within the Cocoons were much higher during the second series of field trials (Table 4), averaging 29.5, 27.0, and 25.0°C for the 48-, 30-, and 24-h treatment, respectively. Because of the higher ambient temperatures, treatment exposures were shortened considerably. Control mortality for most stages was acceptable with the exception of the egg stages during the 48-h treatment. We believe temperatures were again responsible for this high control mortality, as maximum temperatures reached 40°C during this treatment.

We obtained 100% mortality of all test insects in the 48-h treatment. The 30-h treatment, done at slightly cooler temperatures, resulted in nearly 100% mortality, with only a small number (eight) of navel orangeworm eggs successfully hatching. The 24-h treatment, done at a higher pressure level (≈75 mmHg), produced relatively low mortality in diapausing larvae (25.7 and 21.1% for Indianmeal moth and codling moth, respectively) but relatively high mortality for eggs (97.0 and 96.8% for Indianmeal moth and codling moth, respectively).

Discussion

Earlier work on the response of various stored product insects to low pressures has shown that eggs are commonly the most tolerant stage (Bare 1948; Al-Azawi et al. 1983; Mbata and Phillips 2001; Finkelman et al. 2003, 2004). The response to low pressures of diapausing stages, often the most tolerant to fumigants (Bell 1977a,b; Bond 1984, Cox et al. 1984), have not been studied. Results from our laboratory tests show that at the higher mortality level (LT_{95}) the response of diapausing Indianmeal moth larvae is similar to that

Table 3. Mortality of test insects in vacuum-treated shelled almonds held in wooden bins during winter field trials

Temp (°C), mean (max-min.)	Pressure (mmHg)	Exposure (h)	Target insect	Treated		Control	
				n	% mortality	*n*	% mortality
10.5 (9.0–22.5)	43.1	168	Indianmeal moth eggs	271	97.4	340	17.6
			Indianmeal moth diapausing larvae	187	96.8	165	2.4
			Navel orangeworm eggs	198	99.0	208	46.2
			Navel orangeworm larvae	200	100.0	77	13.0
			Codling moth diapausing larvae	189	75.1	188	1.6
8.9 (8.2–12.2)	38.5	216	Indianmeal moth eggs	325	100.0	360	100.0
			Indianmeal moth diapausing larvae	196	88.3	197	1.0
			Navel orangeworm eggs	399	100.0	434	98.8
			Navel orangeworm larvae	202	100.0	147	0.7
			Codling moth diapausing larvae	196	1.5	210	0.5
6.3 (5.0–8.6)	17.7	312	Indianmeal moth eggs	404	100.0	389	100.0
			Indianmeal moth diapausing larvae	201	100.0	195	2.6
			Navel orangeworm eggs	411	100.0	408	100.0
			Navel orangeworm larvae	210	100.0	201	4.0
			Codling moth diapausing larvae	195	99.5	192	0.5

Table 4. Mortality of test insects in vacuum-treated in-shell almonds held in woven polypropylene bags during summer field trials

Temp, (°C) mean (max-min.)	Pressure (mmHg)	Exposure (h)	Target insect	Treated		Control	
				n	% mortality	*n*	% mortality
29.5 (19.0–40.0)	50[a]	48	Indianmeal moth eggs	594	100.0	286	63.3
			Indianmeal moth diapausing larvae	90	100.0	30	3.3
			Navel orangeworm eggs	71	100.0	21	71.4
			Codling moth diapausing larvae	139	100.0	71	0.0
27.0 (22.0–34.0)	50[a]	30	Indianmeal moth eggs	690	100.0	346	2.3
			Indianmeal moth diapausing larvae	145	100.0	70	0.0
			Navel orangeworm eggs	434	98.2	145	17.9
			Codling moth diapausing larvae	132	100.0	70	0.0
25.0 (21.0–29.0)	75	24	Indianmeal moth eggs	595	97.0	329	2.7
			Indianmeal moth diapausing larvae	140	25.7	70	0.0
			Navel orangeworm eggs	689	96.8	330	16.7
			Codling moth diapausing larvae	139	21.1	65	1.5

[a] Average pressures are approximate − pressure ranged from 35 to 120 mmHg.

of Indianmeal moth eggs at 25 and 30°C, whereas codling moth diapausing larvae and eggs are similar at 25°C but eggs are more tolerant at 30°C. Furthermore, our winter field studies indicate that diapausing larvae of both species are tolerant to vacuum treatments at temperatures low enough to be lethal to eggs. Diapausing codling moth larvae in particular were difficult to kill in field trials at low temperatures.

