PRELIMINARY STUDIES TO DEVELOP QUARANTINE TREATMENTS FOR CONTROL OF THE CODLING MOTH IN INSHHELL WALNUTS FOR EXPORT TO JAPAN

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Quarantine restrictions against insects have limited U.S. exports of agricultural commodities to Asian, European, and other overseas markets. The development of effective fumigation treatments for cherries exported from the northwest has opened new markets for this crop in areas where quarantines against codling moth, (CM) *Cydia pomonella* L., have prevented its export. Codling moth does not occur in Japan (1) and is considered a quarantine insect. Entry of walnuts into the Japanese market would be possible if effective and acceptable fumigation treatments were developed.

An acceptable fumigant for control of insects is one that will eliminate an insect infestation and yet not cause injury to the host fruit. Neither should the fumigant impart residues in excess of legal tolerances nor should it present unwarrantable hazards to applicators. Methyl bromide (MB) appears to meet most of these requirements for many commodities if applied at the proper dosage and under prescribed conditions (2,3). The effects of MB as a fumigant against various stages of the codling moth in apples was investigated by Isaac (4), Moffitt (5) and by Morgan, et al. (6), and in pears by Mackie and Carter (7). Methyl bromide as a quarantine treatment of cherries for disinfection from codling moth also has been investigated (8,9,10), and a fumigation schedule for control of this insect on cherries has been subsequently established by the Animal and Plant Health Inspection Service, U.S. Department of Agriculture (11). Methyl bromide is regularly used as a fumigant for various tree nuts including walnuts for the control of other insects.

Since insect mortality is based on fumigant concentration and time, the sorption characteristics of the commodities and their cultivars being fumigated must be studied. The sorptive capacity of a commodity must be defined in order to satisfy the need for maintaining an effective concentration of the fumigant (12). Sorption by the commodity is affected by sorption of the fumigation chamber itself, the quantity and nature of the produce in the chamber (load factor), packaging materials, time and temperature.

**OBJECTIVE:**

1. Obtain an effective artificial diet for use in the laboratory for rearing the codling moth.
2. Conduct walnut infestation studies to determine if dried walnuts can be infested naturally in the laboratory.
3. Develop a procedure to artificially infest the walnuts if natural infestation proves unsatisfactory.
4. Establish fumigation perimeters to study optimum conditions for mortality of all stages (larvae, pupae, and eggs) and conditions (diapausing and non-diapausing) of codling moth on dried walnuts.
(5) Determine if there are any differences in the susceptibility to methyl bromide between codling moth strains found naturally in walnuts and those reared in the laboratory.

(6) Develop optimum rearing conditions for both diapausing and nondiapausing larvae and mortality determination procedures.

(7) Conduct preliminary dosage mortality tests to determine the time required to obtain complete mortality at the lowest dosages possible.

(8) Based on the data derived from the above objective, replicate the optimum mortality dosages. (small scale tests)

(9) As a result of the small scale dosage mortality studies, conduct large scale fumigations. (semi-commercial)

(10) Conduct preliminary codling moth diapausing larvae dosage mortality tests.

(11) Determine concentration vs time during fumigation (sorption)

PROCEDURE:

Since the month of March, the codling moth rearing laboratory was involved in changing from a diet of thinning apples to an artificial diet primarily containing ground lima beans mixed with agar and other nutrients. There were several reasons for this change: The mold problem was the most important factor encountered using thinning apples. Rearing the codling moth under sterile conditions is a primary concern. The use of weakened larvae in fumigation tests may produce inaccurate results. The artificial diet can be made sterile of harmful organisms thus eliminating this problem.

Also, the number of larvae produced in the rearing laboratory can be accurately controlled by infesting neonate larvae into one ounce vials rather than cutting up pieces of waxed paper containing codling moth eggs and spreading them over a tray of thinning apples. The apples themselves were difficult to obtain year round and cold storage proved an ineffective method of holding the apples until needed.

Production of larvae from the artificial diet started out at 800 larvae per week and is now at 1500 larvae per week. Production will be stabilized at this level as it will provide adequate numbers for the fumigation tests and maintenance of the colony.

