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COMBINING CONTROLLED ATMOSPHERE DISINFESTATION WITH NON-CHEMICAL PROTECTIVE TREATMENTS IN AN INTEGRATED PEST MANAGEMENT APPROACH TO INSECT CONTROL IN POSTHARVEST DRIED FRUITS AND NUTS

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ABSTRACT

Current insect control measures for the dried fruit and nut industry depend on fumigation to disinfest large volumes of incoming products during harvest, as well as to control storage infestations. The ban on use of methyl bromide (MB) after 2005, and possible restrictions on phosphine (PH₃) create a critical need for economical alternative systems that provide efficacious control and maintain product quality. Because field pests of dried fruits and nuts do not normally reproduce in storage, initial disinfestation of an incoming product is sufficient to reduce damage by these pests. In contrast, stored-product pests are capable of repeated infestation during storage, so that long-term protective treatments provide the most efficient control. We tested an integrated method; combining initial controlled atmosphere (CA) disinfestations with subsequent protective treatments of microbial agents, low temperature storage or maintenance levels of CA for postharvest insect control of walnuts, almonds and raisins. An initial disinfestation treatment of 0.4% O₂ for 6 days was found to be efficacious against navel orangeworm (*Amyelois transitella*) and raisin moth (*Cadra figulilella*), both common field pests of these products. Indianmeal moth (*Plodia interpunctella*), the most serious storage pest of dried fruits and nuts, was effectively controlled by all storage treatments, including a maintenance CA treatment of 5% O₂. Overall product quality was unaffected by any storage treatment. We conclude that combining treatments in an integrated control program shows promise as an alternative insect control strategy for dried fruits and nuts.

INTRODUCTION

California produces over one million tonnes of dried fruits and nuts, worth nearly US\$ 1.5 billion (Anon. 1998). Costs 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 (MB) or phosphine (PH₃) for postharvest insect control. Processors use fumigants to disinfest large volumes of the incoming product during harvest, and to control storage infestations. Regulatory actions involving MB and PH₃, and the development of PH₃ resistance in stored-product insect pests has

created the need for economical alternative systems that provide efficacious control and maintain product quality throughout processing, storage, and marketing. At present, no single proposed non-chemical method is a suitable substitute for fumigation.

Any postharvest control system for dried fruits and nuts must be targeted against several species of pyralid moths. In particular, Indianmeal moth, *Plodia interpunctella* (Hübner), navel orangeworm, *Amyelois transitella* (Walker), and raisin moth, *Cadra figulilella* Gregson, are the most economically important postharvest pests of these products in California. Because infestations of navel orangeworm and raisin moth originate in the field and are carried into storage, where adults do not normally reproduce, initial disinfestation of incoming product is sufficient to reduce damage by these pests. In contrast, Indianmeal moth attacks the product after harvest, and is capable of repeated infestation during storage, so that long-term protective treatments provide the most efficient control.

To control postharvest insect infestations in dried fruit and nut storages, we proposed an integrated method that combined initial disinfestation using controlled atmospheres (0.4% O₂) with protective treatments of microbial agents (Indianmeal moth granulosis virus), cold storage ($\leq 10^{\circ}\text{C}$) or maintenance levels of controlled atmosphere (5% O₂). Our study demonstrates the efficacy of the proposed combination treatments for walnuts, almonds and raisins, using as the target insects during initial disinfestation treatments navel orangeworm for walnuts and almonds, and raisin moth for raisins. Indianmeal moth was the target insect during subsequent protective treatments of all three commodities.

MATERIALS AND METHODS

Experimental design

After an initial disinfestation treatment (0.4% O₂ for 6 or 9 d after a 2 or 4 d purge), three protective treatments and an untreated control were compared. Test species used during the initial disinfestation treatments were navel orangeworm for walnuts and almonds, and raisin moth for raisins. The protective treatments were a controlled atmosphere of 5% O₂, cold storage at $\leq 10^{\circ}\text{C}$, and Indianmeal moth granulosis virus (IMMGV) applied either as a dust (walnuts) or as an aqueous spray (almonds and raisins). Indianmeal moth was the test insect during the protective treatments for all three commodities.

