Sampling Plans for Cryptophlebia spp. (Lepidoptera: Tortricidae) Attacking Macadamia and Litchi in Hawaii

VINCENT P. JONES

Department of Entomology, University of Hawaii, Honolulu, HI 96822


ABSTRACT The suitability of using the presence of eggs or hatched eggs as a predictor of larval damage from Cryptophlebia spp. in macadamia and litchi was evaluated. Although larval damage or presence was not independent of egg presence, eggs were not suitable as a predictor of damage. Less than 51% of the macadamia nuts with eggs had larvae or larval damage, and <12% of the litchi fruit showed damage. Binomial sequential sampling plans were developed for both crops using the Wald sequential probability ratio test. On macadamia, the peak average sample number was <100 nuts, and when the percentage of infested nuts was <8 or >25%, the average sample number was <50 nuts. Peak average sample number required for litchi was <220 fruit because the thresholds for damage are lower than on macadamia. If damage to litchi is >10%, then the average sample number is <50 fruit.

KEY WORDS Cryptophlebia illepida, Cryptophlebia ombrodelta, sampling, macadamia, litchi

MACADAMIA, Macadamia integrifolia (Maiden & Betche), production is presently centered in Hawaii, Australia, South Africa, Malawi, Costa Rica, Guatemala, and Brazil. The first 4 areas accounted for 92.5% of the world production in 1989, and all have at least 1 Tortricid moth pest in the genus Cryptophlebia Walsingham. Litchi, Litchi chinensis Sonnerat, production is less centralized and smaller, but is an important tropical specialty fruit in Hawaii and Australia. In these areas, Cryptophlebia spp. are important components of the pest complex (Chapman 1984, Jones et al. 1992b).

The litchi fruit moth, Cryptophlebia ombrodelta (Lower), is a native Australian insect found in Australian and Hawaiian macadamia and litchi production areas (Waite 1986, Jones and Caprio 1992, Jones et al. 1992b). The false codling moth, C. leucotreta Meyr, is common to macadamia nuts in South Africa and Malawi (La Croix and Thindwa 1986). The koa seedworm, C. illepida (Butler), is the dominant species in Hawaii in litchi and macadamia (Jones and Caprio 1992, Jones et al. 1992b), and C. batrachopa Meyr is a macadamia pest in Malawi and Zimbabwe (La Croix and Thindwa 1986). Studies from Hawaii, Australia, and Malawi indicate that direct damage to the macadamia kernel is rare following shell hardening (Namba 1957, Ironside 1974, La Croix and Thindwa 1986, Jones and Caprio 1992), and that husk damage is responsible for higher than normal nut abscission rates before nut maturity (Jones 1994a). Damage on litchi from C. ombrodelta and C. illepida is by penetration of the pericarp and tunneling into the fruit.

The importance of C. illepida and C. ombrodelta in Hawaii has made the development of monitoring techniques a priority item for pest management. Adult males of both species can be monitored using traps baited with the oriental fruit moth, Grapholitha molesta (Busck), pheromone, but these traps have a limited use for determining population dynamics on macadamia. For example, Jones and Caprio (1992) reported that in 2 separate locations during the fall, catch of male koa seedworm and litchi fruit moths showed a general increase, while egg deposition decreased to low levels. They suggested that dispersal thus plays an important role in population dynamics of Cryptophlebia spp. This is supported by Namba (1957) who found that damage increased in macadamia after the pods of adjacent Acacia koa dried up. Sinclair (1974) also reported that dispersal of C. ombrodelta from adjacent host plants was important in population dynamics in Australian macadamia nut orchards.

The eggs of Cryptophlebia spp. may be difficult to see, may fall off the nuts, be eaten or parasitized, or larvae emerging may not be able actually to penetrate the husk. Further, in an orchard under mating disruption, unfertilized eggs may be laid. These facts suggest that egg counts would not be suitable for integrated pest-management decision making. To determine accurately the population dynamics in macadamia and litchi, a method which directly measures Cryptophlebia activity on the nuts or fruits is required. My objectives were to determine the relationship between Cryptophlebia spp. eggs and larval damage, and to de-
velop sampling programs that could assist in decision making.

