

## A new method to estimate the population size of coconut mite, *Aceria guerreronis*, on a coconut

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**Abstract.** Studies of the coconut mite, *Aceria guerreronis*, often require accurate assessment of the population density on individual infested coconuts. Here an efficient and accurate method was developed to estimate its population density on an infested coconut. The coconut mites were removed by washing the bracts and surface of an infested coconut with 30 ml of a detergent solution. Shaking the wash for 5 s allowed the mites to distribute uniformly. The number of mites in the first 1 ml of the first wash ( $X$ ) yielded a very accurate predictor of the total number of mites on a coconut ( $Y$ ):  $Y = 30.1X$  ( $R^2 = 0.99$ ;  $p < 0.0001$ ), also confirming that the wash was indeed homogeneous. The advantages and disadvantages of this method are discussed.

### Introduction

The coconut mite, *Aceria guerreronis* (Acari: Eriophyidae), has been one of the most serious pests of coconut in the Americas and Africa for the last three decades (Mariau and Julia 1970; Zuluaga and Sanchez 1971; Mariau 1977; Hall and Espinosa 1981). Its recent introduction into India (Sathiamma et al. 1998) and Sri Lanka (Fernando 1998; Fernando et al. 2002) poses a major threat to the coconut industries in these countries, and other major coconut growing countries in Asia and the Pacific region. Although chemical control is the only known means of controlling the pest effectively, it is not sustainable due to the necessity of frequent and regular application, and high cost. Therefore, other means of control are being explored, mainly the use of entomopathogenic fungus *Hirsutella* spp. (Cabrera 2002; Rabindra and Sreerama Kumar 2003) and predatory mites (Fernando and Aratchige 2003). Further, many aspects of the biology and ecology of *A. guerreronis* have yet to be studied to develop effective control measures.

Ecological and other studies into control measures require assessment of the population density of *A. guerreronis* on coconuts. But its high mobility, microscopic size and hidden lifestyle – colonies aggregate in inner and outer bracts (tepals) and under the bracts of developing coconuts – make such an assessment time-consuming, tedious and often inaccurate. Previous studies have used two methods to estimate the population size of coconut mites on a coconut. In the method developed by Howard et al. (1990), the bracts of the

infested coconuts were removed, and the mites were counted in 1 mm<sup>2</sup> of each 25 mm<sup>2</sup> area of the colonies on the nut surface with over 1000 mites. Moore and Alexander (1987) scored the number of mites on the coconut surface in a log scale (e.g., 0 = 0 mites, 1 = 1–10 mites, 2 = 11–100 mites, 3 = 101–1000 mites, etc.). The bunch with the highest number of mites is scored as 100% and other bunches are recorded as a percentage of the most heavily infested one. Because this is a relative assessment it is not useful to assess the mite density on a single coconut separately. Since coconut mites are not evenly distributed on the coconut surface and bracts, bias in selecting the area for counting could affect the estimated count. The mites in the selected area may not be counted accurately due to movement of the mites during counting and the inability to count the ones in hidden areas. Both methods are time-consuming, difficult and not very reliable.

The present study envisaged developing a new technique to estimate the actual population density of *A. guerreronis* on a coconut by washing off the mites into a solution, distributing the mites homogeneously in the wash by shaking, and counting the mites in a subsample for estimation of the population.

## Materials and methods

### *Development of the wash method*

A solution of 8–10 drops detergent ('Tween 80') in 250 ml tap water was prepared to wash the mites off the coconuts. A translucent silicon tube of 5–8 cm long was connected to the stem of a conical plain funnel having a top diameter of 100 mm and a stem length of 12.5 mm. A Mohr's nickel-plated clip was clamped to the tube to retain the solution in the funnel. The funnel was set on a stand and a test tube was kept directly below the tube to collect the solution.

The bracts (tepals) were removed from the coconut. Approximately 10 ml of detergent solution was poured into the funnel and the meristematic part beneath the bracts of the coconut was thoroughly rinsed in the solution. Then it was removed and sprayed with a little amount of the solution using a wash bottle while holding above the funnel. Bracts were arranged on the funnel in two layers with the curved surfaces up. The solution was sprayed on to the bracts to immerse them and stirred for about 5 s. The outer three bracts were removed using a pair of forceps and the inner three were stirred again for further 8–10 s and removed. Then the clamp was loosened to let the solution ('wash') pass into the test tube. The solution in the tube was shaken for a few seconds (see Experiment 1) on a rotar-mixture at a medium speed and left for 3–4 s until the solution becomes still. Immediately afterwards 1-ml samples of the wash were pipetted out from the middle of the solution into separate Petri

dishes of 4 cm diameter and the mites were counted using a stereo-microscope.