Sixteen commercial raisin bins (1.3 by 1.3 by 0.65 m) filled with product were used for each test. Each bin held about 450 kg of raisins or 225 kg of nuts. After the initial disinfestation treatment, 4 bins of product were isolated in each of 4 separate rooms for subsequent evaluation of protective treatments, resulting in about 1,800 kg of raisins or 900 kg of nuts in each treatment room. Experimental rooms for the CA, IMMGV, and untreated control were specifically built for these tests. They measured 3 m square by 2.4 m high (21.5 m³) and were equipped with heating, air conditioning, and ports for introduction of test insects. A refrigerated, insulated cargo container (6

by 2.4 by 2.4 m), was used for the cold storage treatment. The complete test was done three times for walnuts, twice for almonds and once for raisins.

Description of initial disinfestation treatment

The CA room had 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 Inc. St. Louis, MO) was used to produce the required low O₂ atmosphere. Oxygen levels were monitored with a Servomex 570 paramagnetic oxygen analyzer (Servomex Company, Inc. Norwood, MA). For all walnut tests, the raisin test and the first almond test, only one of the treatment rooms was modified to hold controlled atmospheres. For the second almond test, a second room had been modified as described above, and was available for controlled atmosphere treatments.

All 16 bins of product used in subsequent protective treatment studies were first subjected to the appropriate initial disinfestation treatment. For tests with walnuts and almonds, the initial disinfestation treatment was 0.4% O₂ for 6 d. For all walnut tests and the first almond test, only one treatment room was available. 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 purge time for the first almond test was 2 d. For the second almond test, two treatment rooms were available, allowing all 16 bins to be treated at the same time. Because simultaneous use of two rooms doubled the air space to be treated by the existing equipment, purge time was extended to 4 d for the second almond test.

Initial disinfestation for the single raisin test was targeted against raisin moth. Because preliminary laboratory studies found raisin moth to be slightly more tolerant than navel orangeworm to low O₂ atmospheres, the initial disinfestation treatment for raisins was increased to 9 d at 0.4% O₂. Raisins were treated 8 bins at a time in a single room, with a purge time of 2 d.

Raisin moth and navel orangeworm used to evaluate the initial disinfestation CA treatment were from laboratory colonies maintained on a wheat bran diet (Tebbetts *et al.* 1978) at 27°C, 60% r. h. and a photoperiod of 14:10 (L:D) h. Walnuts to be infested with navel orangeworm were prepared by drilling a small hole through the shell. A single navel orangeworm larva was placed within each walnut, after which the hole was sealed with a plastic plug. About 50 infested walnuts each were enclosed in cheesecloth bags. During each walnut replicate, one bag of infested nuts was buried just under the surface of the walnuts in 8 of the 16 treated bins, for about 400 treated nuts. Four bags (≈ 200) of infested walnuts were left as untreated controls. Walnuts were treated when test insects were 25 days old (late larval instars and pupae). After treatment, walnuts were held for adult navel orangeworm emergence.

For disinfestation tests with almonds and raisins, all insects were held during treatment in glass canning jars (0.48 L) with screened lids. For raisins, 50 raisin moth larvae were placed in the jars with 100 mL of wheat bran diet and 100 mL of

raisins. Tests with almonds used jars containing 50 navel orangeworm larvae in 200 mL of wheat bran diet. Insects were treated when in the late larval or early pupal stage. In all disinfestation tests, jars were buried just below the product surface just before treatment. During each test, 8 jars (400 larvae) were treated. In addition, 4 untreated jars (200 larvae) were used as controls. After treatment, jars were brought back to the laboratory and held for adult emergence.

Description of protective treatments

Temperatures in the controlled atmosphere, IMMGV and control rooms were kept at $25 \pm 2^\circ\text{C}$. Relative humidity was not controlled. Target air temperatures in the cold treatment were $\leq 10^\circ\text{C}$. Relative humidity in the cold room was maintained at 60-80% with a cold dehumidifier. Temperature and relative humidity in the treatment rooms were recorded with data-loggers.

