Methyl Bromide Alternatives for Postharvest Insect Disinfestation of California Walnuts

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Abstract
Before shipment, California inshell walnuts destined for the valuable export market must be disinfested of both field pests (codling moth and navel orangeworm) and common storage pests (Indianmeal moth and red flour beetle). Until recently fumigation with methyl bromide has been the most common disinfestation method, but access to methyl bromide is now greatly restricted in developed countries, causing walnut processors to seek alternative methods. In addition to alternative chemical fumigants, non-chemical physical treatments are being developed. One treatment uses radio frequency energy to rapidly heat walnuts to an average temperature of 60°C for 5 minutes. Large-scale tests using an industrial radio frequency heater successfully disinfested walnuts of navel orangeworm while maintaining good product quality. A second alternative treatment is the use of cold storage (0-5°C) to either disinfest product or prevent reinfestation of clean product. At temperatures of 0 and 5°C, 95% mortality of test insects was achieved at 10 and 18 days, respectively. Storage at 10°C prevented reproduction, product damage and reinfestation, but 127 day exposures were required to kill 95% of Indianmeal moth larvae. A third alternative treatment exposes product to a low pressure (vacuum) environment of 50 mm Hg in flexible polyvinyl chloride containers. Temperature and moisture content of the product strongly affects treatment efficacy, with complete mortality of test insects achieved at treatment exposures of 48 hours or less under optimal conditions.

INTRODUCTION
Each year, about 30% of walnuts (Juglans regia) produced in California are inshell exports. Walnuts may be infested with field pests such as navel orangeworm (Amyelois transitella) and codling moth (Cydia pomonella) or with storage pests such as Indianmeal moth (Plodia interpunctella) or red flour beetle (Tribolium castaneum). Consequently, processors have historically relied on fumigants for disinfestation. Because methyl bromide provides a relatively rapid treatment, allowing processors to meet time sensitive markets in the European Union, it was the fumigant of choice. However, the Montreal Protocol has caused the restriction of the use of methyl bromide for non-quarantine purposes, resulting in a need for suitable alternatives. In addition, the rise in demand for organic produce has increased the need for non-chemical alternatives.

Non-chemical alternatives to fumigants include the use of radio frequency (RF) energy (Mitcham et al., 2004; Wang et al., 2007a), low temperature storage (Johnson et al., 1997, 1998), and low pressure or vacuum (Johnson and Valero, 2005). While none is a substitute for fumigation, each has advantages and disadvantages. The objective of this
work is to evaluate and compare the potential of these three methods for postharvest insect control in inshell walnuts.

MATERIALS AND METHODS

The results of previous studies on RF treatment of inshell walnuts (Wang et al., 2007b) and low temperature storage (Johnson, 2007) were compared to recent studies on the use of vacuum to disinfest inshell walnuts. Vacuum treatments were applied to 70 woven polyethylene bags (polybags) of inshell walnuts (22.7 kg) stacked within a 7.5 m³ GrainPro Cocoon™, an airtight rectangular structure of UV-resistant polyvinyl chloride consisting of top and bottom pieces joined together with a tongue-and-groove zipper. When filled to capacity, the Cocoon™ was 1.5 m high by 2.95 m long by 1.7 m wide. Low pressures (vacuum) were obtained with a 2.24 kW rotary, oil lubricated vacuum pump connected to the Cocoon™ with a 44 mm diameter PVC vacuum hose with quick disconnect. Temperature, relative humidity and pressure were recorded throughout the Cocoon™ using data loggers. A pressure capsule gauge was used to determine the treatment pressure. Twenty polybags of inshell walnuts on a stack of expanded polystyrene foam sheets were used in a second Cocoon as an untreated control.

