FINAL PROJECT REPORT
WTFRC Project Number: PH-05-507

Project Title: Development of an auxiliary cold storage component

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Budget History:

Item	**Year 1:** 2006
Salaries	0
Benefits	0
Wages	19,700
Benefits	1,970
Equipment	0
Supplies	230
Travel	0
Insects	1,300
Fruits	1,200
Miscellaneous	0
Total	24,400

Objectives:

1. Measure mortality of codling moth larvae at three different physiological stages (non-diapause feeding late instars, diapause-destined feeding late instasrs, and diapausing mature larvae) durations of regular air cold storage at 1.1 °C.

2. Develop mathematical models that describe mortality of physiological stage.

Significant findings:

- All non-diapausing larvae were dead within twelve weeks.
- Diaspause-destined feeding larvae were within seven weeks.
- The survival rates between these two stages were not statistically different and the best descriptive mathematical model was: $\ln y = 4.5719 + -0.1253x^{1.5}$, $r^2 = 0.9967$, where y is the percent live and x is the week in storage.
- At least a half of the diapausing larvae survived after eleven weeks, although there was a slight trend towards decreasing survival with cold storage time.
- Diapause occurs outside the fruit and is the mechanism for overwintering.

Introduction:

Taiwan is the third largest importer of United States (U. S.) fresh apples (Taipei Times, 2005). The apples are primarily from Washington and California (The Fruit Growers News, 2002), with 'Fuji' as the predominant cultivar, comprising 80% of those from Washington (Jimenez, 2004). After over 25 years of apple exports to Taiwan, live codling moth larvae were found for the first time in 2002, leading to a temporary ban on U. S. apples (The Fruit Growers News, 2002). To resume exports, the U. S. agreed to increase the numbers of apples inspected and, if live codling moth larvae were found in three consignments, all apple imports from the U. S. would cease (Taipei Times, 2005).

The Systems Approach (SA) is being expanded to meet these increasingly severe phytosanitary regulations for apples. The SA involves the cumulative effect of commercial operations to reduce the risk of possible pest infestation, followed with validation by intense inspection. One area that can be exploited is the cold storage component. Cold storage is already used against the apple maggot [*Rhagoletis pomonella* (Diptera: Tephritidae)] (Hallman, 2004) and the oriental fruit moth [*Cydia molesta* (Lepidoptera: Tortricidae)] (Hansen, 2002) for apples exported to Mexico. A better understanding of the impact of cold storage on codling moth larvae would strengthen this component and improve the overall utility of the SA.

Cold storage (55 d at 2.2 °C or 36 °F) for control of codling moth eggs is a component of the current quarantine treatment against codling moth for apples exported to Japan (Hansen et al., 2000). Thus, 1.1 °C (or 34 °F), the temperature 'Fuji' apples are frequently stored, may also be effective against codling moth larvae. Toba and Moffitt (1991) reported no survivors among 142,000 codling moth larvae after 13 weeks at 1.5 to 2.0% O_2 and < 1% CO_2 and held at 0 °C. However, they based the efficacy of their study on the lack of adult emergence rather than larval mortality.

Furthermore, the insecticidal effect of cold storage may vary due to the physiological condition of the codling moth larvae at the time of harvest when they are undergoing preconditioning for diapause, an inactive state which allows the larvae to overwinter within their cocoons (Newcomer and Whitcomb, 1924). Diapausing larvae do not feed and are freeze-tolerant (Brown, 1991). Cold exposures may be less effective against diapausing destined larvae, but no studies have been done to determine the effect of commercial cold storage, if any. Thus, base line information for all three physiological stages are necessary to understand the cold storage component.

Here we measured larval survival for cumulative durations of regular air (RA) at 1.1 °C, the temperature used for cold storage of 'Fuji' apples, the major export variety. Feeding larvae of both diapaused-destined and nondiapausing were examined separately. Mathematical models were developed to describe the mortality rates at cold temperature exposures and durations for complete control were calculated.

Materials and methods:

The treatments were conducted in a refrigerated room at the USDA-ARS- Yakima Agricultural Research Laboratory (USDA-ARS-YARL) in Wapato, Washington. For each time-temperature exposure, 50 'Fuji' apples were infested by hand with four larvae each, and then held overnight to allow for fruit penetration before placed in cold storage (1.1 °C). Treatment exposures were: 0 (control), 3, 5, 7, 9, 11, 12, 13, 14, 15, 16, 17, and 18 weeks. The shortest period, 3 weeks, represented the time for transoceanic shipment. Weekly exposures were necessary for the long time periods because treatment exposures ceased when no survivors were observed in two successive weeks.

