

## Summary of the Taiwan Protocol for Evaluating the Efficacy of Codling Moth Control Programs in Apple Orchards with Fruit for Export to Taiwan (2006 revised)

**A. Voluntary Initial Screening Guidelines:** If cumulative trap catch in a single codling moth trap during the second and third CM generations meets or exceeds 30 moths per trap in a conventionally treated orchard or 20 moths per trap in a mating disrupted orchard, that orchard or that portion of the orchard should be considered high risk for export to Taiwan and carefully considered before committing the fruit to that market. Use large delta trap; 1 mg. lure in conventional orchards and 10 mg. lure in mating disrupted orchards; WSU suggests 1 trap per 5 acres (or grower practice); based on weekly monitoring.

### **B. Required Codling Moth Damage Pre-Packing Fruit Evaluation:**

#### **On-Tree Sequential Field Sampling Protocol:**

1. The sequential sample should be started in the area with the highest CM trap catches or in area with known codling moth problems. **Sampling personnel must review codling moth trap catch records for the block before beginning the sample.** Samples should be collected on a regular grid throughout the area being sampled. Blocks chosen for sampling should be reasonably uniform in terms of cultivars, growing conditions, and codling moth management program. Any person competent to recognize codling moth damage in the field can do the sampling, **however those without a pesticide consultants' license must participate in a pre-season training session.**
2. Trees should be selected randomly within the area, but separated by roughly 70 feet.
3. Each tree sample will consist of 60 half fruit per tree.
4. The trees should be sampled starting at the top of the tree. Count each fruit of which an entire half plus the calyx can be seen. Continue counting half-fruits from the top working down the tree until a total of 60 fruit are examined.
5. The number of trees to be sampled depends on the codling moth damage status of the orchard. A clean orchard may require sampling as few as 21 trees to complete the evaluation. A fruit with two live larvae is counted as only a single fruit infested. All suspect fruit should be cut to determine if the injury is the result of a successful larval entry or is only a sting (unsuccessful entry). Record only the successful entries as injury.
6. Sampling must be done within two weeks of the beginning of harvest in each variety in each grower lot.
7. The Taiwan codling moth field sampling form included in this packet must accompany the delivery of fruit or be on file in the packing facility for each grower lot to be exported to Taiwan. This form must be presented, along with the cull analysis records to the appropriate state commodity inspection officials at the time a requested phytosanitary inspection takes place.
8. If 0.2% **or more** of the fruit in the sample have live larvae present, the orchard is prohibited for export to Taiwan.
9. See the attached sequential sampling protocol document for more details.

10. If, following a 50 tree sample, no decision can be made to reject or accept the orchard block (detection of 4 to 7 live larvae after 50 trees) (see Taiwan codling moth field sampling form), the field sample can be repeated no sooner than 7 days from the date of the original sample to determine if the block can be accepted or rejected. If the second field sample is again inconclusive or results in the rejection of the block, that variety in that grower lot is rejected for export to Taiwan. Alternatively, the second sample in such blocks may be the bin sample. If the block fails the bin sample, that variety in that grower lot is rejected for export to Taiwan. If the lot passes the second sample all paperwork from both samples must be submitted to state department of agriculture officials to allow phytosanitary certification.

**-or-**

**Bin sampling alternative.** A minimum of 1500 fruit must be inspected per grower lot to look for larvae and insect damage. It is recommended that no more than 25 fruit be sampled randomly from each bin before the lot is submitted for packing for Taiwan. If the lot size is smaller than 60 bins more fruit can be sampled from each bin. Sampling can be done in the orchard or at the packing facility. All suspect fruit **and a minimum of 150 fruit** must be cut. **If fruit without damage symptoms must be cut to meet the minimum fruit cut requirements, that fruit must be cut vertically from stem to calyx to increase the chances of detecting codling moth.** Bins samples should represent fruit from all parts of the orchard. If more than 0.15% of the fruit in the sample have live larvae present (2 live larvae in 1500 fruit), the orchard is prohibited for export to Taiwan. A form to record the results of bin sampling is attached. Any person competent to recognize codling moth damage can do the sampling, **however those without a pesticide consultants' license must participate in a pre-season training session.** Certified cull fruit cutters may also perform the bin sampling.