Yields per vial of diet are now averaging about 75-80% and pupal emergence is averaging 80-85% or better. We have been infesting the cups with two larvae for larger yields of insects.

Rearing conditions and mortality determination procedures:—We have found that the optimum rearing condition for the codling moth is a 16 hrs light/8 hrs dark cycle at 26°C (79°F) with 60% RH. Through experimentation, we have found the optimum conditions for diapause to be 14 hrs dark/10 hrs light cycle at 17°C (62°F) with 55% RH. After the walnuts were fumigated with the 5th instar
codling moth larvae, the cages were then placed in a holding room where mor-
tality counts were conducted. The larvae that were alive or moribund were
placed in a small glass jars with nutmeats as a food source and corrugated
cardboard pieces as a pupation site. The eggs and pupae remained in the vials;
but cotton was dampened and placed inside the vials as a humidity source. A
microscope was used for mortality determination of the eggs. The larvae, eggs,
and pupae were held until there was evidence that the insects were no longer
alive and mortality studies could thereby be completed or the insect completed
its life cycle and could be recorded as such.

Walnut infestation studies:--In order to conduct fumigation studies on walnuts
infested with codling moth larvae, tests were conducted to determine if dried
walnuts could be infested in the laboratory. The shells of one hundred
fifty pounds of dried walnuts were loosened and infested with neonate codling
moth larvae and 24 hr old eggs. The walnuts were re-sealed and held 14 days
under optimum rearing conditions. A zero % infestation rate was achieved from
this study. The results were probably due to the low moisture content of dried
walnuts.

Artificial infestation of dried walnuts:--Due to the unsatisfactory neonate
larvae infestation studies, a procedure was needed to artificially infest the
walnuts. A 4 mm drill was used to bore a hole approximately 2 cm into the
walnut. A 5th instar larvae was placed in the hold and sealed with Fun-Tac,
which is a nontoxic clay-like substance. Twenty Hartleys and twenty Paynes
were infested in this manner and held under optimum conditions for ten days.
From this test 100% survival was obtained with evidence that the larvae were
feeding on the nutmeats. Many of the larvae had pupated and most of the larvae
had spun out.

Fumigation perimeters established.--In this study a range of dosages, times,
and temperatures were required to fully study optimum conditions for mortality
of all stages and conditions of codling moth on dried walnuts. The fumigant
used was methyl bromide. The temperatures of the chambers were 25 C and 15 C,
the times to start at 4 hrs duration and digress according to codling moth
mortality. Natural atmospheric chambers (NAP) were used in the preliminary
studies. The dosages studied were 0, 16, 24, 32, 64, and 96 g/m3. Concen-
tration of methyl bromide was monitored at the start of fumigation, after 0.5
hours and on an hourly basis thereafter. The method of analysis used is
described by Harvey, J. M. et al. 1981 (13).

Susceptibility to methyl bromide of codling moth strains.--To determine if
there was a difference in susceptibility to methyl bromide between field in-
fested codling moth found naturally in walnuts and those reared in the labora-
tory, green walnuts were collected in the field. These walnuts showed evidence
of infestation. Three replications of approximately 3 lbs of walnuts each were
fumigated at 0, 16, 24, 32, 64, and 96 g/m3 for four hours at 250C. The infes-
ted walnuts were conditioned overnight at 250C. Thirty-six per cent of these
walnuts were infested with 4th and 5th instar larvae. Results showed 100%
mortality to all insects fumigated. Control mortality was 9%.

Preliminary dosage mortality tests.--Fumigation trials were conducted using
0, 16, 24, 32, 64 and 96 g/m3 at 250C. The object was to determine the time
required to obtain complete mortality at the lower dosages. Twenty 5th instar
larvae were placed in drilled-out Hartley and Payne walnuts. These infested walnuts were placed in separate brass cages 14.5 cm x 9 cm, mesh size 40. These infested walnuts were placed in the center of 14.5 lbs of Hartley walnuts which constituted an 80% load in 29 litre glass chambers. One hundred codling moth 24 hr old eggs were placed inside four dram vials that were screened at both ends and placed approximately in the middle of the chamber along with the walnuts and cages. The chambers were equipped with air circulation and were pre-conditioned overnight at the fumigation temperature. Three tests were conducted in this manner, but the times of exposure were varied. The first test was done at 4 hours exposure, the second at 3 hours and the third test was at 2 hours. Aeration was the same as the fumigation time.