The stock IMMGV preparation used in the protective treatment was produced as a powder (Vail 1991). For walnuts and raisins, a dose of 28.7 mg/kg of product was selected for application. Because of the porosity of the almond shell, the applied dose was increased to 57.3 mg/kg of almonds. The IMMGV was applied as a dust to walnuts, using ground wheat germ as a carrier. Application to walnuts was done in a modified cement mixer. We applied aqueous suspensions of IMMGV (13.0 g of IMMGV dry preparation in 2.5 L of water per bin of product) to almonds and raisins. Product was spread on a conveyor belt in a thin layer. The aqueous spray was applied to the product through 2 TX8 nozzles at 40 psi placed over the conveyor belt at a height that provided adequate coverage to product.

Indianmeal moths used to evaluate the protective treatments were from a laboratory colony maintained on wheat bran diet at 27°C , 60% r.h., and a photoperiod of 14:10 (L:D) h. Five mated pairs of Indianmeal moth were added each week to the walnuts and almonds, and 15 mated pairs were added each week to the raisins. We added moths beginning immediately after the protective treatments were started, and continued for 11 weeks for the walnut tests and the first almond test, 15 weeks for the second almond test, and 39 weeks for the raisin test.

Protective treatment evaluation

Pheromone trapping: During the protective treatments, all four rooms were monitored continuously with sticky traps baited with Indianmeal moth pheromone lure. In each room, one trap was mounted ≈ 2 m above the floor. Trapped moths were counted each week for each test in the control, IMMGV and cold rooms. Trap bottoms were changed when needed. New lures were applied about every 6 weeks. Because the door to the CA room was sealed during treatment to maintain treatment atmosphere, moths were counted in this trap only after the room was aerated at the end of each test. All other trap data are reported as weekly counts of Indianmeal moth males. Lures were not replaced in the CA treatment room during the walnut or almond tests. For the raisin test, lures were replaced by pulling the trap to an access porthole by means of a string.

Nut Product Sampling: Nut samples were taken from the control, IMMGV and cold treatments at 0, 4, 8 and 12 weeks for the walnut tests and the first almond test and at 0, 4, 8, 12 and 16 weeks for the second almond test. Samples were taken from the controlled atmosphere room at the first and last sampling period for each test. Samples (about 7 kg of walnuts and 4 kg of almonds) were taken from each bin by scooping nuts from the corners and the center of the bin. Each sample was thoroughly mixed by multiple passes through a sample splitter, and then divided into quarters. One quarter was evaluated for insect activity, and one was sent to a commercial laboratory for analysis according to industry standards. The remaining two quarters were held at 4°C in reserve or for ancillary tests.

To evaluate insect activity, we opened 100 walnuts or 500 almonds from each sub-sample and examined 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 presence of live insects was also noted.

Raisin Product Sampling: During the raisin test, we took product samples immediately after the initial disinfestation treatment (0 week) and then every 5 weeks for up to 40 weeks from the control, IMMGV and cold treatments. Samples were taken from the controlled atmosphere treatment at 0 and 41 weeks, because the door seal could not be broken. Two 3.5 - 4.0 kg samples were taken from each corner and the center of each bin and placed in paper bags. One of the two samples was frozen for 3 - 4 d to kill any live insects, allowed to thaw, and sent to an independent laboratory for quality evaluation according to industry standards. The second sample was mixed thoroughly by multiple passes through a sample splitter and then divided in half. One half of each sample was held in glass jars at 25°C for 2 weeks, and then 100 g of wheat bran diet was added over the top of the raisins to accelerate development of any immature Indianmeal moth present within the samples. Samples were held for another 4 weeks and then examined for the presence of adult moths. Raisins from a 1 kg sub-sample from the remaining sample were examined individually for insect and other damage, and for the presence of live or dead insects. Instead of separate categories for minor and serious damage, a single Indianmeal moth damage category was used.

Final Product Samples: Additional product samples were taken at the end of each test to determine potential survival after treatment. Samples of about 1 kg were taken from the surface of each corner and the center of each bin, for a total of 20 samples per room. Nut samples were placed in 2-L plastic buckets. Raisin samples were placed in gallon jars along with a small amount of wheat bran diet. All samples were

held at 25°C for 6 weeks, at which time they were examined for the presence of Indianmeal moth adults.