Three distinct groups of larvae were examined for each cold storage exposures: feeding non-diapausing, feeding diapause destined, and non-feeding diapausing. All were from the rearing colony at USDA-ARS-YARL (Hansen and Anderson, 2006). Diapause destined larvae were induced by altering the rearing conditions, which included lowering the ambient temperatures from the normal regime of 25.5 ± 1.5 °C to 16.5 ± 0.5 °C and reducing the photoperiod from 16 h light: 8 h dark to 8 h light: 16 h dark (Hansen and Anderson, 2006). Feeding diapause destined were collected after three weeks and infested the apples as fourth instars (larval stage after three molts). Diapausing larvae were obtained as fifth instars after five weeks under these temperature and photoperiod conditions. Nondiapausing feeding larvae were acquired in the third week of normal rearing conditions and these were used to infest apples as fifth instars. Late instars were used for evaluating the three physiological stages because cold tolerance increases with larval age (Yokoyama and Miller, 1989).

For each treatment observation, infested fruits were removed from cold storage and held overnight at room temperature (≃20 °C), dissected the following day to procure the larvae, and viability determined. Any larval movement indicated survival. There were four replicates for the non-diapausing larvae, three replicates for the diapause-destined larvae, and two replicates for diapausing larvae.

Data were summarized by using Microsoft® Excel 2002 Spreadsheet (Microsoft Corp., Redmond WA). Data were analyzed by using PROC TTEST with SAS® (Release 6.12, SAS Institute, Cary, NC). Mathematic models describing larval mortality from cold exposures were developed from TableCurve 2D V. 5.01 for Windows (SYSTAT Software Inc., Richmond, CA).

Results;

Both non-diapausing and diapause-destined codling moth larvae were susceptible to cold storage (Table 1). All non-diapausing larvae died by the twelfth week whereas all diapause-destined were dead in the seventh week. The diapause-destined were younger and may have been less tolerant to the cold. Although statistically significant, the average difference in the initial infestation rate (Week 0) between the two groups was less than 2%. There were no further statistical differences in the weekly survival between these types of larvae.

The best simple mathematical model that described mortality in non-diapausing larvae with cold storage was

$$1/y = 0.0104 + 0.00003x^3,\ r^2 = 0.9985,$$

where y is the percent live and x is the week in storage. However, the residuals of this model showed that it consistently over estimated mortality after seven weeks in cold. The simple model that had the best predictions for the long term was

$$\ln y = 4.5719 + -0.1253x^{1.5},\ r^2 = 0.9967,$$

which had the same variables. It was inconsistent with the early storage times (before Week 7), but became more accurate with time. This should be a good predictor of codling moth mortality from cold storage.

Many of the diapausing larvae failed to infest the fruits and stayed outside of the apples (Table 2). Their survivorship slowly decreased during cold storage so that 66.5% were alive by the eleventh week. Larval mortality within fruits remained stable. This test was discontinued because of the high survival rate.

Discussion:

Although the cold storage killed all feeding larvae by the seventh week of cold storage, survival of diapausing larvae was anticipated. To complete the diapause stage in nature, mature fifth instars leave the fruit and quickly seek sites to spin cocoons, such as in bark or on debris lying on the ground (Putman, 1963; Simpson, 1903). By definition, feeding larvae are not diapausing larvae. In our study, we artificially tried to infest fruits with apparent diapausing larvae, but only succeeded with ≃ 25% of them, which probably were in transition from feeding non-diapause to diapause and chewed into apples because of the lack of suitable cocoon sites. The literature reports no incidence of codling moth larvae diapausing within the feeding tunnels inside fruits. Similarly, because diapause allows the larvae to overwinter, even at below freezing temperatures, we expected the larvae to survive our cold storage treatment. A slight mortality dose response was observed over time, but these larvae were not in well protected sites like they would have found in orchards. However, Garlick (1948) reported the annual winter mortality of diapausing larvae in an insectary to range between 16 to 36% over a five year period. For feeding larvae, Yokoyama and Miller (1989) observed that older instars were more tolerant to 0 C° than younger instars, although mean survival for fifth instars was still 67.2% after three weeks, a value similar to our observations (Table 1).

The SA is a practical method for insect control. Jang and Moffitt (1994) described the SA as the integration of commercial practices used in production, harvest, packing, and distribution which cumulatively meet the requirements for quarantine security. Each component of the SA need not be efficacious by itself, but that the effect is additive, so that even with variability among the components, the entire process still results in either complete efficacy (100% mortality) or low likelihood of a mating pair (Landolt et al., 1984). Acceptance of the SA requires a change in institutional philosophy. Instead of using probit-9 (99.9968% mortality per 1 million pest individuals) as the standard for quarantine security, the cumulative effect of many components result in effective quarantine security.

