Methyl Bromide Quarantine Treatment for Codling Moth
(Lepidoptera: Tortricidae) in Unshelled Walnuts

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ABSTRACT Unshelled walnuts were artificially infested with diapausing fifth instar codling moth, *Cydia pomonella* (L.), larvae and fumigated with 56 g/m³ methyl bromide for 4 h at 15.6°C under a reduced pressure of 100 mm Hg. When 34,959 were treated, one larva survived, indicating a survival rate of 2.91 larvae per 100,000 treated. The upper 95% CL for this survival rate was 13.8 larvae per 100,000 treated. When larval survival rates of methyl bromide fumigation tests of a normally applied domestic treatment were combined with survival rates from quarantine vacuum fumigation tests, the 95% CL survival rates were ≤0.4 larva per 100,000 treated. No significant differences were found in mortality of larvae among the four walnut cultivars tested, nor from variation in the size of the walnuts of each cultivar. Whole walnuts sorbed 79.6% of the methyl bromide applied, therefore a relatively high dosage of methyl bromide was required to obtain an efficacious treatment. The treatment was accepted by the Japanese Ministry of Agriculture, Forestry and Fisheries in 1986:

KEY WORDS: Insecta, *Cydia pomonella*, methyl bromide fumigation, unshelled walnuts
UNSHELLED WALNUTS COULD not be exported from the United States to Japan until recently because of quarantine restrictions against codling moth, *Cydia pomonella* (L.) (Anon. 1967). To meet Japanese quarantine regulations, treatments must be developed for fruit which are hosts to codling moth and efficacy must be proven on the least susceptible insect life stage that may be found in the fruit. Quarantine treatments have been developed for codling moth and western cherry fruit fly, *Rhagoletis indifferens* (C.), in cherries (Anthon et al. 1975, 1977, Moffitt et al. 1977, 1983a) and codling moth in nectarines (Yokoyama et al. 1987) which now allow these fruits to be exported to Japan. Research is in progress to develop a quarantine treatment for codling moth in apples (Moffitt et al. 1983b). In all these studies, methyl bromide was the fumigant used at various temperatures and exposure times. Methyl bromide is effective against all stages of codling moth (Gaunce et al. 1980, Tebbets et al. 1986). For many years it has been used extensively to control this pest in walnuts grown in California with no significant deleterious effects on quality or taste of the treated nuts (R. E. Gunnerson, personal communication, Diamond Walnut Growers, Inc.).

Preliminary studies with methyl bromide treatments at normal atmospheric pressure to control codling moth in walnuts proved to be ineffective for quarantine purposes. However, these studies indicated that methyl bromide under a reduced pressure could provide an efficacious quarantine treatment. Although vacuum fumigation is used commercially, practically no literature is available specific to its
use with methyl bromide for codling moth. Lindgren (1936) tested a mixture of methyl bromide and carbon dioxide under partial vacuum to kill codling moth larvae in used, empty walnut sacks. The cause and effect of vacuum fumigation in insect mortality was described by Cotton (1932). Monro (1969) described the improvement of fumigant penetration in the commodity and advantages of shorter exposure times under partial vacuum.

When walnuts are harvested, codling moth instars 1-5 are present from overlapping broods, but only rarely remain in the nuts (Quayle 1926). Also, fifth instars may enter diapause. Due to a shorter photophase beginning in August, diapausing larvae can be found in the walnuts in California. It is, however, a rare occurrence because most mature larvae destined for diapause exit the fruit. Previous methyl bromide concentration/mortality studies on fifth instars in cocoon showed diapausing larvae to be about two times more tolerant of methyl bromide than nondiapausing larvae (Tebbets et al. 1986).

Here we report two phases of the efficacy studies: preliminary work which included normal atmospheric pressure and reduced pressure tests, and large scale tests. Preliminary studies were designed to study efficacy of a constant methyl bromide dosage for diapausing fifth instar codling moth larvae in relation to chamber pressure, temperature, exposure times, cultivar and nut size. Large scale studies were designed to determine efficacy against diapausing fifth instar larvae in unshelled walnuts using one set of fumigation specifications that would meet Japanese quarantine requirements.
Methyl bromide residue results in walnut meats from this study (P.L.H., unpublished data) will be reported separately.