Small scale dosage mortality tests.--Based on the data derived from above tests, three replications were made at 25°C and three at 15.5°C. These tests were conducted at a two hour exposure period. All conditions were the same throughout the duration of these tests, but at 15.5°C, pupae were fumigated also. Ten 12-15 day old pupae were placed in 4 dram vials and placed in the center of each chamber.

Large scale dosage mortality tests.--Based on the results from the small scale studies a dosage mortality study was conducted in 110 cu. ft. chambers. These tests were conducted to determine if the results obtained in the small chambers could be successfully applied to a large scale (semi-commercial) fumigation. Since 100% mortality of the larvae and 99% mortality of the eggs were obtained at 32 g/m³ for 2 hours, this dosage was used in the large scale test. Following are the conditions used in the large scale tests:

(a) Forty 5th instar larvae were placed in three cages as described earlier. The cages were placed in the center of 50 lb bags (22.7 kg) of walnuts which were positioned at the top back, middle center and bottom front of chamber. Ten pupae and 100 eggs in 4 dram vials were placed in the same location as the larvae.

(b) At each insect location, a gas sampling tube and a temperature probe was installed inside the bags and one each in the free air space.

(c) The chamber load was 75% (full chamber) with a total walnut weight of 1406.6 lbs (638.6 kg). Baby and medium grade walnuts were mostly used to create this load and were of a mixed variety.

(d) Gas concentration and temperature readings were taken immediately after dosages, after 0.5, 1 h and 2 h.

(e) Insects and walnuts were conditioned overnight at the fumigation temperature of 25°C.

(f) This test was replicated three times.

Codling moth diapausing larvae dosage mortality tests.--Since the codling moth has a diapausing stage and is considered the most difficult to kill, (14) studies were conducted to determine dosage mortality at 5 dosages, 0, 16, 24, 32, 64, and 96 g/m³. The tests were designed on the same basis as the regular
larvae with the 50 day old diapause larvae placed inside individual walnuts.

RESULTS:

The data presented in tables I, II, and III are from the preliminary dosage mortality tests.

Table 1.--Preliminary dosage mortality test.

<table>
<thead>
<tr>
<th>Four hour fumigation</th>
<th>Percent mortality 25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hartleys (Larvae)</td>
</tr>
<tr>
<td>16 g/m³</td>
<td>100</td>
</tr>
<tr>
<td>24 g/m³</td>
<td>100</td>
</tr>
<tr>
<td>32 g/m³</td>
<td>100</td>
</tr>
<tr>
<td>64 g/m³</td>
<td>100</td>
</tr>
<tr>
<td>96 g/m³</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2.--Preliminary dosage mortality.

<table>
<thead>
<tr>
<th>Three hour fumigation</th>
<th>Percent mortality 25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hartleys (Larvae)</td>
</tr>
<tr>
<td>16 g/m³</td>
<td>90</td>
</tr>
<tr>
<td>24 g/m³</td>
<td>90</td>
</tr>
<tr>
<td>32 g/m³</td>
<td>100</td>
</tr>
<tr>
<td>64 g/m³</td>
<td>100</td>
</tr>
<tr>
<td>96 g/m³</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
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</table>

Table 3.--Preliminary dosage mortality.

<table>
<thead>
<tr>
<th>Two hour fumigation</th>
<th>Percent mortality 25°C</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Hartleys (Larvae)</td>
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<tr>
<td>16 g/m³</td>
<td>80</td>
</tr>
<tr>
<td>24 g/m³</td>
<td>80</td>
</tr>
<tr>
<td>32 g/m³</td>
<td>100</td>
</tr>
<tr>
<td>64 g/m³</td>
<td>100</td>
</tr>
<tr>
<td>96 g/m³</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td>40</td>
</tr>
</tbody>
</table>
The results of the replicated small scale dosage mortality tests at 15.5°C and 25°C are presented here in Tables 4 and 5.