The target treatment pressure was 50 mm Hg absolute, which was normally just above the minimum pressure obtained by running the pump constantly. The time to purge the Cocoon™ to the target pressure was ≤1 hour. Tests were conducted under a shade and rain cover and temperature was not controlled, varying from 13-38°C within the Cocoon™. Test insects were Indianmeal moth, navel orangeworm and codling moth eggs, navel orangeworm non-diapausing larvae, and Indianmeal moth and codling moth diapausing larvae. These stages were selected as those most tolerant to vacuum (Mbata and Phillips, 2001; Johnson and Valero, 2005). Test insects in stainless steel screen vials were placed within two walnut polybags, one on top and one near the bottom of the stack of polybags in the treatment Cocoon™. One polybag with test insects was also placed in the control Cocoon™. After treatment exposures of 48-72 hours, test insects were removed and their mortality evaluated. Six tests were done. After all tests were completed, the nuts from two walnut bags, one from the top and one near the bottom of the stack, were evaluated for damage and compared with nuts from an untreated bag. Only nuts with no cracks and solid shell sutures were considered unbroken (sound).

RESULTS

As expected, pressures throughout the treatment Cocoon™ were uniform while temperatures varied considerably (Fig. 1). The greatest variation in temperature within the Cocoon™ was seen in the data logger placed high in the stack of walnut polybags, which received the least insulation from outside air temperatures. Temperatures from the data loggers placed low in the stack were far more uniform.

Mortality levels from test insects and corresponding treatment parameters for each of the six tests are summarized in Table 1. Average mortality was less than 4% for untreated larvae and IMM eggs, while average mortality for untreated NOW and CM eggs was about 30%. Average pressure levels in Test 1 were above the target treatment level of 50 mm Hg, due to minor leaks that were eventually patched; all other tests were <50 mm Hg. Only Test 2 (72h exposure and 27.2°C average temperature) provided complete mortality for all test insects. Very little survival was noted in Test 3 (48h exposure and 23.6°C average temperature), but there was survival in all test stages in Test 4 (48h exposure and 21.0°C average temperature). The last two tests, both with 72h exposures but with average treatment temperatures below 20°C, had relatively high survival. The lowest mortality was found in the two diapausing larval stages in Test 6, which had the lowest average treatment temperature (16°C).

Walnuts treated under vacuum had a higher proportion of broken nuts than did untreated nuts (Table 2). Much of the damage noted in treated nuts was slight, consisting of minor cracks or loose shell sutures. However, many of the treated nuts showed cracked
indentations in their shells, apparently due to pressure from the tip of another nut. These indentations were not seen in untreated nuts.

**DISCUSSION**

Temperature was critical to the success of vacuum disinfestation treatments; complete or nearly complete mortality was obtained after 48-72h exposures when average temperatures were above 23°C, but were less successful at temperatures <21°C. Diapausing larvae in particular seem to be more difficult to kill at lower temperatures. These results indicate that successful disinfestation may be achieved with exposures as short as 48 hours, provided temperatures are above 25°C, and 72 hour treatments may be more suitable. Extended exposures are needed at temperatures below 20°C, particularly when diapausing larvae may be present.

Another non-chemical alternative for fumigation of inshell walnuts is low temperature storage. Clean, insect-free product may be effectively protected from infestation when stored under temperatures ≤10°C (Johnson et al., 1998), but lower temperatures are needed for disinfestation. As with vacuum treatments, diapausing larvae were the most tolerant stage to low temperatures, with 20 days of exposure to -10°C being needed to obtain 95% mortality (Johnson, 2007). Commercial freezer temperatures of around -18°C, however, provided control of this cold-tolerant stage after 48h of exposure. Common storage temperatures used to maintain product quality (about 5°C) also may disinfest product after 3 weeks of exposure, provided diapausing larvae are not present. Temperatures of 0 and 5°C required 10 and 18 days of exposure, respectively, to achieve 95% mortality of non-diapausing insects (Johnson, 2007).