Materials and Methods

Test insects were obtained from a colony maintained in our laboratory. Rearing diet (BioServ #9370), procedures, and diapause inducement parameters were described previously by Tebbets et al. (1986). For all fumigation tests, walnuts were prepared by drilling a hole 4 mm ± 1 mm in diameter to a depth of 20 mm ± 3 mm through the shell. One larva that had been in diapause for 4 to 8 weeks was placed in each hole. The holes were sealed with a nontoxic clay-like substance. About 95% of the larvae were cocooned and were removed from the cocoon for ease of placement in the walnuts. This can cause metabolic disturbances which affects the degree of susceptibility to methyl bromide (Tebbets et al. 1986). However, the walnuts were infested 24 to 96 hours before treatment, which should have allowed some metabolic stabilization by the time treatment occurred.

The nuts were contained in 22.6 kg commercial bags of open mesh polymer fibers. All fumigations were conducted in 2.84 or 3.12 m³ controlled temperature chambers meeting performance standards established by USDA-APHIS (1976). For fumigation, the chambers were loaded with 18-21 bags, depending on bag shape. This constituted a ≥50% vol/vol load factor. About 2.2 kg of infested walnuts were isolated by placing them in smaller bags of the same type construction as the large bags. These small bags were placed among the walnuts in
the large bags that were then placed at the top back, middle center and bottom front of the chambers. Before fumigation, the uninfested and infested walnuts were conditioned at 15.6°C for 24 and 18 hours, respectively. For vacuum fumigation, chamber pressure was reduced to 100 mm Hg as observed on a pressure gauge (Model 61C-ID-0410, Penwalt, Belleville, N.J.). Pressure readings were taken just before the methyl bromide was introduced, 0.5 h afterwards and at hourly intervals thereafter. Immediately after reaching the desired pressure (about 4 minutes), the methyl bromide was introduced through a standard commercial volumetric dosimeter and was uniformly distributed in the chamber with fans operated throughout the exposure periods. Temperature was monitored with probes in the center of bags of walnuts in approximately the same location as the larvae-infested walnuts. One additional probe was placed in the open void space at the top of the vacuum chamber and in the air return duct of the normal atmospheric pressure chamber. Temperature readings were made on a scanning tele-thermometer (YSI Model 47, Yellow Springs, Ohio) just before introduction of the methyl bromide, at 0.5 h, and at hourly intervals thereafter. Gas concentration samples were drawn through 4 mm ID plastic lines, located in the same positions as those for temperature readings, at time intervals of 2-5 min., 0.5, 1, 2, 3, and 4 h post methyl bromide introduction.

For normal atmospheric pressure fumigations, methyl bromide concentration and temperature readings were made after 2-5 min., 0.5, 1, 2, 4, 8, 12 and 24 h. During vacuum fumigation, a modification of
the procedure described by Childs et al. (1976) for attaining ethylene oxide gas samples from vacuum fumigation chambers was used. In our modification, we found that maximum methyl bromide was recovered by vacuum purging the gas collecting tubes for 15 seconds before the air was evacuated from the gas sampling tubes. Concentration was determined by GLC flame ionization analysis as described by Tebbets et al. (1983). At the end of the fumigation periods, normal atmospheric pressure chambers were aerated for 24 h by drawing outside air through the walnut load and out the exhaust stacks. The vacuum chambers were washed once with air to remove the fumigant, i.e., the chambers were returned to atmospheric pressure with outside air, the pressure was again reduced to 100 mm Hg and again returned to atmospheric pressure. The doors were opened and the chambers aired under passive conditions for 48 h.

At the end of the 48 h aeration period, the insects were removed from the chambers and placed under diapause breaking conditions as described by Tebbets et al. (1986). Insects used as controls were handled and treated in the same manner as treated ones, but were not fumigated. Surviving insects or emerging adults were observed weekly. Mortality was determined by response to mechanical stimuli, larval color, and texture.

