Integration of Nonchemical, Postharvest Treatments for Control of Navel Orangeworm (Lepidoptera: Pyralidae) and Indianmeal Moth (Lepidoptera: Pyralidae) in Walnuts

J. A. JOHNSON, P. V. VAIL, E. L. SODERSTROM, C. E. CURTIS, D. G. BRANDL, J. S. TEBBETS, AND K. A. VALERO

Horticultural Crops Research Laboratory, 2021 South Peach Avenue, Fresno, CA 93727

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ABSTRACT We propose a treatment strategy combining an initial disinfestation treatment with 1 of 3 protective treatments as an alternative for chemical fumigation of walnuts for control of postharvest insect populations. The initial disinfestation treatment (0.4% O_2 for 6 d) was designed to disinfest walnuts of field populations of navel orangeworm, *Amyelois transitella* (Walker). The protective treatments were low temperature (10°C) storage, controlled atmosphere (5% O_2) storage, and application of the Indianmeal moth granulosis virus, and were designed to prevent establishment of Indianmeal moth, *Plodia interpunctella* (Hübner). The initial disinfestation treatment was effective against laboratory populations of navel orangeworm. Efficacy of protective treatments was determined by exposure to Indianmeal moth population levels far higher than those found in commercial walnut storage facilities. All 3 protective treatments prevented development of damaging Indianmeal moth populations as measured by pheromone trap catches and sample evaluation of the walnuts. No Indianmeal moths were trapped, nor were any seriously damaged walnuts (nuts with obvious damage that rendered the nutmeat less marketable or unmarketable) recovered from either low temperature or controlled atmosphere storage. Very low numbers of moths (≤21/wk) were trapped from walnuts treated with virus, and only 0.2% of the walnuts were seriously damaged. In contrast, large numbers of moths (119–793/wk) were trapped from untreated nuts, and 35% of the sampled walnuts showed serious damage. Quality analysis by a commercial laboratory showed that overall walnut quality for all protective treatments was maintained at levels acceptable by industry standards.

KEY WORDS Indianmeal moth, navel orangeworm, integrated pest management, controlled atmospheres, low temperatures, granulosis virus

THE AVERAGE ANNUAL production of dried fruits and nuts from California is >1 million metric tons and is worth nearly U.S. $1.5 billion (USDA 1997). Postharvest processing increases product value still further. Postharvest insects cause commodity losses during storage through direct damage, product contamination, and creation of favorable conditions for mold growth and product degradation. Costs to the dried fruit and nut industry due to insect-related product loss and control measures are substantial. Currently, the dried fruit and tree nut industry depends on fumigation with methyl bromide or phosphine for postharvest insect control. Processing plants use fumigants to disinfest large volumes of the incoming product during harvest, and to control storage infestations.

After action taken in 1992 by >100 signatory nations of the Montreal Protocol, methyl bromide was designated an ozone depleter (UNEP 1992). Under the provisions of the U.S. Clean Air Act (USEPA 1993), use of methyl bromide will be severely restricted or eliminated. Insect resistance to phosphine, the only currently available alternative fumigant, has been documented in other commodities (Zettler et al. 1990). Thus, the need for economical alternative systems that provide efficacious control and maintain product quality throughout processing, storage, and marketing is critical. At present, no single proposed nonchemical method is a suitable substitute for fumigation. By applying an initial disinfestation treatment to incoming products followed by long-term protective measures during storage, infestations may be reduced or eliminated. Such an integrated control system would have most of the advantages of fumigation without the liabilities. The diversity of the dried fruit and nut industry requires that several combinations of treatments be considered to ensure that the most efficient system for each situation is available.

Walnuts were selected to evaluate integrated alternative control methods to replace methyl bromide because walnut quality is relatively sensitive to changes in processing or the storage environment (Ryall and Pentzer 1982). Indianmeal moth, *Plodia*

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interpunctella (Hübner), and navel orangeworm, *Amyelois transitella* (Walker), are the most economically important postharvest pests of tree nuts in California. Because infestations of navel orangeworm originate in the field and are carried into storage, where adults do not normally reproduce (Simmons and Nelson 1975), initial disinfestation of incoming product is sufficient to reduce damage by this pest. In contrast, Indianmeal moth attacks the product after harvest and is capable of repeated infestation during storage (Simmons and Nelson 1975), so that long-term protective treatments provide the most efficient control.