A third non-chemical alternative is the use of radio frequency energy to rapidly heat (average temperature of 60°C for 5 minutes) and disinfest the product. Although walnuts are notoriously heat sensitive, radio frequency (RF) is capable of heating the product throughout, so that the exposure to elevated temperatures is kept to a minimum and quality is unaffected (Mitcham et al., 2004). Temperature uniformity can be maximized by putting the product through two RF units, with a mixing step in between (Wang et al., 2005, 2007a). Complete mortality of test insects was achieved and product quality of treated nuts was very similar to untreated nuts when this system was tested under commercial conditions (Wang et al., 2007b).

Each of these three alternatives has distinct advantages and disadvantages. Vacuum treatments in flexible structures such as the GrainPro Cocoon™ are safe and simple to use, have relatively low initial capital costs, and minimum power requirements for the pump. However, treatments may damage nutshells and treatment times are lengthy at low temperatures. Low temperature storage requires greater initial capital costs, but this becomes irrelevant when processors already use cold storage to maintain product quality. Treatment times to disinfest product with refrigeration (0-5°C) require at least 3 weeks, and is not feasible when diapausing larvae are present. Freezing at -18°C is used by some organic processors (Klonsky et al., 1994) to disinfest walnuts of insect pests, but 2-3 weeks may be needed to reach lethal temperatures throughout bulk-stored nuts. Radio frequency treatments which make use of commercially available, industrial scale RF units show the most promise for quickly disinfesting large amounts of product. While the initial capital costs are significant, the energy cost per kg of treated product is comparable to the current cost of methyl bromide fumigation (Wang et al., 2007a).

**ACKNOWLEDGEMENTS**

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**Literature Cited**


Johnson, J.A. 2007. Survival of Indianmeal moth and navel orangeworm (Lepidoptera:
Pyralidae) at low temperatures. J. Econ. Entomol. 100:1482-1488.
Table 1. Mortality of navel orangeworm (NOW) non-diapausing larvae (NDL), NOW eggs, Indianmeal moth (IMM) diapausing larvae (DL), IMM eggs, codling moth (CM) diapausing larvae and CM eggs during vacuum disinfection tests of inshell walnuts using a GrainPro Cocoon™.

<table>
<thead>
<tr>
<th>Test#-Exp (hrs)</th>
<th>Average Treatment Parameters</th>
<th>% Mortality$^1$ ($n$)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pressure (mm Hg)</td>
<td>Temp (°C)</td>
</tr>
<tr>
<td>1-48</td>
<td>57.4</td>
<td>25.7</td>
</tr>
<tr>
<td>2-72</td>
<td>46.8</td>
<td>27.2</td>
</tr>
<tr>
<td>3-48</td>
<td>46.9</td>
<td>23.6</td>
</tr>
<tr>
<td>4-48</td>
<td>46.7</td>
<td>21.0</td>
</tr>
<tr>
<td>5-72</td>
<td>47.3</td>
<td>19.1</td>
</tr>
<tr>
<td>6-72</td>
<td>42.1</td>
<td>16.0</td>
</tr>
</tbody>
</table>

$^1$Values corrected for control mortality using Abbott’s formulae (Abbott, 1925)

* Insects in this stage were unavailable for test
Table 2. Weight of broken and unbroken nuts from untreated and vacuum-treated walnuts.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Sound nuts (kg)</th>
<th>Broken nuts (kg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vacuum 1</td>
<td>17.7</td>
<td>5.5</td>
</tr>
<tr>
<td>Vacuum 2</td>
<td>19.5</td>
<td>3.8</td>
</tr>
<tr>
<td>Untreated Control</td>
<td>22.7</td>
<td>0.8</td>
</tr>
</tbody>
</table>

**Figures**

Fig. 1. Typical pressure and temperature levels recorded during vacuum treatments by data loggers in walnut bags placed high (High) and low (Low) in the stack, in between walnut bags (Between) placed low in the stack, and attached to a small inlet monitoring valve (Outside).