Research Reports

Effect of Cold Temperature Treatments on the Mortality of Eggs and Feeding Larvae of the Oriental Fruit Moth

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SUMMARY. Two cold storage treatments were evaluated against eggs, early instars, and late instars of the oriental fruit moth [*Cydia molesta* (Lepidoptera: Tortricidae)] a quarantine pest for Mexico of stone and pome fruit from the United States. In the first, 'Delicious' apples (*Malus domestica*) were infested with these life stages and treated for 13 weeks in cold storage at 38 °F (3.3 °C) in replicated studies. In the second, the same life stages infesting 'Delicious' apples were exposed to air temperatures slightly above freezing, 33.3 ± 0.7 °F (0.7 ± 0.4 °C), up to 7 weeks to simulate near commercial storage conditions. Weekly subsamples of the life stages were

examined for survival. At 38 °F, complete mortality was obtained for eggs and early instars by the eighth week, and for late instars by the tenth week. At near freezing temperatures, eggs and early instars died by the fourth week, and late instars eliminated by the sixth week. This study demonstrated that the treatments were effective against the infesting life stages.

The oriental fruit moth (OFM) is a quarantine pest of stone and pome fruits in the United States. The seasonal history of OFM is similar to that of codling moth [*Cydia pomonella* (Lepidoptera: Tortricidae)] (Chapman and Lienk, 1971). OFM has a several generations during the growing season and overwinters in cocoons as mature larvae. Like the codling moth, the diapausing larvae forms cocoons outside of fruit on tree trunks and debris on ground (Chapman and Lienk, 1971; Dustan, 1963; Rothschild and Vickers, 1991). Furthermore, because survivorship of non-diapausing codling moth has been accepted as indicators of efficacy from postharvest treatments (Hansen et al., 2000a, 2000b), postharvest treatment tests on OFM should involve only eggs and feeding larvae, the life stages found with fruit at harvest.

Mexico is the United States' largest foreign market for apples (*Malus domestica*). For example, the 1994 apple crop exported from the U.S. to Mexico was valued at nearly \$90 million (U.S. Department of Agriculture, 1998) and 4.5 million boxes [≈42 lb (19 kg) per box] of domestic apples were sold to Mexico in 1997–98 season (Washington Apples, 1999). However, Mexico has phytosanitary regulations involving domestic apples to prevent the intro-

duction of OFM into their tree fruit growing areas (Secretaría de Agricultura, Ganadería y Desarrollo Rural-Dirección General de Sanidad Vegetal, 1995). For the United States to export apples into Mexico, the fruit must be treated at 38 °F for at least 90 continuous days or 32 °F (0.0 °C) for at least 40 continuous days. Although it has long been accepted that these treatments are efficacious, there is a lack of documentation in the scientific literature.

The purpose of this study was to verify that the first option treatment, at 38 °F, was efficacious for all OFM egg and feeding larval stages and to explore appropriate length of treatment when temperatures are lowered to near freezing conditions.

Materials and methods

INSECTS. The OFM colony at the USDA-ARS Yakima Agricultural Research Laboratory in Wapato, Wash., originated with eggs from an established colony at the USDA-ARS Horticultural Crops Research Laboratory in Fresno, Calif. The insects were reared on a soya-wheat germ-starch laboratory diet (Toba and Howell, 1991), which is also used for the codling moth. The colony was maintained at ≈80 °F (27 °C), 40 to 50% relative humidity, at a 16/8 h light/dark photoperiod.

INFESTATION. Host apples were organic unwaxed 'Delicious,' size ≤138 (138 fruits per 42-lb box), locally obtained, then stored in a ≈36 °F (2 °C) cold room before experimentation. Apples were infested with OFM eggs by placing ≈70 apples in a cardboard tray, 22.5 × 22.5 inches (57.5 cm), with a screen top, adding ≈500 moths containing both sexes, and maintaining them in a 77 °F (25 °C) rearing room for 24 h. Four days later, the apples were divided into groups, each containing about the same number of eggs in the blackhead stage. This stage was used because it indicated egg viability and high probability of eclosion. The eggs were counted using a microscope and marked on the fruit with a circle. Observed hatched larvae were destroyed and younger egg stages were not marked. The number of eggs used varied among the replications.

Apples were infested with OFM larvae similar to the procedure for eggs. Apples were first infested with OFM eggs by placing ≈70 apples in a screened cardboard tray, 22.5 × 22.5 inches, with ≈500 moths containing both sexes and holding them in a 77 °F rearing room

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Table 1. Average hourly treatment temperatures for fruit, air, vial of water, and fruit for two cold storage treatments.