One proposed alternative disinfestation method for dried fruits and nuts is the use of controlled atmospheres. Soderstrom et al. (1986) showed that 0.5% O_2 can provide 95% mortality of mature larvae and pupae of navel orangeworm after exposures of 48–171 h, depending on temperature and relative humidity. Preliminary studies (E.L.S. and D.G.B., unpublished data) indicated that maintenance levels of O_2 (5%) were effective at preventing reinfestation by Indianmeal moth. Low temperature storage is another well-known method of managing postharvest insects (Fields 1992), including Indianmeal moth (Johnson et al. 1997), on dried fruits and nuts. Use of the Indianmeal moth granulosis virus to protect against infestations in stored nuts (Hunter et al. 1973) and raisins (Vail et al. 1991) in small-scale tests has been demonstrated.

To obtain acceptable control throughout the postharvest system, we proposed an integrated method that combined initial disinfestation using controlled atmospheres with protective treatments of microbial agents, low temperature storage or maintenance levels of controlled atmosphere. In the study described here, we evaluated the efficacy of the proposed combination treatments, using navel orangeworm as the target insect during initial disinfestation and Indianmeal moth during subsequent protective treatments.

Materials and Methods

Experimental Design. After an initial disinfestation treatment of 0.4% oxygen for 6 d, 3 protective treatments and an untreated control were compared (Fig. 1). Test species were navel orangeworm for the initial disinfestation treatment and Indianmeal moth for the protective treatments. The protective treatments were a controlled atmosphere of 5% oxygen, low temperature storage at ≤10°C, and Indianmeal moth granulosis (IMMG) virus applied as a dust (28.7 mg virus per kilogram of nuts). Four commercial raisin bins (1.3 by 1.3 by 0.65 m), each with ≈225 kg of either 'Hartley' or 'Franquette' walnuts, were used for each of the 3 protective treatments and untreated control. Each treatment was isolated in a separate room. Experimental rooms for the controlled atmosphere, IMMG virus, and untreated control were specifically built for these tests, measured 3 m square by 2.4 m high (21.5 m^3) and were equipped with heating, air conditioning, and ports for introduction of test insects. A refrigerated,

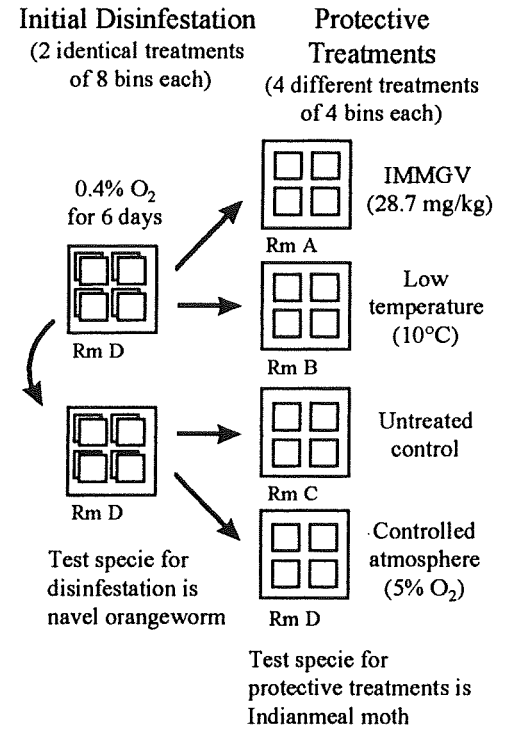

Fig. 1. Experimental design combining initial disinfestation of walnuts with 3 different protective treatments.

insulated cargo container (6 by 2.4 by 2.4 m), was used for the low temperature storage. The protective treatments were maintained and evaluated for 13 wk. This design was replicated 3 times.

Description of Initial Disinfestation Treatment. The disinfestation treatments for the 1st, 2nd, and 3rd replicates were begun on 15 April 1994, 31 August 1994, and 18 January 1995, respectively. We were unable to get unfumigated walnuts for the 1st replicate. Walnuts for the 2nd and 3rd replicates were not fumigated and may have contained field populations of navel orangeworm.

The controlled atmosphere room was equipped with pressure relief valves, a standard air expansion bag, and was sealed to a pressure half-life of 1 min. A hollow fiber membrane gas separation system (Prism Alpha Nitrogen System CPA-5, Permea, St. Louis, MO) was used to produce the required low oxygen atmosphere. Oxygen levels were monitored with a Servomex 570 paramagnetic oxygen analyzer (Servomex, Norwood, MA)

All 16 bins of walnuts used in subsequent protective treatment studies were first subjected to the initial disinfestation treatment of 0.4% O_2 for 6 d (after purging the room of O_2 for 2 d) in the controlled atmosphere room. This treatment schedule was determined to be efficacious against navel orangeworm in laboratory studies (E.L.S. and D.G.B., unpublished data). Because only 8 bins could be treated at a time, half the bins were treated and moved to the appropriate protective treatment rooms before the remaining nuts

were treated. The 8 bins were arranged in 2 rows of 2 bins each, and stacked 2 bins high.