Table 4.—Small scale dosage mortality tests.

<table>
<thead>
<tr>
<th>Dosage g/m³</th>
<th>25°C (2-hours)</th>
<th>Average percent mortality of 3 reps 25°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hartley (Larvae)</td>
<td>Payne (Larvae)</td>
</tr>
<tr>
<td>16</td>
<td>80</td>
<td>73</td>
</tr>
<tr>
<td>24</td>
<td>93</td>
<td>100</td>
</tr>
<tr>
<td>32</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>64</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>96</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td>13</td>
<td>3</td>
</tr>
</tbody>
</table>

Table 5.—Small scale dosage mortality tests.

<table>
<thead>
<tr>
<th>Dosage g/m³</th>
<th>15.5°C (2-hours)</th>
<th>Average percent mortality of 3 reps 15.5°C</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Hartleys (Larvae)</td>
<td>Paynes (Larvae)</td>
</tr>
<tr>
<td>16</td>
<td>83</td>
<td>93</td>
</tr>
<tr>
<td>24</td>
<td>90</td>
<td>93</td>
</tr>
<tr>
<td>32</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>64</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>96</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td>00.00</td>
<td>3</td>
</tr>
</tbody>
</table>

In the following table (Table 6) are the results from the large scale semi-commercial dosage mortality tests.

Table 6.—Average percent mortality from the large scale tests (3 reps) 32 g/m³ at 25°C for 2 h.

<table>
<thead>
<tr>
<th>Percent mortality</th>
<th>Larvae</th>
<th>Pupae</th>
<th>Eggs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bottom front</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Top back</td>
<td>99</td>
<td>98</td>
<td>100</td>
</tr>
<tr>
<td>Middle center</td>
<td>99</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td>2</td>
<td>12</td>
<td>18</td>
</tr>
</tbody>
</table>
Three small scale replications have been completed on diapausing larvae at 25°C for 2 hour duration. Since diapause mortality determination takes several weeks to complete, only the results from the first test is shown in Table 7.

Table 7.—Dosage mortality of diapausing CM larvae to methyl bromide in Hartley walnuts at 25°C for 2 hours.

<table>
<thead>
<tr>
<th>Dosage</th>
<th>Percent mortality from one test</th>
</tr>
</thead>
<tbody>
<tr>
<td>g/m³</td>
<td></td>
</tr>
<tr>
<td>16</td>
<td>40</td>
</tr>
<tr>
<td>24</td>
<td>45</td>
</tr>
<tr>
<td>32</td>
<td>90</td>
</tr>
<tr>
<td>64</td>
<td>100</td>
</tr>
<tr>
<td>96</td>
<td>100</td>
</tr>
<tr>
<td>Control</td>
<td>20</td>
</tr>
</tbody>
</table>

Concentration and time curves of methyl bromide during the small scale, large scale and diapause fumigation tests are presented in figures 1 through 4. The curves presented here show that the starting concentration is higher than the indicated dosage. This is the result of the displacement of the walnuts in the chamber. The sorption (methyl bromide retained by the walnuts) were as follows:

- Small scale 25°C, average of three replications: 82.2%
- Small scale 15.5°C, average of three replications: 69.6%
- Large scale 25°C, average of three replications:
  - Bottom front of chamber: 58.4%
  - Top back of chamber: 74.5%
  - Middle center of chamber: 49.8%
  - Free air space of chamber: 56.5%
- Small scale 25°C, (diapause) one replication: 75.1%

CONCLUSIONS:

The conversion from thinning apples to an artificial diet and the development of techniques for dependable rearing of consistent numbers of diapausing codling moth larvae have been the most significant achievement in the first year of this project.

Since we found that dried walnuts could not be naturally infested with codling moth, the development of an artificial infestation technique has provided the means to study the effects of methyl bromide treatments on codling moth larvae inside the walnuts.
With the implementation of these techniques, we have developed some dosage mortality data, on all stages of the codling moth.