Percentage sorption was calculated from the original dosage and end methyl bromide concentration. Concentration multiplied by time products (CXT products), i.e., the area under each resulting methyl bromide gas concentration curve, were calculated in a manner similar
to that described by Monro (1969). CxT products are expressed in gram
hours per cubic meter (g.h/m³). The statistical procedures of Couey
and Chew (1986) were used to evaluate codling moth survival rates.

Preliminary Tests. Soon after harvest, walnuts are normally
fumigated with methyl bromide to control codling moth and other field
insect populations. Therefore, a preliminary test was conducted to
determine control of diapausing fifth instar codling moth larvae using
methyl bromide at 56 g/m³ for 24 h at 15.6°C, with normal atmospheric
pressure. This is one of the label schedules (EPA Reg. No. 8536-15).
Three chambers were treated simultaneously, each containing 300
diapausing larvae, and 300 were held as controls. The walnuts were
mostly 'Hartley' cultivar.

The preliminary vacuum study included four cultivars, 'Hartley',
'Payne', 'Eureka', and 'Franquette', each represented by jumbo, large,
and medium size nuts. For each of these tests, the chamber was loaded
with about equal amount of each cultivar and size. Fumigation
temperatures were 15.6, 21.1 and 26.6°C, with exposure periods of 4, 3
and 2 h, respectively. A dose of 56 g/m³ was used for all tests. For
each temperature/time test, 360 infested walnuts were split into 3
lots for the 3 chamber locations.

Large Scale Tests. To expedite the acquisition of data required
for development and approval of a quarantine treatment, only one
cultivar ('Hartley') and one set of treatment parameters were used.
To meet the Japanese quarantine security requirements, a total of
35,000 diapausing fifth instar larvae were treated and 1,853 were held as controls in three replications; each replication consisted of six tests. In each test, the total number of larvae treated were divided equally, placed inside three 22.6 kg bags of walnuts and placed in the three locations of the chamber as previously described. The methyl bromide dose was 56 g/m³ for 4 h at 15.6°C. This treatment was chosen to represent the least favorable conditions for efficacy (15.6°C) for California warehouses during the winter months.

Results and Discussion

Table 1

Preliminary Tests. Table 1 shows that 14 of 849 diapausing larvae survived the domestic normal atmospheric pressure methyl bromide schedule, which is an unacceptable survival rate for quarantine purposes. In contrast, when 988 larvae were subjected to vacuum fumigation none survived (Table 2). Under these vacuum test conditions, cultivar differences and walnut size had no apparent effect on larval mortality.

Table 2

Large Scale Tests. Table 3 shows the response of diapausing fifth instar larvae to large scale methyl bromide vacuum fumigation. After correction for control mortality, 33,972 treated larvae and the corrected sum of those treated at 15.6°C in Table 2 (preliminary) were included for a total of 34,306 larvae treated under the same vacuum conditions. One larva survived these combined studies. Based on statistics of Couey and Chew (1986), calculated survival rate for the
combined treatments is 2.91 larvae per 100,000 treated and the upper 95% CL survival rate is 13.8 larvae per 100,000 treated. Couey and Chew (1986) also demonstrated a procedure to evaluate confidence limits of survivors, "true survival proportions", by combining response to separate, independent treatments of the same population. This procedure seemed applicable for walnuts since they are routinely fumigated with methyl bromide shortly after harvest and in addition, subjected to the quarantine treatment for export. Using equation 8 (Couey & Chew 1986) to combine the survival rates of the domestic methyl bromide treatment (Table 1) and the large scale quarantine vacuum treatment (Table 3), survival rate is ≤0.4 larva per 100,000 treated at the 95% CL.

Mean values from the large scale study for methyl bromide concentration, CxT product, and sorption are shown in Fig. 1. The mean CxT product of 77.9 g·h/m³ was considered an approximate minimum value for quarantine efficacy. Tebbets et al. (1986) showed vacuum fumigation with methyl bromide was more than twice as effective (based on CxT products) as normal atmospheric pressure fumigation in controlling diapausing codling moth larvae. However, the high methyl bromide sorptive properties of walnuts (≥79.6%) required a relatively high dose (56 g/m³) and long exposure time (4 h) to obtain a CxT product that was efficacious for codling moth.