Description of Protective Treatments. Temperatures in the controlled atmosphere, IMMG virus and control rooms were kept at 25 ± 2°C. Target air temperatures in the low temperature treatment were ≤10°C. Relative humidity in the cold room was maintained at 60–80% with a low temperature dehumidifier (Ebco, Columbus, OH). We did not attempt to control relative humidity in either of the remaining treatment rooms or in the control room.

During the protective treatments, temperature, and relative humidity in the control, IMMG virus and controlled atmosphere rooms were recorded with Datapod DP-220 dataloggers (Omnidata, Logan, UT). In each room, 1 sensor was mounted between bins to measure the air temperature and 2 sensors were buried ≈10 cm deep in the nuts, each in different bins. Measurements were taken every 4 h. A similar datalogger was used to record air temperatures and relative humidity in the low temperature treatment. In addition, temperatures in the walnut bins of the low temperature treatment were recorded with thermocouples of 36 gauge copper-constantan attached to a Polycorder datalogger (Omnidata). Two thermocouples were placed in each bin; 1 was at the center of the nuts and 1 was ≈5 cm below the nut surface. Another thermocouple was placed at the center of the cargo container ≈0.3 m above the bins. Temperatures were recorded every 15 min.

The stock IMMG virus preparation used in the protective treatment was produced as a powder (Vail 1991). Bioassay of the stock showed that the LC_{50} was 0.1 μg/g of diet (95% CL = 0.13 μg/g; slope = 1.06 ± 0.05) as estimated by probit analysis (POLOPC, LeOra Software 1994). A dose of 28.7 mg/kg, equivalent to the upper 95% CL of the estimated LD_{99} for the above IMMG virus preparation was selected for application. The IMMG virus dust formulation was prepared by mixing 26.0 g IMMG virus (AI) with 881.2 g ground, 60-mesh, stabilized wheat germ (Bioserv no. 1661, Bioserv, Frenchtown, NJ). The formulated dust was applied to 907.2 kg walnuts. This provided rates of 0.1% (wt:wt) of the formulated dust, equivalent to 28.7 mg IMMG virus per kilogram of nuts as the applied dose of virus.

The IMMG virus formulation was applied to the walnuts on the same day that nuts were removed from the initial controlled atmosphere disinfestation treatment. Application of the formulation was done in lots by placing 113.4 kg of walnuts into a 0.26-m^3 cement mixer. The mixer had been modified with additional vanes welded to the existing paddles to ensure adequate mixing. The mixer was started and set to run at 10 rpm, 113.4 g of IMMG virus dust was added to the walnuts and the mouth of the mixer was covered with an aluminum foil lid. The nuts were tumbled for 20 min and then poured back into bins. This process was repeated until 907.2 kg of walnuts had been treated and placed into 4 bins, each containing ≈226.8 kg. Bins were then placed in the treatment room as described above. To determine coverage provided by the formulation, a fluorescent powder (FP Tracerite, Metronics, Palo Alto, CA) was thoroughly mixed into the formulation using a ball mill. Treated walnuts were observed under UV light to determine that coverage by the virus was adequate.

Method of Infestation. Navel orangeworm used to evaluate the initial disinfestation controlled atmosphere treatment were from a laboratory colony originally obtained in 1966 from the University of California, Berkeley, and maintained on a wheat bran diet (Tebbets et al. 1978) at 27°C, 60% RH, and a photoperiod of 14:10 (L:D) h. Walnuts to be infested with navel orangeworm were prepared by drilling a hole (4 mm in diameter) through the shell. A single 21-d-old navel orangeworm larva was placed within each walnut, after which the hole was sealed with a plastic plug. Approximately 50 infested walnuts each were enclosed in cheesecloth bags and held 4 d. This procedure resulted in walnuts infested with insects that were at the most tolerant lifestage (late larvae to early pupae, Storey and Soderstrom 1977).

In each disinfestation treatment, 1 bag of infested nuts was buried just under the surface of the walnuts in each of 4 of the 8 treated bins. During treatment of all 16 bins, a bag was placed at each of the 8-bin locations, for a total of ≈400 treated nuts. For each treatment, 2 bags of infested walnuts (≈100 nuts) were untreated and used for controls, for a total of ≈200 untreated nuts.