Materials and Methods

Litchi Data Sets. Trials were conducted in the summer of 1992 at 4 commercial orchards with 4 different cultivars. Twenty-five fruit representing the full range of fruit size present on a tree on a given sampling day were collected from 3 to 4 randomly selected trees per orchard per cultivar (75–100 fruit per cultivar per sampling day). Fruit were collected from early season just after fruit set at 2-wk intervals until harvest from early March to late May, and at weekly intervals afterwards until harvest in mid-July to early August at the Kilauea and Kapaa sites (Kauai Island). At the Kona sites (Hawaii Island), fruit collection started in mid-May at weekly intervals using the same methods previously described. Fruit from a given tree were placed in a paper bag and transported to the laboratory on Oahu for processing where fruit were examined for Cryptophlebia spp. eggs or damage. Eggs were classified as either old (hatched, destroyed by natural enemies, unhatched but dead) or new (<4–5 d old and viable). For analysis of the relationship between eggs and larval damage, data were considered only when the litchi size >18 mm diameter because Cryptophlebia females rarely oviposit on smaller fruit (Jones 1994b).

Macadamia Data Sets. Macadamia data were collected from several locations on the island of Hawaii over a 4-yr period. For the first 4 data sets, hatched and unhatched eggs were not recorded separately. For the 1st data set, nuts were collected from the University of Hawaii Kona Experiment Station over a 13-mo period between November 1990 and December 1991 using ‘Purvis’, ‘Kau’, ‘Mauka’, ‘Pahala’, ‘Makai’, and 2 unnamed cultivars ‘816’ and ‘856’. These experiments, described in detail by Jones and Caprio (1992), used a maximum of 50 nuts per tree collected monthly from under 3 trees of each cultivar between November 1989 and August 1990; 4 trees of each cultivar sampled between September 1990 and December 1990. Thus, a maximum of 150 and 200 nuts per cultivar per month were collected during their respective periods. No insecticides were applied in the orchard throughout the study period. Nuts were frozen at −10°C for a minimum of 3 d, then shipped to the laboratory in Honolulu where each nut was examined for Cryptophlebia eggs and larvae.

The 2nd data set (island survey) was collected from seven orchards on the island of Hawaii during the summer of 1991. This data set was also used to examine the relationship between eggs and larvae. Ten nuts were randomly collected from under 10 randomly chosen trees in each orchard once a month from July to December (n = 100 nuts per month per orchard). No insecticides were applied during the study period, and nuts were evaluated as previously discussed. Cultivars sampled were mixed, but included predominantly ‘Keaouhi’, ‘Kau’, and ‘Ikaila’.

The 3rd macadamia data set (Keaouhi 1) came from a 10-ha unsprayed macadamia orchard of the cultivars Keaouhi and Kau near Keaouhi, HI. Thirty nuts were collected randomly from under each of 5 randomly chosen trees at approximately monthly intervals between September and December 1990, and between June and December 1991. The 4th data set (Keaouhi 2) was collected from a 2-ha unsprayed orchard 600 m from Keaouhi 1. The cultivars sampled were a mixture of Keaouhi and Kau. This orchard was under a mating disruption program using 1,000 oriental fruit moth dispensers per hectare (Biocontrol, Davis, CA). Nut samples were taken on the same day and used the same methods as in Keaouhi 1.

The final data set (Honomalino) came from the control portion of a pesticide trial for Cryptophlebia spp. conducted between 31 March and 21 July 1992. Treatments were arranged in a randomized block design with 8 replicates in a 1-ha plot within a 1,456-ha orchard near Honomalino. Each treatment was applied to 3 consecutive trees in a row and was separated from the other treatments in a row by a single untreated tree buffer. Tree spacing was 7.7 by 7.7 m. Fifty recently fallen nuts in each replicate (400 nuts per sampling day) were collected every 2 wk from early May to early August and examined for eggs and larval damage caused by Cryptophlebia spp. Eggs were recorded as new and old as described previously.