Treatment	Life stage	Replications (no.)	Air [$^{\circ}\text{F}$ ($^{\circ}\text{C}$)] ^z		Vial [$^{\circ}\text{F}$ ($^{\circ}\text{C}$)] ^y		Fruit [$^{\circ}\text{F}$ ($^{\circ}\text{C}$)] ^x	
			Avg	SD	Avg	SD	Avg	SD
38 $^{\circ}\text{F}$ (3.3 $^{\circ}\text{C}$)	Egg	8	37.6 (3.1)	0.7 (0.4)	38.8 (3.2)	0.2 (0.1)	38.8 (3.2)	0.9 (0.5)
	Early instar	5	37.6 (3.1)	0.5 (0.3)	38.8 (3.2)	0.0 (0.0)	38.8 (3.2)	0.2 (0.1)
	Late instar	5	37.6 (3.1)	0.5 (0.3)	38.8 (3.2)	0.0 (0.0)	38.8 (3.2)	0.2 (0.1)
34 $^{\circ}\text{F}$ (1.1 $^{\circ}\text{C}$)	Egg	8	33.3 (0.7)	0.9 (0.5)	33.4 (0.8)	0.2 (0.1)	33.3 (0.7)	0.4 (0.2)
	Early instar	8	33.3 (0.7)	0.7 (0.4)	33.4 (0.8)	0.4 (0.2)	33.1 (0.6)	0.9 (0.5)
	Late instar	8	33.3 (0.7)	0.9 (0.5)	33.6 (0.9)	0.4 (0.2)	33.3 (0.7)	0.5 (0.3)

^zAverage and standard deviation of hourly temperatures from two air probes.^yAverage and standard deviation of hourly temperatures of probe in vial of water.^xAverage and standard deviation of hourly temperatures from four fruit.

for 24 h. Four days later, the apples were divided into groups, each containing about the same number of eggs in the blackhead stage. This assured that all larval treatment groups had equivalent initial populations. The apples then were returned to the 77 $^{\circ}\text{F}$ rearing room and held for an appropriate time to obtain eclosion and the desired instar stage. In an earlier study, we found that all blackhead eggs hatched within 3 d at 77 $^{\circ}\text{F}$ resulting in infestation of early instars. Likewise, late instars (Instar-IV and -V) were obtained if infested apples were held for 11 d. Thus, infested apples were maintained in the 77 $^{\circ}\text{F}$ rearing room for these times depending on the desired life stage.

TREATMENT. Eggs, early instars, and late instars were cold treated the same for each replicate. Each container, a labeled screened plastic box, had the same initial number of insects for each treatment within a replicate. The control groups were maintained in the 77

$^{\circ}\text{F}$ rearing room. The cold storage treatments were conducted in a cold room, 7.2 ft long $\times$ 6.2 ft wide $\times$ 8.9 ft high (2.2 $\times$ 1.9 $\times$ 2.7 m) where the temperature was either 38 $^{\circ}\text{F}$ or 33.3 $\pm$ 0.7 $^{\circ}\text{F}$, depending on the treatment.

Both rooms were monitored with temperature sensors. The rearing room had a micrologger (model 21X; Campbell Scientific, Inc., Logan, Utah), which recorded hourly fruit and room temperatures. The cold room had thermistors in fruit and in a vial of water, connected to a personal computer and the temperatures were read every hour. The probe in the water is a standardized method of recording temperature in cold rooms and compensates for the temporal temperature fluctuations from the cooling and defrosting phases, and opening of the cooling room door.

Every week, a subsample, a proportion based on the total number of treatment weeks, was removed and placed in the 77 $^{\circ}\text{F}$ room until all weeks

of cold storage samples were collected. After a week, the egg samples were observed for hatch. For both larval stages, cardboard strips, used by larvae as pupation sites, were added to each cage. The larvae in cages were held for more than 1 month to allow for completion of the life cycle. The numbers of emerged adults were recorded. Tests were terminated when there was no survival after 2 consecutive weeks of treatment.

DATA ANALYSIS. Average hourly temperatures and other univariate statistics were obtained for each replicate by using data from two probes for air and four for fruit. Egg survival was based on egg hatch. Larval survival was based on the number of pupae. The initial treatment population per apple was determined by the number of observed blackhead eggs. Percent survival was calculated using the number of survivors and the initial observed population. The control group was used to determine cohort survival. Data were managed using Quattro Pro

Table 2. Survival (%) of oriental fruit moth life stages from a cold storage treatment 38 $^{\circ}\text{F}$ (3.3 $^{\circ}\text{C}$).