Indianmeal moths used to evaluate the protective treatments were from a laboratory colony originally obtained from a walnut packing house in Modesto, CA, in November 1967 and were maintained on wheat bran diet. Rearing conditions were 28°C, 60% RH, and a photoperiod of 14:10 (L:D) h. Strips of corrugated cardboard (≈25 mm wide) were placed along the inside of rearing jars to provide pupation sites. Pupae were carefully removed from the strips, sorted by sex and held in 1-liter containers until adult emergence. Approximately 4 h before Indianmeal moths were to be added to the treatment rooms, adults of both sexes were released into a glove box. Mating pairs were gently collected in plastic tubes (10 by 1.5 cm) capped at both ends with plastic culture tube caps. After pairs had separated, the moths were placed in the treatment and control rooms.

To avoid disturbing the treatments, Indianmeal moths were introduced into the rooms through access ports. The plastic tubes were uncapped and fixed to the end of a blow-gun made from plastic tubing. A barrier of organdy cloth supported by a plastic ring inserted into the blow-gun and a tethered stopper placed in the tube kept the moths from escaping. The tip of the blow-gun was inserted through the access port and air was blown through at a force sufficient to propel both the stopper and the moths out of the tube. Five pairs of Indianmeal moths were added to each room each week for 11 wk, beginning immediately after the protective treatments were started. As an external control, when moths were added to the treatment rooms, an additional 5 pairs of moths were placed into 1-liter containers with 250 g of diet and held at

normal rearing conditions. All adult progeny produced in these containers were removed and counted.

Evaluation of Initial Disinfestation Treatment. To determine the efficacy of the initial disinfestation treatment against navel orangeworm, infested nuts were removed from the cheesecloth bags and held in glass jars at 25°C until adults emerged. Nuts were then opened to determine the stage at which test insects died.

Normally, mature navel orangeworm chew through walnut shells, produce a silken exit tube, and then retreat back into the nut to pupate. Occasionally, mature larvae will chew out of the nut and wander in search of a pupation site. Because some time was required for the atmosphere in the treatment room to reach levels that would prevent movement of larvae, this behavior resulted in some of the treated larvae escaping from the bags. Because the fate of escaped larvae could not be determined, we estimated survival of treated navel orangeworm from the number of live and dead insects recovered from the walnuts (i.e., not on the initial number of infested nuts).

Although adult navel orangeworm that emerged from untreated control walnuts were removed from the jars as soon as possible, some viable eggs were laid. Larvae from these F_1 eggs reinfested the walnuts, feeding on and destroying any dead test larvae, pupae, or entrapped adults still present in the nuts, making it impossible to recover and account for all insects. Controls were moved from the cheesecloth bags to jars soon after the treatment began. Some control larvae escaped the nuts but did not leave the bags. Very few of the control insects were considered to be missing. Thus, survival of control insects was estimated based on adult emergence from the initial number of infested nuts.

Evaluation of Protective Treatments. During the protective treatments, all 4 rooms were monitored continuously with Pherocon 1C sticky traps (Trece, Salinas, CA); each trap was baited with Indianmeal moth pheromone lure (Consep Membranes, Bend, OR). New dispensers were applied at the beginning and at the midpoint (6 wk) of each test. In each room, 1 trap was mounted ≈2 m above the floor. Trapped moths were counted each week for the first 5 wk and then 3 times per week for the last 7 wk for each test in the control, IMMG virus and low temperature rooms. Trap bottoms were changed when needed. Because the controlled atmosphere room was inaccessible during treatment, moths were counted in this trap only after the room was aerated at the end of the test. All other trap data are reported as weekly counts of Indianmeal moth males. Any incidental insects caught in the sticky traps were identified after paint thinner was used to remove the sticky trap material.

Nut samples were taken from the control, IMMG virus and low temperature treatments at 0, 4, 8, and 12 wk to evaluate quality and insect damage during the protective treatments. Samples were taken from the controlled atmosphere room at 0 and 13 wk. A total of ≈27 kg of nuts were sampled from each room by randomly scooping 6.5–7.5 kg of nuts from the surface of each bin. Each bin sample was run through a modified raisin shaker and the resulting debris examined for insects. Each sample was then divided into 4 equal subsamples by multiple passes through a sample splitter. The subsamples were stored in onion bags at 4°C until needed. One subsample was evaluated for damage and live insects by our laboratory personnel. A 2nd subsample was sent to a commercial laboratory for analysis according to industry standards. The remaining 2 subsamples were held at 4°C in reserve or for ancillary tests.