While conducting tests with the five dosages and two temperatures, our aim has been to determine the minimum dosage and time required to effect 100% mortality on all stages of the codling moth. In the small scale tests, we were able to obtain 100% mortality of normal larvae and pupae with a dosage of 32 g/m$^3$ for 2 hours. When this treatment was applied to a large scale test (semi-commercial) we did not obtain 100% mortality in some areas of the chamber.

Although we did not obtain 100% mortality of codling moth eggs in the small scale test at 32 g/m$^3$, 100% mortality was obtained in the large scale tests. Since we found that larvae hatched from eggs that were placed on or in the dried walnuts would not survive, complete mortality of eggs may not be required. This will depend on the Japanese acceptance of this data.

In the one dosage/mortality test that was completed on 50 day old diapausing larvae, we found that higher dosages are required to obtain 100% mortality. The dosage that will cause 100% mortality falls somewhere between 32 and 64 g/m$^3$ for 2 hours. In future work, time and dosages will be refined to fill the existing gaps to find an effective treatment for both normal and diapausing larvae. In the small scale tests, overall sorption was 75.9%, while average sorption for the large scale chambers was 59.8%. The small chambers had an 80% load, the large chambers a 75% load. The main difference here was the walnuts in the large chamber were in sacks and the interstitial space was not accounted for as load factor is normally based on gross displacement.

It is important to develop treatments that will not only effect a 100% mortality to all stages of the codling moth but also not impart excessive residues to the nutmeats. Walnut nutmeats have a high oil content (64%) and, therefore, are subject to high inorganic bromine residues as well as the long retention time of organic bromide residues. In future work, we will be focusing more attention on this aspect.

1. Complete dosage mortality of 50 day old diapause larvae at 15.5°C in small scale test.
2. Complete dosage mortality of 50 day old diapause larvae at both 25°C and 15.5°C in large scale tests.
3. Develop vacuum fumigation of diapausing larvae in first the small scale chambers and then develop this data into large scale chambers.
   (This work will be done only if NAP fails or is inappropriate).
4. Study aged diapause for dosage mortality from 50 days through at least 6 months of age.
5. Start conducting organic and inorganic bromide residues on walnut fumigation that show promise of becoming acceptable quarantine treatments.
6. In the past year the Environmental Protection Agency (EPA) has focused considerable attention on residues from fumigants. With this in mind, it might be to our advantage to develop dosage mortality studies on normal and diapausing larvae using phosphine ($\text{PH}_3$). Phosphine takes much longer to kill.
the insect but does not leave residues.

(7) Prepare first report for Japanese government on efficacy.
Figure 1. Average Concentrations of Methyl Bromide during Small Scale Fumigation of Codling Moth in Walnuts at 25°C. Dosages: 16, 24, 32, 40, 46 g/m³ CH₃Br for 2 hr. duration.
Figure 2. Average concentrations of methyl bromide during small scale fumigation of codling moth in walnuts at 15°C. Dosages: 16, 24, 32, 44, 96 g/m³ CH₃Br for 2 hr duration.

- Empty chamber dosed with 48 g/m³ CH₃Br.
**Figure 3: Average Concentrations of Methyl Bromide at Four Different Locations within One Chamber during Large Scale (Semi-Commercial) Fumigation of Codling Moth in Walnuts at 25°C.**

Dosage: 32 g/m³ CH₃Br.

- **Bottom Front Location**
- **Top Back**
- **Chamber Air**
- **Middle Center**

![Graph showing the decline of methyl bromide concentration over time with different locations labeled.]
Figure 4. Concentrations of Methyl Bromide during Small-Scale Fumigation of Diapausing Codling Moth Larvae in Dried Walnuts at 25°C.
DOSAGES: 16, 24, 32, 64, 96 g/m³ CH₃Br for 2 hr duration.

Empty chamber dosed with 48 g/m³ CH₃Br.
References


References (Cont'd)

(13) Harvey, J. M., C. M. Harris, and P. L. Hartsell. 1981. Commodity Treatment: Response of nectarines, peaches, and plums to fumigation with methyl bromide (Accepted for publication - ARS series 1982)