Reliability of Eggs as an Indicator of Damage.

To determine the relationship between eggs and larvae, data from the litchi and macadamia data sets were analyzed separately using 2 methods: (1) 2 × 2 G tests to determine if larval presence (or damage) was independent of egg presence (SAS Institute 1994), and (2) grouping all nuts within a data set which had the same number of eggs present and determining the proportion of nuts within that group which had larvae or larval damage present. The latter method provides a direct measurement of the accuracy of using eggs as a predictor of damage. Egg data for the 1st 4 macadamia data sets did not distinguish between new and old eggs. Therefore, all data sets were subjected to both analyses using total eggs. However, because unhatched eggs cannot cause damage, the analysis was repeated using just-hatched eggs to evaluate the effect of including new eggs on the observed relationship in all the litchi data sets and the Honomalino macadamia data set.

Sampling Plans for Cryptophlebia Larvae. Although sampling plans based on the mean number of larvae per nut could be developed, previous studies have shown that Cryptophlebia spp. feeding in macadamia nut husk early in the season causes early abscission and the resulting nuts have an unacceptably low oil content, which results in rejection by the processor (Jones 1994a).
Table 1. Relationship between Cryptophlebia damage and the presence of Cryptophlebia eggs on litchi

<table>
<thead>
<tr>
<th>Site</th>
<th>Kona 1</th>
<th>Kona 2</th>
<th>Kapaa</th>
<th>Kilauea</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs present, damage absent</td>
<td>96.4 (96.2)*</td>
<td>100.0</td>
<td>98.8 (98.7)</td>
<td>89.4 (88.5)</td>
</tr>
<tr>
<td>Eggs present, damage present</td>
<td>3.6 (3.8)</td>
<td>0.0</td>
<td>1.3 (1.2)</td>
<td>10.6 (11.5)</td>
</tr>
<tr>
<td>Eggs absent, damage present</td>
<td>1.7</td>
<td>0.1</td>
<td>0.8</td>
<td>1.7</td>
</tr>
<tr>
<td>G**</td>
<td>6.0 (4.4)</td>
<td>0.14 (0.12)</td>
<td>0.25 (0.56)</td>
<td>&lt;0.0001 (&lt;0.0001)</td>
</tr>
<tr>
<td>P</td>
<td>0.04 (0.01)</td>
<td>0.71 (0.73)</td>
<td>0.62 (0.45)</td>
<td>Kwai Mi, Kaimana, Bowworth 3, Groff</td>
</tr>
<tr>
<td>Cultivars sampled</td>
<td>Kaimana, Bowworth 3</td>
<td>Kaimana, Bowworth 3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*, Percentage of time when both new and old eggs are combined; value in parenthesis is when only old eggs were used. **, Test of H_0: larval presence is independent of egg presence; value in parenthesis is when only old eggs were used.

larval penetration through the pericarp directly damages the fruit and, secondarily, introduces microorganisms that speed decay. Sampling plans were therefore developed using binomial methods which classify the proportion of nuts or fruit damaged as being above or below a predetermined threshold. Classification was chosen over estimation because the sample size required to reach a decision is minimized compared to estimation procedures and the procedures are easier to explain and implement in the field (Binns and Nyrop 1992).

Classification procedures set up 2 hypotheses to be tested at α and β error rates:

\[ H_1: \theta_1 \leq p_1 \]

and

\[ H_2: \theta_2 \geq p_2, \]