We evaluated the samples by cracking open 100 walnuts from each subsample and examining them for damage and the presence of insects. Damage was considered minor if only pinhole or other damage unlikely to be noticed by the consumer was present. Insect damage that was obvious and rendered the nutmeat less marketable or unmarketable was considered to be serious. We distinguished fresh damage by Indianmeal moth from other pretreatment insect damage. Total damage, including mold, shrivel or mechanical damage, was also recorded. The commercial laboratory quality evaluation recorded moisture content, water activity, peroxide values, free fatty acids, insect damage, and total damage.

Additional samples were taken at the end of each test (13 wk) to determine potential survival after treatment. Approximately 100 nuts were taken from each corner and the center of each bin, for a total of 20 samples per room. Nuts were placed in 2-liter plastic buckets closed with organdy cloth held in place by snap-on plastic lids with a 75-mm-diameter hole cut in the center. The nuts were held at 25°C for 6 wk, at which time they were examined for the presence of Indianmeal moth adults.

Statistical Analyses. For samples taken during the protective treatments and evaluated by laboratory personnel or the commercial laboratory, damage and quality values for each treatment were compared using the SAS general linear model (GLM) analysis of variance (ANOVA) procedure (SAS Institute 1989). With the exception of live Indianmeal moths, an arcsine transformation was done for all parameters. Analyses were done independently for each sample date, with bin samples treated as independent observations within each treatment. Where ANOVA showed significant differences ($P \leq 0.05$), means were separated using Bonferroni t-test (SAS Institute 1989).

Results

Initial Disinfestation. Efficacy of the initial disinfestation treatment against the navel orangeworm is shown in Table 1. Survival of control insects ranged from 77.5 to 84.5%. In the first 2 replications, no treated larvae survived. In the 3rd replication, 4 navel orangeworm survived the treatment (1.1% survival). We attributed this result in part to technical problems that resulted in the O_2 level rising to 0.7% for 2 d while the second 8 bins were being treated. Three of the 4 surviving adults came from that portion of the treatment. Efficacy of the disinfestation treatment over all

Table 1. Survival of navel orangeworm in walnuts after an initial disinfestation treatment of 0.4% O_2 for 6 days at 25°C

Replication	Infested nuts	Adults emerged	Live larvae	Dead larvae	Dead pupae	Missing	% Survival
			Control[a]				
1	204	163	—	—	—	—	79.9
2	200	155	—	—	—	—	77.5
3	200	169	—	—	—	—	84.5
			Treated[b]				
1	400	0	0	263	113	24	0
2	398	0	0	306	83	9	0
3	396	3	1[c]	256	99	37	1.1

[a] Survival of control insects was based on the number of adults emerging from the number of infested nuts; it was not possible to determine the number of dead larvae and pupae because of feeding by F1 progeny.
[b] Survival of treated insects was based on the number of live and dead insects recovered from the walnuts; missing test insects were not considered in the calculation.
[c] Larva was discovered when nuts were examined; successfully pupated and emerged as an adult.

3 replications was 99.6%; if the results from the 2nd half of the 3rd replication are not included, overall efficacy was 99.9%.

In the 3rd replication, we found navel orangeworm adults within the control room during the protective treatment phase. The most likely source of these insects was nuts infested in the field. The nuts in the control room were from the second 8 bins to be treated, and thus had experienced 2 d of slightly elevated O_2 levels. We found no navel orangeworm in the other 3 treatment rooms during the 3rd replication, nor in any of the rooms during the first 2 replications.

Temperature and Relative Humidity. During the protective treatments, temperature and relative humidity levels in the control and IMMG virus treatment rooms fluctuated during the first 2 replicates because of problems with air conditioning equipment (Table 2). However, mean temperatures of the control, IMMG virus and controlled atmosphere treatment rooms remained within 1°C of the desired target temperature of 25°C. Both air and bin temperatures in the low temperature treatment room were less than or equal to the target temperature of 10°C. Relative humidity in the control and IMMG virus treatment room were comparable, with mean levels of between 47 and 65%. Relative humidity in the controlled atmosphere treatment room was lower (37–46%), because of the imput of dry 5% O_2 atmosphere, whereas it was higher (70–72%) in the low temperature treatment room.

Estimation of Indianmeal Moth Population Level. The mean numbers (±SE) of adult progeny produced per female Indianmeal moth on wheat bran diet under laboratory conditions were 333.2 ± 23.0, 358.0 ± 27.7, and 322.1 ± 12.5 for the 1st, 2nd, and 3rd replication, respectively. Given that egg to adult survival for our laboratory isolate under these conditions is ≈95% (Johnson et al. 1995), the estimated number of eggs produced by a single female was 365. Because 5 females were added to each treatment room each week for 11 wk, 1,825 eggs were deposited each week for a total of 20,075 eggs for each test. The total product surface area for the 4 bins was 5.88 m^2, so egg deposition was estimated to be 310 eggs per square meter per week, or 3,410 eggs per square meter for each test.