RESULTS

Initial disinfestation treatment

The efficacy of the initial CA disinfestation treatment is summarized in Table 1. Survival of test insects was very low (10% or less) in all treatments. All survival of navel orangeworm in treated walnuts occurred in a single replicate, and is believed to be due to a technical problem that allowed the O₂ level to rise during the treatment. The 10% survival of navel orangeworm in the first almond replicate may have been due to the occurrence of diapause in test larvae.

TABLE 1
Efficacy of CA disinfestation treatments against navel orangeworm and raisin moth in walnuts, almonds and raisins

Test Insect	Commodity	Treated		Control	
		<i>n</i>	% Survival	<i>n</i>	% Survival
Navel orangeworm	Walnut ^a	1194	0.4	604	81
	Almond (Rep 1)	400	10	200	95
	Almond (Rep 2)	400	0	200	100
Raisin moth	Raisin	800	0	400	63

^a Value for walnut tests is the average for three replicates. All other values are single replicates.

Protective treatments

Pheromone traps: The numbers of Indianmeal moths caught each week in the pheromone traps are presented in Figs 1-4. No Indianmeal moths were caught in pheromone traps in either the CA maintenance or the cold storage treatment rooms in any of the commodity tests. For this reason, only trap numbers from the control and the IMMGV treatment room are given in the graphs. Results from the walnut test (Fig. 1) are presented as a single weekly average of three replicates.

The highest numbers of Indianmeal moths were found in the untreated walnuts, where trap numbers peaked at about 800 moths per week. Indianmeal moth numbers peaked at about 100 moths per week in the first almond replicate (Fig. 2). The test was discontinued at this point because poor ventilation of the control room caused a lethal build up of CO₂, resulting in high mortality of test insects. The second almond replicate (Fig. 3) was continued for 4 weeks longer, and Indianmeal moth numbers peaked at nearly 250 moths per week.

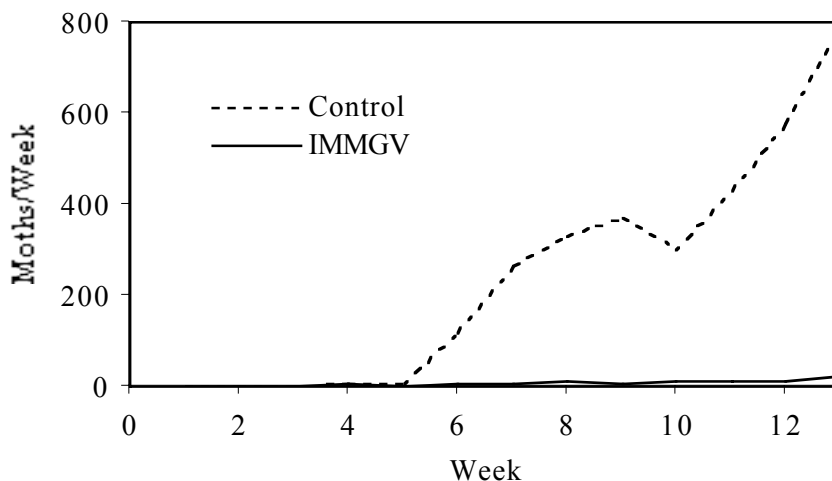


Fig. 1. Average weekly pheromone trap results for three replicates of walnut tests.

Control Indianmeal moth numbers were lowest in the raisin test (Fig. 4), even though the test was continued for 40 weeks and 15 pairs of Indianmeal moths were added each week. Trap numbers peaked at about 150 moths per week at 33 weeks, and then dropped suddenly. During this decline in moth numbers we found as many as 10 *Habrobracon hebetor* Say per week in the control room pheromone traps. We believe activity by *H. hebetor*, a parasitoid attacking pyralid larvae, was largely responsible for the decline in the moth population.

Product samples: In all tests, no live Indianmeal moth or damage caused by Indianmeal moth feeding was found at the beginning of the protective treatments (Table 2-4). At the end of each test, Indianmeal moth damage was significantly higher in control samples than in samples from any of the protective treatments. No Indianmeal moths or Indianmeal moth damaged product was found in any of the samples taken from the CA maintenance treatments. Cold storage was nearly as effective as CA in protecting product from damage. IMMGV was the least effective of the protective treatments, but still provided acceptable protection.