## Background on the Binomial Sequential Sampling Plan for CM Orchard Evaluation

### Protocol:

1. The sequential sample will be started in the area with the highest CM trap catches.
2. Trees should be selected randomly within the area, but separated by roughly 70 feet.
3. Each tree sample will consist of 60 half fruit per tree.
  - a. The trees should be sampled by looking at the top initially. Count each fruit of which an entire half plus the calyx can be seen. Continue counting half-fruits from the top working down the tree until a total of 60 fruit are examined.
  - b. Movement around the tree is allowed as long as the same fruit are not sampled twice. If a total of 60-half fruit cannot be obtained from one tree due to small tree size, then continue sampling fruit on the adjacent tree.
  - c. Record the total number of fruit with successful entries by codling moth on each tree.  
*IMPORTANT – a fruit with two live larvae is counted as only a single fruit infested!*
4. It is highly recommended that an extendable pruning sampler be used to collect fruit that is suspected to have codling moth injury. The suspect fruit should be cut to determine if the injury is the result of a successful larval entry or is only a sting (unsuccessful entry). Record only the successful entries as injury.
5. Do not sample for codling moth injury in the middle of the day when the sun is high in the sky.
  - a. The reflection of light of fruit and the intensity of light makes it difficult to observing codling moth injury.
  - b. Sample so that you are not looking directly into the sun.
  - c. Do not sample for more than 4 hours (continuous) at any one time.
6. Samples should not be concentrated on the edges of the block alone. Samples should be collected on a regular grid throughout the area being sampled, starting in the area with the highest CM trap catches.
  - a. Blocks should be reasonably uniform in terms of cultivars, growing conditions, and codling moth management program. If they are not, each area needs to be sampled separately.

### Binomial Sequential Sample

#### Background:

A binomial sequential sample allows us to greatly reduce the number of samples needed when the population of codling moth is high or low compared to our target thresholds. Typically, these sequential sampling plans are a bit more complicated to explain, but are relatively simple to use and can reduce overall sampling effort by about 30-50%.

The codling moth sequential sample detailed below is based on field data from 24 orchards and validated by use of 27 other orchards. Our analysis used the most conservative approach, which is to use the tenth percentile of the clumping parameter calculated from the 24 orchards (*i.e.*, we used a very high estimate of clumping). We then used simulation studies with the 27 orchards to validate how accurate the sampling protocol would be and adjusted our error rates to achieve the best sampling program.

The thresholds used are 0.2% as the upper threshold, which would eliminate the orchard from export (unless further sampling down the line were performed? Wasn't this what we said?), with a lower threshold of 0.04% (which would allow us to stop sampling and accept the orchard was ok for export).

**How to use the Sampling Table:**

The sampling table has three columns with numbers and 2 blank columns:

1. The number of trees sampled (60 half fruit/tree)
2. The cumulative number of infested fruit that allows you to stop sampling – if this number is exceeded then the upper threshold (0.2%) has been exceeded.
3. The cumulative number of infested fruit that would allow you to stop sampling – if the number of infested fruit is less than this number then the damage is less than 0.04%.
4. The first blank column is the number of fruit infested with live larvae on that tree
5. The second blank column is the cumulative number of fruit infested with live larvae.
6. To use the table, you sample the first tree and put the number of fruit with live larvae present in the 4<sup>th</sup> column. The fifth column is used to calculate the running sum (cumulative number) of fruit with live larvae present.
7. If the cumulative number of fruit with live larvae is greater than or equal to the second column, you stop sampling and reject the orchard from further consideration for export.
8. The third column is the number of fruit with live larvae that are allowed before you stop sampling. The cumulative number of fruit with live larvae must be less than or equal to this value to stop sampling. Notice that you must sample at least 21 trees before you can stop because the number of larvae is less than the value in the below column.