where \( \theta \) is the true population level. The lower threshold, \( p_1 \), is the lowest level of husk or pericarp damage that would ever require intervention and the upper threshold (\( p_2 \)) is the lowest level that would always require some pest-control action. For macadamia, \( p_1 \) and \( p_2 \) were set to 0.12 (12% infested nuts) and 0.20, and \( \alpha \) and \( \beta \) were set to 0.10. These values were chosen because the correlation between Cryptophlebia damage and nut abortion is relatively low unless infestation level exceeds the natural nut abortion rate (Jones 1994a). The levels of \( p_1 \) and \( p_2 \) for litchi were set at 0.03 and 0.06, because larvae feeding on litchi directly damage the fruit, and thus even low levels of feeding directly contribute to lost yield. The sequential sampling plans, their operating characteristics, and average sample number were developed using the equations from Wald's sequential probability ratio test (SPRT) (Wald 1947). The Wald SPRT was used because the approximate equations for aggregate sample number and operating characteristics are simple, and among all sequential tests with equal operating characteristics values for \( \theta_1 \) and \( \theta_2 \), the average is minimized (Wald 1947). The equations used were taken from Fowler and Lynch (1987).

Sequential sampling plans are best evaluated by examination of the average sample number and operating characteristics (Binns 1993). The operating characteristics curve is a plot of the probability of no intervention versus the true population density and varies between 1 and 0. The operating characteristics curve has its value =1 when the true density \( \theta \leq p_1 \), =0.5 when it is near \( p_1 + p_2/2 \), and =0 when \( \theta \geq p_2 \). The steepness of the curve between \( p_1 \) and \( p_2 \) is a function of the precision desired. As \( \alpha \) or \( \beta \) is made numerically smaller, the sample size required to make a decision increases (Jones 1993).

Results

Reliability of Eggs as an Indicator of Damage.
Larval presence (or larval damage) was independent of the presence of eggs in 2 litchi orchards, probably because very little damage or larvae were present even though large numbers of eggs were present (Table 1). At the other 2 orchards, G tests showed that larval presence was not independent of the presence of eggs (\( G = 4.4, 64.6, df = 1, P < 0.04 \) and \( P < 0.001 \)). The presence of eggs was associated with larvae or their damage <10.6% of the time at all locations (Table 1), suggesting that eggs are a poor indicator of damage which would occur later. Even when \( \geq 3 \) eggs were found on a fruit, average infestation was <15% at all locations (Fig. 1).

Fig. 1. Percentage of litchi fruit damaged when different numbers of Cryptophlebia spp. eggs were found at 4 different locations during summer of 1992. Error bars are 95% CL.
Table 2. Relationship between Cryptophlebia damage and the presence or absence of Cryptophlebia eggs on macadamia nuts

<table>
<thead>
<tr>
<th>Condition</th>
<th>Keahou 1</th>
<th>Keahou 2</th>
<th>Honomalino</th>
<th>UH KES</th>
<th>Island survey</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eggs present, damage absent</td>
<td>64.9</td>
<td>71.0</td>
<td>60.0 (56.8)*</td>
<td>63.8</td>
<td>49.5</td>
</tr>
<tr>
<td>Eggs present, damage present</td>
<td>35.1</td>
<td>29.0</td>
<td>40.0 (43.2)</td>
<td>36.2</td>
<td>30.5</td>
</tr>
<tr>
<td>Eggs absent, damage present</td>
<td>5.8</td>
<td>7.3</td>
<td>7.0</td>
<td>13.2</td>
<td>9.0</td>
</tr>
<tr>
<td>G**</td>
<td>146.0</td>
<td>77.8</td>
<td>71.9 (79.9)</td>
<td>618.5</td>
<td>284.1</td>
</tr>
</tbody>
</table>

* Percentage of time when both new and old eggs are combined; value in parenthesis is when only old eggs were used. ** Test of H0: larval presence is independent of egg presence. All values P < 0.0001.

There was little effect of using old eggs only versus using the combination of new and old eggs in the relationship of eggs and resulting damage. This is probably because eggs of C. illepidata hatch in ≈4–5 d at temperatures commonly found in Hawaiian fruit and macadamia growing areas (unpublished), whereas egg cases tend to remain on the fruit for a much longer period of time. Combining all the litchi data sets together, 2,295 eggs were found, of which 230 were new eggs (10%).