Final product samples: The number of Indianmeal moth adults that were recovered from the large samples taken at the end of each test is given in Table 5. No moths were found in any of the CA treatments, and very low numbers were found in the IMMGV and cold storage treatment. In contrast, large numbers of Indianmeal moths were recovered from untreated walnut (1,271) and almond (172) samples. Although

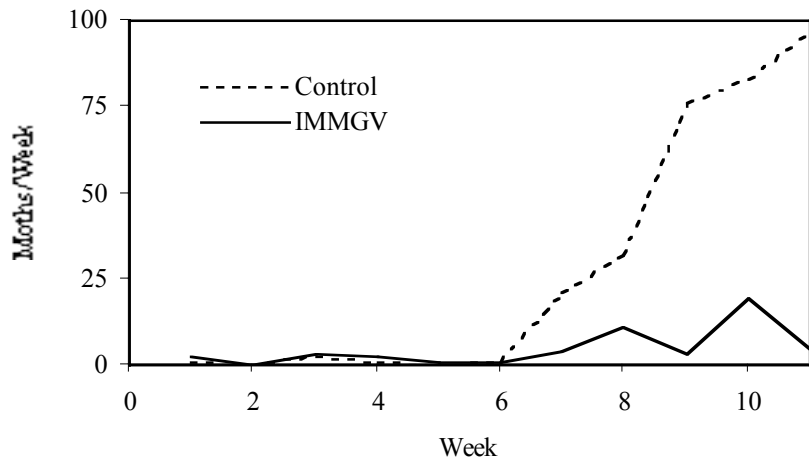


Fig. 2. Weekly pheromone trap results for the first almond replicate.

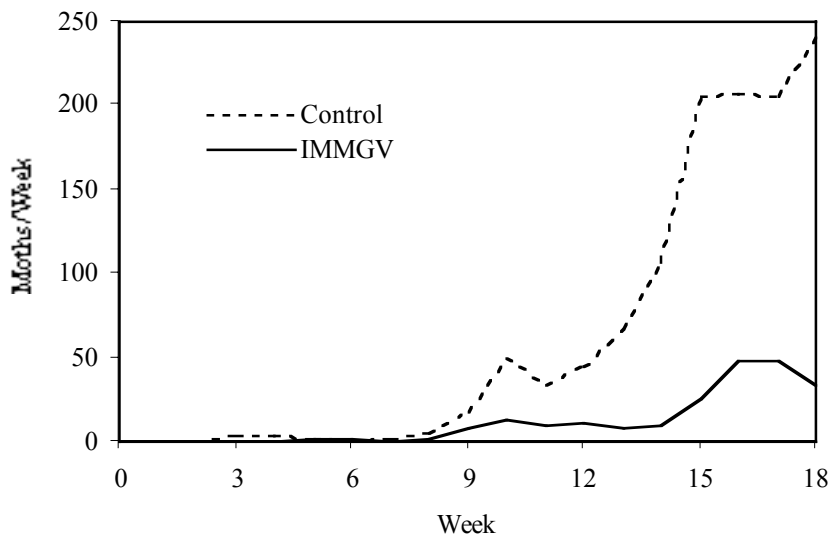


Fig. 3. Weekly pheromone trap results for the second almond replicate.

the number of Indianmeal moth found in the untreated raisin samples (31) was not as impressive as those found in the nuts, it was significantly greater than that found in the treatments.

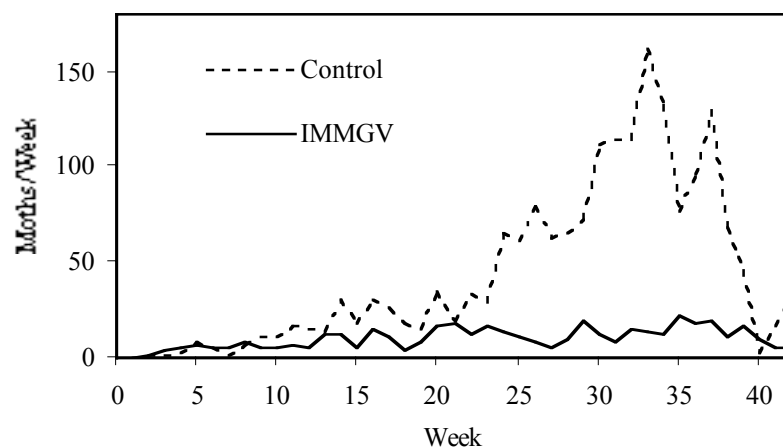


Fig. 4. Weekly pheromone trap results for the single replicate of the raisin test.