Pheromone Traps. High numbers of moths were caught in the untreated control room starting 6 wk after the beginning of the tests (Fig. 2). Peak counts of moths in the control room were 566, 468, and 847 per week for replicate 1, 2, and 3, respectively. Low numbers of moths were caught in the IMMG virus treatment room; peak numbers were 12, 7, and 31 per week in replicate 1, 2, and 3, respectively. Only 1 moth was caught in the controlled atmosphere treatment room during replicate 3; this moth was probably 1 of the laboratory-reared moths released into the room each week. No moths were trapped in the low temperature treatment room.

Walnut Samples. At the beginning of each replicate (0 wk), no walnut samples from any of the treatment rooms or the untreated control room showed any live Indianmeal moth larvae or adults or damage caused by Indianmeal moth (Table 3). In these initial samples,

Table 2. Mean (±SD) air temperature, bin temperature and relative humidity during protective treatments

Variable	Replicate	Control	Controlled atmosphere	IMMGV	Low temperature
Air temperature (°C)	1	24.2 ± 2.1	24.6 ± 0.2	24.1 ± 2.0	8.4 ± 0.7
	2	23.9 ± 1.2	24.9 ± 0.2	24.2 ± 1.2	9.2 ± 0.6
	3	24.8 ± 0.8	25.0 ± 0.2	24.5 ± 0.5	9.6 ± 0.3
Bin temperature (°C)	1	25.6 ± 2.4	—*	24.3 ± 1.7	9.0 ± 0.6
	2	24.3 ± 1.4	24.4 ± 0.3	24.1 ± 0.9	9.9 ± 0.5
	3	25.6 ± 1.6	24.4 ± 0.2	24.5 ± 0.5	10.3 ± 0.2
Relative humidity (%)	1	59.8 ± 8.8	37.4 ± 2.6	57.1 ± 2.6	70.7 ± 8.5
	2	50.4 ± 2.6	38.9 ± 4.3	46.9 ± 2.9	71.8 ± 9.6
	3	54.6 ± 2.7	46.1 ± 5.7	64.7 ± 2.6	69.8 ± 1.8

* Data lost because of recorder malfunction.

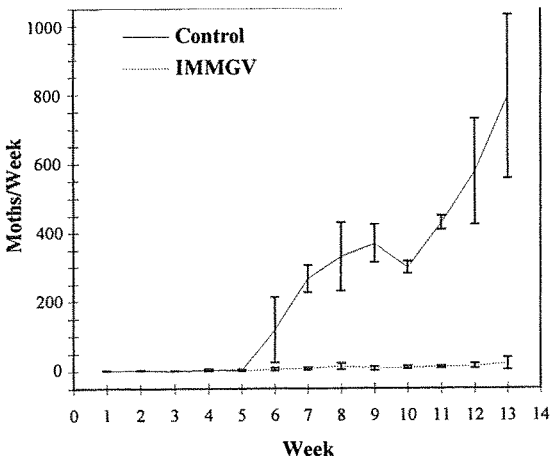

Fig. 2. Average number of Indianmeal moths caught each week in the control and IMMG virus treatment rooms. (A single moth was caught in the controlled atmosphere treatment room, none were caught in the low temperature treatment room.)

total damage before treatment was similar for all nuts. In subsequent samples from the control room, insignificant levels of Indianmeal moth damage and live Indianmeal moth were detected in the sample at 4 wk. At 8 wk, samples from the control room had significantly higher levels of live Indianmeal moths (F = 7.36; df = 2, 33; P = 0.002) and both minor (F = 17.53; df = 2, 33; $P \leq 0.0001$) and serious (F = 6.37; df = 2, 33; P = 0.005) damage caused by Indianmeal moth compared with that found in the treatment rooms. At 12 wk, samples from the control room continued to show significantly higher levels of live Indianmeal moths (F = 39.04; df = 3, 44; P = 0.0001) and both minor (F = 26.86; df = 3, 44; $P \leq 0.0001$) and serious damage (F = 131.24; df = 3, 44; $P \leq 0.0001$). In the final (12 wk) sample, 35% of the nuts from the control room had serious Indianmeal moth damage and 13% had minor damage. An average of 81 live Indianmeal moth were found. Total damage was also significantly greater (F = 36.25; df = 3, 44; $P \leq 0.0001$) in nut samples taken from the control room.