Mbata et al. (2004) showed that younger Indianmeal moth eggs are more tolerant to vacuum than older eggs and suggests that our study may be underestimating the lethal times. However, because egg development is strongly affected by temperature, it is difficult to directly compare egg age between studies. Mbata et al. (2004) ended treatment of Indianmeal moth eggs at 48 h, just before hatch, but under our laboratory conditions, eggs did not hatch until ≈72 h (Johnson and Wofford 1991, Johnson et al. 1995). We also treated all test insects with product (in-shell walnuts), whereas Mbata et al. (2004) did not include product. We have found that the moisture content of product may have an effect on insect mortality during vacuum treatments (Johnson, unpublished data).

The mode of action of low pressure treatments has been shown to be largely due to low oxygen tensions at high humidities (Navarro and Calderon 1979). Diapausing stages are normally characterized as having reduced respiration and oxygen demands and are more tolerant to low oxygen environments (Kukal et al. 1991). As such, diapausing stages also should be more tolerant of the low oxygen environment found in vacuum treatments. Insect mortality under low pressures is increased at low humidities by increasing the moisture loss normally found under reduced oxygen environments (Navarro 1978). Life stages that are tolerant to cold are often tolerant to desiccation as well (Ring and Danks 1994, Appel et al. 1999). This characteristic also may give them tolerance to low pressures. Although Indianmeal moth eggs are less cold tolerant than other stages (Johnson 2007), egg hatch is unaffected by humidity (Morrison and Crawford 1970), indicating that eggs are tolerant of desiccation. This may partially explain the relative tolerance of eggs to low pressures.

Because of the variable treatment pressures and temperatures experienced in the field, it is difficult to compare results from our field tests with our lab studies. Although relative mortality of life stages in field tests was similar to that found in the lab, there were inconsistencies. During the winter tests with shelled almonds in bins, diapausing codling moth survival was consistently higher than diapausing Indianmeal moth larvae, whereas response of diapausing codling moth in the lab was consistently lower than diapausing Indianmeal moth larvae. This suggests that diapausing codling moth may be more tolerant than Indianmeal moth at temperatures lower than those studied in the lab. In the summer treatments of bagged in-shell almonds, the only survival after the 30-h treatment was navel orangeworm eggs, the stage identified as most tolerant in the lab at 30°C. However, mortality of both diapausing codling moth and diapausing Indianmeal moth was considerably lower than either Indianmeal moth or navel orangeworm eggs in the 24 h treatment. This treatment was anomalous in that the pressure never dropped below 75 mmHg and may account for the apparent discrepancy.

The development of inexpensive flexible containers has increased the practicality of vacuum treatments for durable commodities such as tree nuts. Our summer field tests showed good levels of control after 1.5–2 d treatments at temperatures of 27–30°C. This is an improvement over modified atmosphere treatments, also suggested as an alternative to fumigation and similar to vacuum treatments in mode of action, which require exposures of 3–7 d, in addition to initial purging (Kader 1996). Johnson et al. (2002) used a 6-d treatment after a 2-d purge to O_2 levels of 0.4% to control navel orangeworm and raisin moth in almonds and raisins. Summer vacuum treatments also compare favorably to phosphine, where recommended exposures for almonds are 2–3 d plus aeration (Nelson et al. 1980, Kader 1996). Results from our winter field tests show vacuum to be slightly less favorable compared with phosphine at low temperatures. We achieved incomplete control after a 7-d exposure to vacuum at average temperatures of 10°C, whereas a 5-d exposure is recommended for phosphine at 10°C (Bond 1984).

Successful treatments also may require some experience in loading, sealing, and protecting the Cocoons. We were able to treat shelled nuts in wooden bins only after completely filling the bins to prevent the sides from buckling under the pressure. To avoid rodent damage to the Cocoons, it is recommended that sides of the Cocoons be pulled taut with straps, reducing folds of material at points of contact with the floor (Donahaye et al. 1991). In spite of taking these precautions, we found during our summer tests that rodents were able to travel under the Cocoons down a small groove between the stacked bags, and eventually succeeded in chewing holes in the material.

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