**Table 1.** Sequential sample table to determine if the damage level is greater than or equal to 0.2% or less than or equal to 0.04%

Stop if No. Fruit w/Live Larvae is				
Tree	Above or equal to	Below or equal to	No. Fruit w/Live Larvae	Cum no.
1	3	A minimum of 21 trees must be sampled before stopping in orchards with low risk of codling moth infestation.		
2	3			
3	3			
4	3			
5	3			
6	3			
7	3			
8	3			
9	4			
10	4			
11	4			
12	4			
13	4			
14	4			
15	4			
16	4			
17	4			
18	4			
19	5			
20	5	–		
21	5	0		
22	5	0		
23	5	0		
24	5	0		
25	5	0		
26	5	0		
27	5	0		
28	6	0		
29	6	0		
30	6	0		
31	6	1		
32	6	1		
33	6	1		
34	6	1		
35	6	1		
36	6	1		
37	6	1		
38	7	1		
39	7	1		
40	7	1		
41	7	2		
42	7	2		
43	7	2		
44	7	2		
45	7	2		
46	7	2		
47	7	2		
48	8	2		
49	8	2		
50	8	3		

## Questions About the Performance of the Binomial Sequential Sample:

*How many samples will be required?*

In general, the farther the orchard-wide percentage of fruit with live larvae is from the upper or lower threshold, the lower the sample size. This is particularly true if the orchard-wide percentage is above the upper threshold – you can literally make the decision within just a few trees. For the case where the value is below the threshold value of 0.04%, you cannot stop sampling until at least 21 trees are sampled, so clean orchards are a bit harder to trigger the lower stopping point. The place where it is hardest to determine if you are above or below the threshold is if the orchard-wide percentage of fruit with live larvae is between 0.2% and 0.04%, and the sample size is highest in this area. Our data and simulation studies show that the highest average number of samples taken in this area is typically 34 trees, but it may be higher or lower than that based on chance alone.

*This seems complicated; why not use a fixed sample size?*

The sequential sample should result in 30-50% fewer samples being taken than using a fixed sample size of 50 trees per block.

*What is the chance that I'll miss a hot spot using this sampling system?*

On average, 5% of the time you will make a type I error (thought the orchard was higher than 0.2%, when it was lower) and 5% of the time will make a type II error (thought the orchard was lower than 0.04% when it was higher). In fact, we've skewed these percentages to even lower numbers two ways. First, we're starting in the area with the highest trap catch. Second, we're using an estimate of population clumping that is so high only 10% of the orchards we've surveyed exhibit that value. Both of these factors actually make our sample more sensitive and reduce the chance of a type I error – where we get cut off from export because we have live larvae present. We are also sampling a long distance between the samples so we are covering a large part of the orchard.

**Example Tables:**

The first table (Table 2) shows what would happen if the average percentage fruit with live larvae is 0.4%. The data were generated with a negative binomial random number generator. As you can see, with this block, only 9 trees needed to be sampled ( $60 \times 9 = 540$  fruit total) to reach a decision. In the next table (Table 3), the average percentage infested is 0.01% and we can't reach a decision until 21 trees ( $21 \times 60 = 1260$  fruit) have been sampled. The final example table (Table 4) shows when the full 50 tree sampling fails to reach a decision. The final count falls between 4 and 7 meaning that the infestation level falls between the cut off thresholds. In this case the orchard will require a follow-up sampling either by repeating the orchard sampling no sooner than 7 days, or by performing a bin sampling.

**Table 2.** Example of sequential sample when the average percentage of fruit with live larvae is 0.4%.

Stop if No. Fruit w/Live Larvae is			Example Data		
Tree	Above	Below	No. Fruit w/Live Larvae	Cum no.	Decision
1	3	-	0	0	continue
2	3	-	0	0	continue
3	3	-	0	0	continue
4	3	-	1	1	continue
5	3	-	0	1	continue
6	3	-	0	1	continue
7	3	-	0	1	continue
8	3	-	1	2	continue
9	3	-	1	3	Reject