In contrast, we recovered almost no live Indianmeal moth from any of the treatment room samples. A single Indianmeal moth larva was found in 1 IMMG virus treatment sample at 12 wk, and no live Indianmeal moths were found in any of the low temperature or controlled atmosphere treatments (Table 3). No Indianmeal moth damaged nuts were found in the controlled atmosphere treatments. A single nut with minor Indianmeal moth damage was recovered from a low temperature treatment sample at 12 wk, and low levels of damage were detected in the IMMG virus samples. Walnuts from the treatment rooms for all sampling dates showed no significant differences in number of live Indianmeal moth or Indianmeal moth caused damage.

We observed large differences in moth emergence between the control and the protective treatments from walnut samples taken at the end of each test and held for 6 wk. From ≈2,000 walnuts sampled per room, an average (±SE) of 1,270.7 (±468.6) moths emerged from those taken from the control room, compared with an average (±SE) of 3.3 (±1.8) moths that emerged from samples taken from the IMMG virus treatment room. Samples taken from the low temperature treatment room produced an average of 1 (±1) moths and no moths were recovered from the controlled atmosphere treatment room.

Industry Standard Quality Analysis. The results from the commercial laboratory on quality of the treated walnuts are given in Table 4. All quality values were similar in all treatments at the beginning of the test (0 wk). After 12 wk, values for moisture content,

Table 3. Mean (±SD) insects and damage found in walnut samples

Treatment	Live Indianmeal moth	Indianmeal moth damage		Total serious damage
		Minor	Serious	
		0 Week		
Control	0	0	0	8.1 ± 1.1
Controlled atmosphere	0	0	0	8.3 ± 1.6
IMMGV virus	0	0	0	7.2 ± 1.3
Low Temperature	0	0	0	9.9 ± 1.4
		4 Week		
Control	0.5 ± 0.4	0.2 ± 0.1	0.8 ± 0.8	9.8 ± 1.2
IMMGV virus	0	0	0.1 ± 0.1	7.7 ± 1.4
Low Temperature	0	0	0	7.8 ± 0.8
		8 Week		
Control	9.2 ± 3.4a	5.3 ± 1.6a	4.8 ± 2.3a	10.8 ± 2.2
IMMGV virus	0b	0b	0b	9.7 ± 2.1
Low Temperature	0b	0b	0b	9.9 ± 1.3
		12 Week		
Control	81.0 ± 13.0a	12.6 ± 3.2a	35.1 ± 4.7a	40.4 ± 5.2a
Controlled atmosphere	0b	0b	0b	6.2 ± 0.4b
IMMGV virus	0.1 ± 0.1b	0.2 ± 0.1b	0.2 ± 0.1b	9.8 ± 2.1b
Low Temperature	0b	0.1 ± 0.1b	0b	7.4 ± 0.9b

Among treatments for each variable and sample date, values followed by a different letter are significantly different (Bonferroni t test for mean separation, $P \geq 0.05$).

Table 4. Commercial evaluation of walnut quality parameters (mean ±SD)

Treatment	Moisture content (%)	Water activity	Peroxide value	Free fatty acid (%)	Insect damage (%)	Total damage (%)
			0 Week			
Control	3.1 ± 0.03	0.48 ± 0.01	0.45 ± 0.09	0.20 ± 0.02	3.3 ± 0.9	7.5 ± 1.7
Controlled atmosphere	3.1 ± 0.04	0.49 ± 0.01	0.34 ± 0.09	0.21 ± 0.03	2.6 ± 0.9	7.5 ± 1.8
IMMGV virus	3.1 ± 0.04	0.48 ± 0.01	0.36 ± 0.07	0.24 ± 0.03	2.8 ± 0.7	7.8 ± 1.8
Low Temperature	3.1 ± 0.04	0.48 ± 0.01	0.28 ± 0.08	0.20 ± 0.02	2.2 ± 0.7	7.8 ± 1.2
			12 Week			
Control	3.2 ± 0.03	0.50 ± 0.01	0.60 ± 0.09a	0.30 ± 0.05	58.7 ± 4.5a	62.1 ± 4.4a
Controlled atmosphere	3.1 ± 0.03	0.45 ± 0.01	0.61 ± 0.11a	0.25 ± 0.03	5.0 ± 1.0b	9.9 ± 1.4b
IMMGV virus	3.1 ± 0.04	0.47 ± 0.02	0.65 ± 0.09a	0.30 ± 0.02	5.2 ± 1.4b	8.5 ± 2.0b
Low Temperature	3.2 ± 0.05	0.49 ± 0.02	0.31 ± 0.26b	0.28 ± 0.04	3.7 ± 1.0b	9.4 ± 1.8b