The 2 × 2 G tests showed that larval presence was not independent of presence of eggs on macadamia at any location. However, as with litchi, analysis of the 2 × 2 tables showed that presence of eggs is not a good indicator of larval damage. When eggs were absent, husk damage from larvae was found between 5.8 and 13.2% of the time (Table 2). Presence of eggs also coincided with larval presence (or larval damage) <50.5% of the time in all cases (Table 2). This can be demonstrated by plotting the number of eggs found on a nut versus the proportion of times larval damage was found (Fig. 2); examination of this figure shows that using eggs as a threshold overestimates the damage for nuts with >1 egg and underestimates the damage from nuts with no eggs present.

As with the litchi data, using old eggs alone compared with the total of both new and old eggs at the Honomalino orchard showed little improvement on the correlation of damage and the presence of eggs (Table 2).

**Sampling Plans for Cryptophlebia Larvae.**

Larval sampling plans on macadamia typically require <50 nuts when ≤8 or ≥25% of the nuts are infested (Fig. 3a). The peak average sample number is only ≈100 nuts between $p_1$ and $p_2$ where maximum sample sizes are found (Fig. 3a). The operating characteristics curve shows that when the fruit are ≥25 or ≤8% infested, the probability of making an incorrect decision is extremely low even though the average sample number is <50 nuts. Sequential sampling decision rules are shown in Fig. 3b. The Wald SPRT produces parallel lines and theoretically sampling could continue for excessively large numbers of fruit if the true population level ($\theta$) is between $p_1$ and $p_2$ (the average sample number is the average sample number over a large number of trials; on any given trial the sample number required may be considerably more or less than the average sample number. Binns (1993)

![Graph](image-url)

**Fig. 2.** Percentage of macadamia nuts damaged when different numbers of Cryptophlebia eggs were found at 5 different locations. Error bars are 95% CL.

**Fig. 3.** Sequential sampling plans for Cryptophlebia spp. larvae on macadamia using a lower threshold of 0.12 and upper threshold of 0.20, $\alpha = \beta = 0.10$. (a) Average sample number and operating characteristics functions; (b) sequential decision rules.
suggested that if more than \(2\times\) the average sample number is required to reach a decision, it is probably best to terminate sampling and resample the orchard a short time later. For Cryptoplebia spp., the generation time is a minimum of 33 d at 25°C (unpublished data); therefore, sampling 1 wk later should be sufficient to detect the extent of the infestation before too much additional damage occurs.

Sampling plans for litchi require a higher average sample number than those for macadamia (Fig. 4a). This is because \(p_1\) and \(p_2\) are much lower and the difference between \(p_1\) and \(p_2\) for the litchi sampling plans is smaller than for macadamia (Jones 1993). However, even though the average sample number is higher, if \(\theta > 0.10\), the average sample number is still \(<50\) fruit. As with the macadamia plan, the operating characteristics curve shows good discrimination with a reasonable average sample number (Fig. 4b). As mentioned previously, if the number of fruit is 2-fold higher than the average sample number, sampling should be terminated and the orchard should be resampled 1 wk later.

**Discussion**

My data indicate that egg sampling is useful in determining if female Cryptoplebia spp. are ovipositing in the orchard, but the presence of eggs is not an accurate predictor of Cryptoplebia damage. This is particularly true on litchi where \(<12\%\) of the fruit with \(>3\) eggs present had larval damage or live larvae present. On macadamia, the correlation is higher, but even when \(>3\) eggs are present, \(<60\%\) of the nuts have larval damage or live larvae present. When the problems of egg sampling are combined with the deficiencies of pheromone traps noted previously, larval sampling plans are the most reliable method of determining treatment thresholds.

The sampling programs presented in this article do not have to be implemented until nuts or fruit are \(=18\) mm diameter, because previous studies have shown that Cryptoplebia females do not oviposit on macadamia or litchi before that size with any frequency (Jones 1994b). The larval damage to macadamia is also only important during the period of oil accumulation (between reaching full size and harvest—approximately late May to early October in Hawaii) because nuts dropping during this period are immature, and if harvested, increase the grower damage tally at the processor. Nuts dropping after full oil accumulation simply remain on the ground for longer periods before harvests (several harvest rounds occur per season, generally by hand picking from the ground). Although this may cause a problem in the drier areas of Hawaii where Hypothenemus obscurus (F.) (Coleoptera: Scolytidae) is found in large numbers (Jones et al. 1992a), in other areas it will have little or no effect on final nut quality.