Temperature just before fumigation was 15.6°C, but after the chamber door was closed, temperatures rose slightly and remained almost constant thereafter (16.7°C ± 0.16°C). The reduced pressure at
the start (just before methyl bromide introduction) was 100 mm Hg in all tests. The expansion of methyl bromide gas increased the pressure in the chamber to 116-118 mm Hg after 0.5 h and gradually increased to \( \geq 132 \text{ mm Hg} \) at end of the 4 h exposure period.

A fumigation of 56 g/m\(^3\) methyl bromide for 4 h at \( \geq 15.6^\circ\text{C} \) and 100 mm Hg with a \( \geq 50\% \) walnut load was proposed to the Japanese Ministry of Agriculture, Forestry and Fisheries for consideration as a quarantine treatment to disinfect unshelled walnuts of codling moth. A successful commercial demonstration of this treatment was performed for the officials of the Japanese Ministry at a northern California walnut plant. In addition, a demonstration of the proposed treatment for codling moth efficacy on the cultivars 'Franquette' and 'Payne' was successfully completed for the Japanese officials. In 1986, the quarantine ban was lifted by the Japanese government allowing export of the three unshelled cultivars 'Hartley', 'Franquette' and 'Payne'.


Acknowledgment

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Table 1. Survival of diapausing fifth instar codling moth larvae in unshelled walnuts fumigated with 56 g/m³ methyl bromide for 24 h at 15.6°C, normal atmospheric pressure and a load factor of ≥50%.

<table>
<thead>
<tr>
<th>Replication</th>
<th>Control</th>
<th>Treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>Percent</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mortality</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
<td>4</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>5</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>8</td>
</tr>
</tbody>
</table>

849 14 98.4

*Adjusted for natural mortality.
Table 2. Survival of diapausing fifth instar codling moth larvae in unshelled walnuts\textsuperscript{a} fumigated with 56 g/m\textsuperscript{3} methyl bromide at 100 mm Hg at different temperatures and exposure times with a load factor of \( \geq 50\% \)

<table>
<thead>
<tr>
<th>Temperature (°C)</th>
<th>Exposure (h)</th>
<th>Control n</th>
<th>Percent mortality</th>
<th>Treated n\textsuperscript{b}</th>
<th>Survivors</th>
<th>Percent mortality</th>
</tr>
</thead>
<tbody>
<tr>
<td>15.6</td>
<td>4</td>
<td>120</td>
<td>7.2</td>
<td>334</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>21.1</td>
<td>3</td>
<td>120</td>
<td>10.0</td>
<td>324</td>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>26.6</td>
<td>2</td>
<td>120</td>
<td>8.6</td>
<td>329</td>
<td>0</td>
<td>100</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Each test contained 30 infested walnuts from each 'Hartley', 'Payne', 'Eureka' and 'Franquette' cultivar in each of the three chamber locations.

\textsuperscript{b}Adjusted for natural mortality.
Table 3. Survival of diapausing fifth instar codling moth larvae in unshelled walnuts fumigated with 56 g/m³ methyl bromide for 4 h at 100 mm Hg and 15.6°C with a load factor of ≥50%.

<table>
<thead>
<tr>
<th>Replication</th>
<th>n</th>
<th>Percent mortality</th>
<th>n① Survivors</th>
<th>Survival rate (per 100,000)</th>
<th>Upper 95% CL (per 100,000)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>602</td>
<td>1.5</td>
<td>8,896</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>601</td>
<td>2.5</td>
<td>7,638</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>650</td>
<td>3.8</td>
<td>17,438</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>From Table 2</td>
<td></td>
<td></td>
<td>334</td>
<td>0</td>
<td></td>
</tr>
</tbody>
</table>

34,306 1 2.91 13.8

①Adjusted for natural mortality.
Figure Caption

Figure 1. Methyl bromide concentrations (x ± SEM for CxT product and percent sorption) during large-scale fumigation of diapausing fifth instar codling moth larvae in unshelled walnuts fumigated with 56 g/m³ methyl bromide for 4 h at 15.6°C under a reduced pressure of 100 mm Hg and a load factor of ≥ 50%.
Running head: Hartsell et al.: Methyl bromide for codling moth in walnuts.