**Table 3.** Example of sequential sample when the average percentage of fruit with live larvae is 0.01%.

Stop if No. Fruit w/Live Larvae					
Tree	Above	Below	No. Fruit w/Live Larvae	Cum no.	Decision
1	3	-	0	0	continue
2	3	-	0	0	continue
3	3	-	0	0	continue
4	3	-	0	0	continue
5	3	-	0	0	continue
6	3	-	0	0	continue
7	3	-	0	0	continue
8	3	-	0	0	continue
9	4	-	0	0	continue
10	4	-	0	0	continue
11	4	-	0	0	continue
12	4	-	0	0	continue
13	4	-	0	0	continue
14	4	-	0	0	continue
15	4	-	0	0	continue
16	4	-	0	0	continue
17	4	-	0	0	continue
18	4	-	0	0	continue
19	5	-	0	0	continue
20	5	-	0	0	continue
21	5	0	0	0	Accept

**Table 4.** Example of sequential sampling when the average percentage of fruit with live larvae falls between 0.01% and 0.4%. This block will require a second sampling to make a final determination. If it occurs a second time, the lot is rejected.

Stop if No. Fruit w/Live Larvae is:					
Tree	Above or equal to	Below or equal to	No. Fruit w/Live Larvae	Cum. No.	Decision
1	3	<b>A minimum of 21 trees must be sampled</b>	1	1	continue
2	3		0	1	continue
3	3		0	1	continue
4	3		0	1	continue
5	3		0	1	continue
6	3		0	1	continue
7	3		0	1	continue
8	3		0	1	continue
⋮	⋮	⋮	⋮	⋮	⋮
47	7	2	1	4	continue
48	8	2	0	4	continue
49	8	2	0	4	continue
50	8	3	2	6	Resample

## Taiwan Export Program-Orchard Codling Moth Control Worksheet

Sequential sampling table to determine if the fruit from a specific grower lot is eligible for export to Taiwan  
(i.e. damage level is greater than 0.2% or less than or equal to 0.04%)

**Stop if No. Fruit  
w/Live Larvae is:**

Tree	Above or equal to	Below or equal to	No. Fruit w/Live Larvae	Cum. no.
1	3	<b>A minimum of 21 trees must be sampled before stopping in orchards with low risk of codling moth infestation.</b>		
2	3			
3	3			
4	3			
5	3			
6	3			
7	3			
8	3			
9	4			
10	4			
11	4			
12	4			
13	4			
14	4			
15	4			
16	4			
17	4			
18	4			
19	5			
20	5			
21	5	0		
22	5	0		
23	5	0		
24	5	0		
25	5	0		
26	5	0		
27	5	0		
28	6	0		
29	6	0		
30	6	0		
31	6	1		
32	6	1		
33	6	1		
34	6	1		
35	6	1		
36	6	1		
37	6	1		
38	7	1		
39	7	1		
40	7	1		
41	7	2		
42	7	2		
43	7	2		
44	7	2		
45	7	2		
46	7	2		
47	7	2		
48	8	2		
49	8	2		
50	8	3		

**Grower/Orchard Name** \_\_\_\_\_ **Variety/Grower Lot Number** \_\_\_\_\_

**Sampled by** \_\_\_\_\_ **Date** \_\_\_\_\_

*This document must accompany the first load of fruit from this lot upon delivery to the packing facility. Lots that do not have documentation verifying low risk of codling moth presence are not eligible for export to Taiwan. If no decision is reached after sampling 50 trees, orchard may be resampled once in the field in no less than 7 days to verify infestation levels or once using the bin sample. If the lot does not pass following these resamples, the lot is disqualified for export to Taiwan.*



# Taiwan Apple Inspection Log

## Detection of Codling Moth During Bin Sampling

Packer: \_\_\_\_\_

Exporting Company: \_\_\_\_\_

Producer	Lot Number	Variety	Date	Total # of bins	# of bins sampled	# of fruit sampled	# of fruit cut	Number of live larvae	Identification

Note: a minimum of 150 fruit must be cut per each grower lot

\_\_\_\_\_

Name of Technician

\_\_\_\_\_

Signature