*Effect of volume and duration of shaking on distribution of mites in the wash*

A preliminary study suggested that the amount of detergent used and the duration of shaking the wash have an impact on the mites' distribution. Further, it was found that the homogeneity of the distribution did not remain constant until all aliquots were drawn out from the wash, but remain so until a minimum of 10 samples were drawn out. To determine the most suitable combination of volume and duration of shaking samples of detergent solution 20, 30 and 40 ml were shaken for 5, 10 and 15 s and another sample was used unshaken. Five 4-month-old infested coconuts selected randomly from different palms were used for each combination of volume and duration. After shaking the wash of each nut, 10 1-ml samples were pipetted into separate Petri dishes and the mites in each 1 ml were counted.

Data were analysed using the CATMOD procedure in SAS (1996) to describe the interactive effect of volume and shaking duration on the number of mites in the wash. The coefficient of variation was used to quantify homogeneity of the numbers of mites in the washed samples.

*Estimation of the number of mites on a coconut*

The bracts and surface of each of 18 four-month-old infested coconuts selected randomly from palms at different locations were washed separately in 30 ml of detergent solution, shaken for 5 s ('first wash') as described above, and the mites in each of the 1 ml subsamples of the wash were counted. Since mites could have been left on the bracts and coconut surface after the first wash, the procedure was repeated once for each nut ('second wash') to count the remaining mites. The bracts and surface were then examined under a stereo-microscope for the presence of mites, and any remaining ones were counted. Thus the actual total number of mites on a coconut ( $Y$ ) is given as follows:

$$Y = \sum_{i=1}^{30} (\text{mites counted in 1ml of } i\text{th sample of first wash}) + \sum_{i=1}^{30} (\text{mites counted in 1ml of } i\text{th sample of second wash}) + \text{mites counted on coconut surface and bracts through stereo-microscope.}$$

For the estimation of the total number of mites ( $Y_{\text{est}}$ ) it is convenient and less time consuming to use the number of mites in any 1 ml of the first wash. To

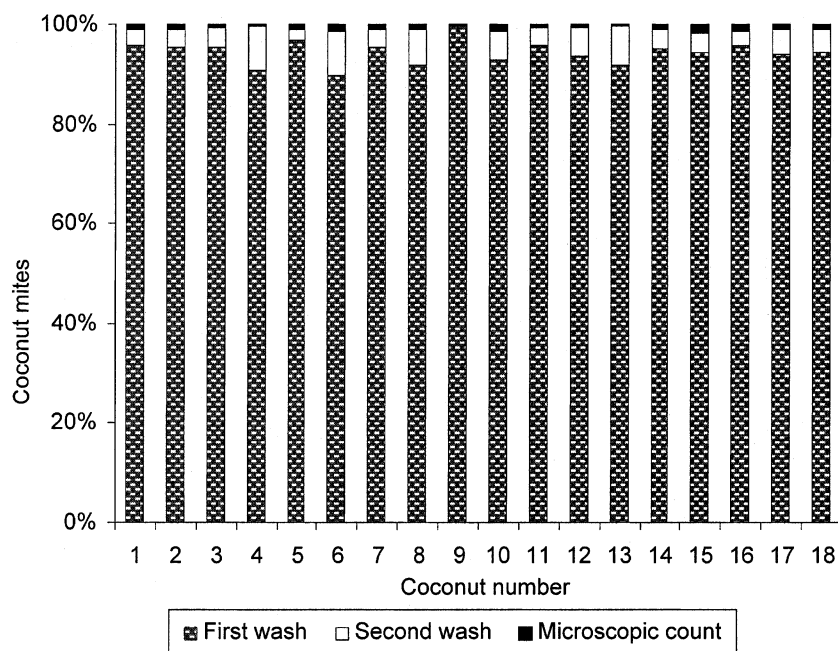


Figure 1. Average distribution of total coconut mites counted in the first and second wash and under the stereo-microscope ( $n = 18$ ).

determine the suitable predictor variable ( $X$ ) for estimating  $Y_{est}$  the correlation coefficients were computed between  $Y$  and the number of mites in each subsample of the first wash. Of the 30 predictors, the one, which gave the highest correlation coefficient was used to establish a statistical relationship with  $Y$ . The validity of the relationship was tested using the coefficient of determination ( $R^2$ ) and correlation between actual and estimated values.