Macadamia should require no more than \(5\) samples to be collected over the 5-mo period that nuts are susceptible to damage. Because a typical yield for Hawaiian macadamia nut trees is \(=6,000\) nuts per tree, nuts can be picked off the tree and placed in a bag for later counting without yield loss. For each block sampled, randomly select 10 nuts from each of 10 randomly selected trees. Nuts should be collected from the lower canopy for both ease of collection and because there is no difference in damage between the lower and middle canopy of large trees (Jones and Shearer 1995). The lower level of damage found in the upper canopy should not bias the overall estimate of damage within the orchard because nut density is typically lower than that found lower in the tree canopy.

For litchi, the cultivar is important in determining the number of times sampling must be performed. Jones (1994b) found that the period between 20-mm diameter (minimum oviposition size) and harvest varied from 2 to 8 wk depending on the cultivar. When the generation time of the insect is taken into consideration, it is unlikely that \(>2\) generations will occur in the susceptibility window. Therefore, \(>2\) samples should rarely be required if only a single cultivar is found within the orchard. In Hawaii, it is fairly common for several cultivars to be grown within a 0.5- to 1.0-ha orchard, suggesting that more samples may be required depending on the cultivars grown and their fruit phenology.

The lower thresholds \((p_1\ and\ p_2)\ used\ for\ the\ litchi\ sampling\ plan\ require\ a\ greater\ number\ of\
fruits be sampled than the macadamia plan. If a particular litchi cultivar must be sampled multiple times and the trees are young, then fruit should be examined without removal from the tree to prevent removing a significant amount of the total fruit per tree. However, on older and taller trees, this is unlikely to be a problem because it is often difficult to pick all the fruit before it is overripe.

The effect of height on Cryptophlebia spp. damage on litchi is similar to that found on macadamia (Jones et al. 1992b). Damage is greatest in the lower canopy, but density of the fruit is highest in the upper canopy when trees reach full size. Sampling the lower canopy therefore biases the sample estimate so that higher mean damage levels are found than actually exist if all fruit were sampled. However, it is impractical to sample full-size litchi trees (10–12 m) by collecting from the top of the canopy. The bias can be overcome by increasing the damage levels ($p_1$ and $p_2$) to levels approximating those levels used in the macadamia nut sampling program. This has the advantage of reducing the average sample number required to make the decision, while maintaining simplicity of sample collection.

Acknowledgments

I thank Carrie Tome, Lois Caprio, Kris Kajiwara, Dayna Mizunaka, Gary Fowler, Joanne Morisato, and Brad Moriyama for technical assistance. I also thank Bill Budd, Mike Strong, Brian Paxton, George Shattauer, Phil Ito, Hillary Brown, Alan Yamaguchi, Randy Aluna, Dave Reitow, and Bill Taylor for access to their orchards. The reviews of Peter Shearer, Skip Bittenbender, and Marshall Johnson, University of Hawaii at Manoa, contributed greatly to this manuscript. This research was supported in part by Governor's Agricultural Coordinating Committee grants 89-37 and 92-14, and a grant from the Hawaii Tropical Fruit Growers Association. This is journal series number 4086 of the College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa, Honolulu.

References Cited


1994a. Feeding by Cryptophlebia illepidula (Butler) and C. ombrodelta (Lower) (Lepidoptera: Tortricidae) on macadamia nut abortion. J. Econ. Entomol. 87: 781–786.

1994b. Oviposition patterns of koa seedworm and litchi fruit moth (Lepidoptera: Tortricidae) on macadamia and litchi in Hawaii. J. Econ. Entomol. 87: 1278–1284.


Received for publication 17 August 1994; accepted 12 June 1995.