TABLE 2
Live Indianmeal moth and damage caused by Indianmeal moth feeding found in walnut samples^a

Treatment	Live IMM	% Indianmeal moth damage	
		Minor	Serious
Initial (0 week)			
Control	0	0	0
CA	0	0	0
IMMGV	0	0	0
Cold	0	0	0
After 12 weeks			
Control	81.0 ± 13.0 a	12.6 ± 3.2 a	35.1 ± 4.7 a
CA	0 b	0 b	0 b
IMMGV	0.1 ± 0.1 b	0.2 ± 0.1 b	0.2 ± 0.1 b
Cold	0 b	0.1 ± 0.1 b	0 b

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

Product quality

The industry standard commercial evaluation of treatment samples showed that all three protective treatments had no negative effects on product quality. In particular, insect activity levels by the commercial laboratory were comparable to those found in our evaluations. Peroxide levels in walnut samples from all treatments except cold storage increased gradually over time, but were never above acceptable limits. Moisture content of raisins in cold storage increased over time, although there was no associated increase in mold. The increase in moisture content was due to faulty door seals in the refrigerated container. For walnuts a more detailed report of the commercial evaluation is provided in Johnson *et al.* (1998).

TABLE 3
Live Indianmeal moth and damage caused by Indianmeal moth feeding found in almond samples^a

Treatment	Live IMM	% Indianmeal moth damage	
		Minor	Serious
Initial (0 week)			
Control	0	0	0
CA	0	0	0
IMMGV	0	0	0
Cold	0	0	0
After 12 weeks			
Control	71.6 ± 45.6 a	4.4 ± 3.1 a	13.7 ±
CA	0 b	0 b	0 b
IMMGV	0.6 ± 0.7 b	1.0 ± 1.1 b	1.2 ± 1.0 b
Cold	0 b	0 b	0 b
After 16 weeks			
Control	482.5 ± 289.7 a	4.4 ± 1.0 a	28.0 ± 6.3 a
CA	0 b	0 b	0 b
IMMGV	4.0 ± 2.2 b	0.7 ± 0.7 b	2.0 ± bc
Cold	0 b	0 b	0.3 ± 0.4 c

^a Values at 0 and 12 weeks combine data from both replications, values at 16 weeks use data from second replicate only. Among treatments for each variable and sample date, values followed by a different letter are significantly different (Bonferroni *t* test for mean separation, $P \geq 0.05$)

DISCUSSION

The overall efficacy of the initial CA disinfestation treatment was acceptable, and at times produced 100% mortality. The least successful disinfestation treatment (10%

navel orangeworm survival) occurred during the second almond replicate, and was probably due to the occurrence of a diapause-like condition in the test larvae. Diapause is not well documented in navel orangeworm, but is known to increase tolerance to CA in other insects. If diapause does occur in navel orangeworm populations, a longer treatment exposure may be necessary.

During the protective treatments, the large numbers of Indianmeal moths found in pheromone traps, the high damage levels, and high numbers of Indianmeal moth recovered in product samples in the untreated storage indicate that the Indianmeal moth population was far larger than that normally found in commercial dried fruit and nut storages. The fact that all three protective treatments 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.

Each of the three protective methods has advantages and disadvantages. Storage under CA was the most efficacious, but the sealed storage created logistical problems and reduced ready access to the product. Low O₂ atmosphere conditions present worker safety considerations that do not exist for the other methods. Extensive sealing of facilities and equipment for generating CAs would be required to provide the needed storage conditions, and may result in considerable expense.

Low temperature storage was nearly as efficacious as CA. 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 weeks 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, as storage of nuts at low temperatures may help improve shelf life, some processors may already use some refrigerated storage. For these processors, added costs would be limited.

Application of IMMVG is probably the least expensive and easiest to apply of the three methods. Although the IMMVG kept Indianmeal moth populations at acceptable levels under very high pest pressure, it was not as effective as either CA 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. Although the IMMVG 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 material to bulk quantities of product.

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