## Results

### *Effect of volume and duration of shaking on distribution of mites in the wash*

The maximum likelihood ANOVA indicated a significant interaction ( $p < 0.001$ ) between detergent solution volume and shaking duration, suggesting that both factors simultaneously influenced the homogeneity of the wash. The coefficient of variation of the number of mites among 10 subsamples ranged from 24.05 (40 ml wash, shaken for 10 s) to 5.82 (30 ml, 5 s shaken). Therefore, it was decided to use 30 ml of the solution to wash the coconut surface and bracts and shake the wash for 5 s to prepare the wash for estimation of the mite density.

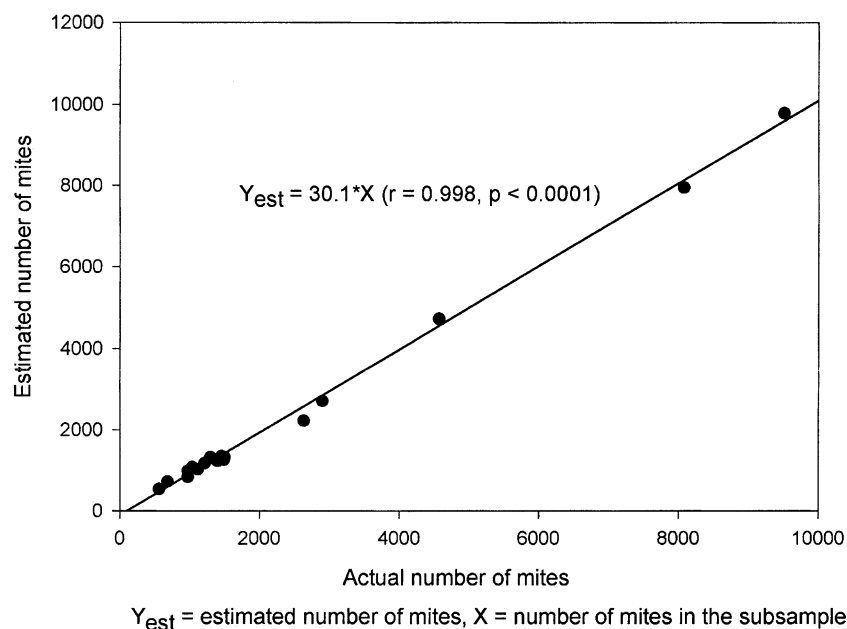


Figure 2. Relationship between actual and estimated number of coconut mites on coconuts ( $n = 18$ ).

#### *Estimation of the number of mites on a nut*

On average 94.3% of all mites were extracted in the first wash (range: 90.1–99.8%) and 4.7% (0.3–8.8%) in the second wash (Figure 1). The numbers of mites remaining on the bracts and nut surface after the second wash (microscopic count) were negligible (Figure 1). This confirmed the reliability of using a sample from the first wash only to estimate the total mite density on a coconut.

It was found that all 30 correlation coefficients were highly significant ( $p < 0.001$ ), indicating that any subsample would suit as a predictor for the estimation of the total mite count on a coconut. However, the highest correlation coefficient was found for the first 1-ml subsamples. The differences between the actual and the estimated values were low and the percentage error varied from –6 to 15% (Figure 2). These results indicate that the sampling method is accurate.

#### **Discussion**

The wash method extracts almost all coconut mites from the coconut surface and bracts rapidly and effectively and distributes them evenly in the wash. The

30 ml solution shaken for 5 s gave the most homogenous wash. The number of mites in the first 1-ml subsamples of the first wash (30 ml) could be used to estimate the population density of coconut mites on a coconut with high accuracy, and the finding that the subsample mite numbers must be multiplied by 30.1 to yield the total numbers reconfirms that the mites are distributed evenly in the 30-ml samples.

This method has many advantages over other methods that are presently practiced. It needs simple equipment. Counting mites in 1 ml of the wash makes the handling of many samples feasible. Further, taking the mite count in the first sample is convenient and rapid. Thus, from both statistical and practical point of view, the first 1 ml of the first wash was determined as the best predictor. It is important that the 1-ml subsample is drawn out immediately after the shaken wash becomes still because the mites sink with time affecting the homogeneity of the wash. The main disadvantage of this method is that live and dead mites cannot be distinguished in the wash since live mites also tend to be still in the wash.

The method could be further improved to distinguish between dead and live coconut mites by using suitable stains and complemented by development of an image processing technique to replace manual counting of the mites.

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