Week ^z	Alive (%)								
	Eggs			Early instars			Late instars		
	n ^y ^x	Avg	SE	n ^x	Avg	SE	n ^x	Avg	SE
0	1,661	80.1	2.5	1,769	33.2	14.3	1,227	49.1	16.5
1	1,566	50.2	2.2	1,540	30.4	3.9	1,133	57.4	12.0
2	1,517	10.3	2.6	1,540	27.9	5.8	1,133	46.9	8.3
3	1,517	4.5	1.2	1,540	9.9	2.5	1,133	32.9	7.2
4	1,517	4.8	0.7	1,540	3.1	1.2	1,133	26.5	5.1
5	1,522	2.2	0.8	1,540	0.9	0.4	1,133	7.0	1.4
6	975	0.9	0.4	1,540	0.0	0.0	1,133	3.7	0.6
7	975	0.6	0.3	1,540	0.1	0.1	1,133	0.9	0.4
8	388	0.0	0.0	1,540	0.0	0.0	1,133	1.8	1.2
9	388	0.0	0.0	1,540	0.0	0.0	1,133	0.6	0.4
10	388	0.0	0.0	1,540	0.0	0.0	1,137	0.0	0.0
11	388	0.0	0.0	1,540	0.0	0.0	1,133	0.0	0.0
12	401	0.0	0.0	1,540	0.0	0.0	1,133	0.0	0.0
13	399	0.0	0.0	1,540	0.0	0.0	1,130	0.0	0.0

^zWeek 0 = control.^yn = total number of individuals.^xNumber of eggs declined with progressive weeks because replicates were discontinued that had no egg hatch for two consecutive observations or had detected premature egg hatch.

Table 3. Survival (%) of oriental fruit moth life stages from a cold storage treatment 34 °F (1.1 °C).

Week ^z	Alive(%)								
	Eggs			Early instars			Late instars		
	n ^{yx}	Avg	SE	n ^x	Avg	SE	n ^x	Avg	SE
0	1,521	81.0	2.7	1,314	26.3	3.2	1,327	29.6	5.4
1	1,512	64.0	2.4	1,314	14.4	1.2	1,327	30.7	3.5
2	1,516	15.3	2.2	1,314	0.6	0.3	1,327	13.4	3.1
3	1,514	0.5	0.2	1,314	0.0	0.0	1,327	0.6	0.3
4	1,519	0.0	0.0	503	0.0	0.0	1,132	0.1	0.1
5	1,206	0.0	0.0				454	0.2	0.2
6							161	0.0	0.0
7							161	0.0	0.0

^zWeek 0 = control.^yn = total number of individuals.^xNumber of insects declined with progressive weeks because some replications were discontinued that had no survivors for two consecutive observations or had detected premature egg hatch.

v. 7 (Corel, 1996). Univariate statistics of the survival data were obtained using the PROC MEANS procedure of SAS (SAS Institute, 1990).

Results

COLD STORAGE AT 38 °F. The average hourly fruit temperatures were within 1.2 °F (0.1 °C) of the intended treatment temperature of 38 °F (Table 1). The standard deviations of the hourly vial temperature were lower than those of the air temperatures. The standard errors of all hourly temperatures were less than 0.09 °F (0.05 °C).

Egg survival greatly declined during the first 2 weeks of cold storage; no eggs nor early instars survived beyond treatment week 7 (Table 2). The late instars were more cold tolerant than the early instars. The decrease in survival for both instar groups during cold storage was more gradual than for eggs. However, no larvae were collected beyond 9 weeks of cold storage.

COLD STORAGE NEAR FREEZING. Average hourly temperatures showed more variability than with the 38 °F study because the refrigeration unit was reaching its cooling capacity. However, the standard deviation of the air and vial temperatures never exceeded 0.9 °F (0.5 °C) (Table 1).

Nearly all the early instars were dead by the second week of treatment. Practically all eggs and all but eight larvae were destroyed by the third week of treatment of late instars (Table 3). The 2-week treatment extension assured no survivors among eggs and early instars by the fifth week. As before, the late instars were the most cold tolerant life stage. Survival extended to the fifth week, followed by 2 weeks with complete mortality. The number of

treated larvae declined after the fourth week because tests were discontinued in most replicates when there were no survivors for 2 consecutive weeks.

Discussion

Cold temperatures have been known to be detrimental to OFM. Dustan (1963) killed all second to fourth instars within eight weeks at 32 °F in a treated population of several hundred. Yokoyama and Miller (1989) obtained complete mortality for early OFM instars within 21 d at 32 °F; both their data and this study indicate that late instars are the most cold tolerant stage. Using controller-atmosphere storage, Dustan (1963) reported OFM survivors, among populations of about 50 larvae, after 140 d at 38 °F. We used considerably more insects, but could not validate his observation for insects at that temperature in standard storage.

The current treatment protocol involves cold storage at 38 °F for 13 weeks. This study's data demonstrate that the treatment exceeds total efficacy by several weeks. All viable OFM eggs and larvae were eliminated on apples subjected to 38 °F for 10 weeks, and all OFM died by the sixth week on apples stored near freezing, 33 °F. Thus, reducing the treatment time of domestic apples exported to Mexico market allows flexibility in marketing and provides a fresher product to the consumer.

The issue of OFM in apples of the Pacific northwestern U.S. may be academic. Between 1993 and 1997, the Washington State Department of Agriculture inspected 274,525,552 apples without any incidence of OFM (Washington State Dept. of Agriculture, 1998). Other recent surveys, from confidential sources, validate these results.

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