Among treatments for each variable and sample date, values followed by a different letter are significantly different (Bonferroni t test for mean separation, $P \geq 0.05$).

water activity, and free fatty acids remained nearly identical to the initial samples, and no significant differences among treatments were detected. Peroxide values for walnuts from the control, IMMG virus and controlled atmosphere rooms at 12 wk were nearly twice those in the sample at 0 wk. Peroxide values for walnuts from the low temperature treatment were significantly less ($F = 3.05$; df = 3, 44; $P = 0.38$) than those from the control and the other treatments. However, peroxide levels for all treatments were well within the industry standards of ≤1.0 for good quality nuts (Dried Fruit Association, personal communication).

Damage levels in the control walnuts at 12 wk were significantly higher than those found in the treatments for both serious insect damage ($F = 99.7$; df = 3, 44; $P \leq 0.0001$) and total serious damage ($F = 76.8$; df = 3, 44; $P \leq 0.0001$). Although levels of serious insect damage in walnuts from the treatments determined by the commercial laboratory were similar to those of our analysis, the commercial laboratory found higher damage levels in the control walnuts. All treatments kept insect damage below 6%, the level that may cause rejection by inspectors (Dried Fruit Association, personal communication).

Discussion

The disinfestation treatment of 0.4% O_2 for 6 d was very effective in controlling navel orangeworm in the first 2 replications. Because the O_2 level was not maintained at 0.4% for part of the treatment, problems occurred during the 3rd replication. However, only 3 of the surviving 4 insects may be attributed to this anomaly. Still, the overall efficacy of the initial disinfestation treatment proved to be above 99%.

Only in the 3rd replication were live navel orangeworm adults found in any of the treatment rooms after the disinfestation treatment. We assume that these adults were from larvae present in the walnuts at harvest and were only found in the untreated control room. The fact that no navel orangeworm were found in the IMMG virus room, which contained nuts that had received the correct treatment, supports our conclusion that the survival of field populations of navel orangeworm was the result of anomalous conditions.

The large numbers of Indianmeal moths found in pheromone traps, the high damage levels, and high numbers of Indianmeal moth recovered in nut samples in the untreated storage indicate that the Indianmeal moth population was far larger than that normally found in commercial walnut storages. The fact that all 3 protective treatments, storage under 5% O_2, storage at 10°C, or application of IMMG virus before storage, were able to protect the walnuts by keeping moth populations and damage at such low levels under such high pest pressure proves the efficacy of these methods. Because facilities and practices for the storage of dried fruits and nuts vary within the industry, the availability of several efficacious pest management methods from which processors may choose is advantagous.

Each of the 3 protective methods has advantages and disadvantages. Storage under controlled atmosphere was the most efficacious, but the sealed storage created logistical problems and reduced ready access to the product. Low oxygen atmosphere conditions present worker safety considerations that do not exist for the other methods. Extensive sealing of facilities and equipment for generating controlled atmospheres would be required to provide the needed storage conditions, and may result in considerable expense.

Low temperature storage was nearly as efficacious as controlled atmosphere, and, by keeping peroxide levels low, was more effective at maintaining product quality. Low temperature storage was readily accessible and there was no worker safety concerns. While low temperatures kept Indianmeal moth population growth to a minimum, moths exposed to 10°C for <3–4 wk are capable of recovery (Johnson et al. 1997). Low temperature storage may require insulation of existing storages or building of new facilities, with an associated increase in capital expenditure. Energy requirements for running refrigeration units add to the costs. However, because storage of walnuts at low temperatures is recommended (Ryall and Pentzer 1982), most walnut processors have some refrigerated stor-

age. For these processors, added costs would be limited.

Application of IMMG virus is probably the least expensive and easiest to apply of the 3 methods. Although the IMMG virus kept Indianmeal moth populations at acceptable levels under very high pest pressure, it was not as effective as either controlled atmosphere or low temperature storage. However, because the protection is applied to the nuts themselves, and is independent of physical plant configurations, the product remains protected as it moves through the processing chain. Earlier studies with raisins (Vail et al. 1991) showed that high levels of control (>90%) of Indianmeal moth on raisins were obtained for up to 3 mo of storage at 27°C. Although the IMMG virus preparation is not immediately available, registration of a commercial product is expected soon. The success of this method will depend on future availability and the ability of processing plants to apply the dust to bulk quantities of inshell walnuts.

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