The SA to quarantine security has the greatest promise in maintaining fresh fruit and vegetable exports in the face of increasing phytosanitary barriers around the world. This procedure is now used by the vast majority of importing countries for codling moth and other pests in Northwest tree fruits. The SA has been used to export citrus fruits to Japan from fruit fly-free zones in Florida (Simpson, 1993) and Mexican avocados (*Persea americana*) into northeastern U. S. (Animal and Plant Health Inspection Service, 1997). Deciduous tree fruits from the Pacific northwest U. S. are other likely commodities. Thus, high quality products can be distributed to foreign lands without fear of spreading quarantine pests.

The SA for meeting quarantine requirements for codling moth is based on: insect pest control measures in the orchard before harvest in order to eliminate pests in harvested fruit; initial inspection of the fruit upon arrival at the packinghouse; postharvest grading, sorting, and packing procedures, with emphasis upon removal of insect-infested or damaged fruit; and inspection and certification of packed fruit (Moffitt, 1989; Jang and Moffitt, 1994). A high degree of quarantine security can be provided for codling moth on apples, sweet cherries (*Prunus avium*), and nectarines (*Prunus persica*) using such a system (Curtis et al., 1991; Moffitt, 1989; Vail et al., 1993). Sorting and culling along the packing line have been effective in removing cherries infested by surface pests (Hansen et al., 2003b), apples infested by codling moth (Hansen and Schievelbein, 2002; Knight and Moffitt, 1991;), and surface pests from apples (Hansen et al., 2003a).

Previous studies indicate that cold temperatures can be efficacious against codling moth larvae. Morgan et al. (1974) reported > 67.5% mortality in codling moth larvae infesting 'Delicious' apples when held 5 weeks at 0.5 °C RA. Moffitt and Burditt (1989) killed > 35,000 codling moth eggs at red-ring stage after 55 d at ≤ 2.2 °C in RA. Moffitt and Albano (1972) reported an increase in codling moth mortality after 60 days for CA (or "controlled atmosphere" where CO_2 ranges between 0.8 to 1.6% and O_2 ranges between 2.2 and 3.0% [Moffitt, 1971]) over RA cold storage. Knight and Moffitt (1991) found only dead larvae after CA and RA cold storage. Simmons and Hansen (1999) found > 97% mortality of third and fourth instar codling moth after four weeks in cherries packed with 10% O_2 and 2% CO_2 and held between 1.0 and 2.5 °C. Toba and Moffitt (1991) observed no adult emergence among 40,000 fifth instar codling moth when held in a commercial CA (1.5 to 2.0% O_2, < 1% CO_2) after 13 weeks at 0 °C. Other apple pests that have been controlled by cold temperatures are: the oriental fruit moth (Hansen, 2002); mealybugs [*Pseudococcus affinis* (Homoptera: Pseudococcidae)] (Hoy and Whiting, 1997); apple maggot (Glass et al., 1961); and plum curculio [*Conotrachelus scutellaris* (Coleoptera: Curculionidae)] (Glass et al., 1961).

Our data show that feeding codling moth larvae can be controlled by cold storage at commercial temperatures used for 'Fuji' apples. In addition to field pest management programs to reduce codling moth populations, verified for intense inspection, cold storage can be used as a supplemental component to the SA for codling moth in apples. Young larvae that may escape detection can still be controlled within seven weeks of cold storage at 1.1 °C. Even at five weeks, the probability of larval survival is reduced to less than 20%. Hence, the marketability of 'Fuji' apples to Taiwan can be sustained with the option of using short term cold storage.

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Table 1. Comparisons by Student's *t* test of the weekly percent (mean ± SE) live codling moth larvae between non-diapausing (four replicates per treatment) and diapaused destined (three replicates per treatment) in 'Fuji' apples held at 1.1 °C for 13 weeks.

	% Live		
Week	Non-diapause	Diapause destined	*t*
0	95.7 ± 0.3	97.6 ± 0.1	4.53**
3	53.6 ± 11.4	67.7 ± 7.4	0.94
5	19.9 ± 5.3	12.6 ± 0.6	1.16
7	9.1 ± 4.4	0	2.08
9	2.7 ± 1.8	0	1.55
11	3 ± 0.3		
12	0		
13	0		

Initial infestation was at four larvae/fruit with 50 fruits per replicate.
** Significant at $P < 0.01$.

Table 2. Weekly percent (mean ± SE) of live diapausing codling moth larvae in 'Fuji' Apples and all found within the container holding the fruits at 1.1 °C for 11 weeks.

	% Live	
Week	In fruit	Total
0	28.6 ± 3.3	98.5 ± 1.5
3	20.7 ± 2.4	84.2 ± 4.9
5	25.0 ± 1.1	88.5 ± 1.0
7	29.0 ± 5.4	81.9 ± 1.3
9	29.4 ± 6.3	74.8 ± 10.0
11	22.7 ± 3.8	66.5 ± 4.1

Initial infestation is at four larvae/fruit with 50 fruits/replicate and two replicates per treatment.