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Oviposition Periodicity, Egg Morphology and Life History of Large Cabbage Moth *Crocidolomia Pavonana* Population in Samoa

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Abstract

A study on the biology and behaviour of the Samoan population of Crocidolomia pavonana was carried out through a series of experiments. The study showed that C. pavonana completes its life cycle in 24-35 days. Female emerge, mate and oviposit (as egg mass) during the scotophase. The average size of egg mass was 9.0 ± 0.48 mm^2 *and the mean number of eggs oviposited were significantly (P<0.05) greater in scotophase than photophase. The age of the female was correlated negatively to daily oviposition and a decrease in oviposition was recorded after 15 days, which was also less than 1 egg mass per day. The colour of newly oviposited egg mass was light-green changing yellow after two days and black on the subsequent day. The larvae passed through four instars before it was found to undergo pupation. The duration of the first instar was smaller than the fourth instar. Males took a slightly longer time to develop from pupae (10.19 ± 0.22 vs 9.52 ± 0.17 days), and lived longer than females (27.0 ±* 1.01 vs *19.8* ± 1.14 days). *The Samoan C. pavonana oviposits small egg masses. This information could be used to develop effective pest management using the recently identified egg parasitoid Trichogramma chilonis.*
Keywords: *Large cabbage moth LCM; Trichogramma chilonis; Life stages; Diel behaviour*

1. Introduction

Brassica vegetable crops are among the most widely cultivated leafy vegetables worldwide, and approximately 86 million tons of Brassica vegetable are grown annually (FAO, 2013). However, production of these crops is greatly constrained by a complex of Lepidoptera pests including the Diamondback moth (DBM) *Plutella xylostella* L, cabbage webworm (CWW) *Hellula undalis* Fab., Army worm *Spodoptera litura* and Large cabbage moth (LCM) *Crocidolomia pavonana* (Ebenebe *et al.,* 2006; Waterhouse and Norris, 1987). All these pests attack Brassica vegetables including head cabbage and Chinese cabbage in Samoa (Ebenebe *et al.,* 2006). However, the damage inflicted by *C. pavonana* has a serious impact on growing brassicas in Samoa.

The moth *C. pavonana* F. also known as cabbage cluster caterpillar, belong to family Crambidae, and is considered native to Asia and Africa (Waterhouse and Norris, 1987). However, the pest is widely distributed throughout the tropics and subtropics including the Pacific region (Sastrosiswojo and Setiawati, 1992; Uelese *et al.,* 2014; Waterhouse and Norris, 1987). Adults lay eggs on the lower surface of the cabbage leaves and larvae hatch out and damage the young buds (Sulifoa *et al.,* 2016). *C. pavonana* larvae damage a wide range of Brassica plants including head cabbage, Chinese cabbage, broccoli, cauliflower, radish and mustard by feeding on leaves, inflorescences and even fruits and pods. Heavily infested plant can be completely defoliated (Freres and Atalifo, 2000; Hsiao, 1984).

In Samoa, it is a major problem in all the head and Chinese cabbages growing areas (Ebenebe *et al.,* 2006). Currently, farmers utilise a large amount of insecticide to grow cabbage and meet the high market demand (Fangupo *et al.,* 2016). ACIAR and Ministry of Agriculture and Fisheries (MAF) Samoa are working together to develop viable pest management strategies in Samoa. A recent survey reported that Samoa has established a population of the egg parasitoid *Trichogramma chilonis* which could be a potential biological control for *C. pavonana* (Uelese *et al.,* 2014). However, detailed study of the biology and behaviour of *C. pavonana,* especially the oviposition behavior, is required to develop effective management of *C. pavonana* using the egg parasitoid. Recent literature suggests limited information on the biology and oviposition behaviour of *C. pavonana* from the Pacific region while information on *C. pavonana* biology from other parts of the world is contradictory (Table 1). This might be explained by the presence of distinct biotypes of *C. pavonana,* with the biology and ecology of insects varying with location (Kant, 2012). Thus, our research reported here endeavors to study the biology and oviposition behaviour of the Samoan population of *C. pavonana* which will be essential for developing the most effective local management strategies.

Table 1. Information on the biology and life stages of *C. pavonana* extracted from studies carried out in different parts of the world. Symbol * indicates where data was not available.

Country/ location	Temperature	TLC	Egg, larval, pupal stages (days)	References
China	Predicted*	18.5-36	2.5-6, 8-13, 8-17	Gao *et al*., 2007
Indonesia	16-22	27-37	4-5, 10-14, 13-18	Van de Oever, 1973
	26-32	41	3-6, 10-14, 13-18	Sastrosiswojo and Setiawati, 1992
Mauritius	20-24	60	6, 10, 12	Fagoonee, 1980
Malaysia	Predicted*	25.4	4.1, 11.7, 9.6	Ooi and Kelderman, 1979
India	24-34	17.5-27	2-4, 8.5-13, 7-10	Kannan *et al.*, 2011
	Predicted*		6.4, 23.4, 15.5	Singh and Rawat, 1980
Taiwan	25	*	*, 9.75, 6.84	Hsiao, 1984

2. Materials and Method

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2.1. Insect Rearing and Experimental Site

Insects were reared in 2014 in the Entomology Laboratory, University of the South Pacific, Alafua campus, Alafua, Samoa. A laboratory culture of *C. pavonana* was established from a commercial Chinese cabbage farm near Apia, Upolu Island, Samoa. The insects were reared on cabbage plants in plastic cages (40 x 40 x 40 cm) made of fine net cloth. Chinese cabbages (cultivar - *Pakchoi*) were grown in a glass house to meet the continuous supply of cabbages for this study. Plants used for oviposition experiment were grown in 1L polybags filled with a mixture of soil and commercial substrate (Yates thrive) at the ratio of 2:1. The plants were not sprayed with any insecticide. The bioassays were conducted in a controlled room set at normal day light (12 h photoperiod) with light intensity of 438 ± 0.01 lux (Extech LT300 digital light meter), 27 ± 1 ^{o}C and 65 ± 3 % relative humidity. Insects used in this study were reared for 5-8 generations under the above conditions.

2.2. Biology and Life History of *C. pavonana*

Late instar *C. pavonana* of larvae were kept in containers until adult emergence, and later males and females were separated. *C. pavonana* adults (one male and one female) were released into oviposition cages (40 × 40 × 40 cm) with two 2-3 weeks old potted Chinese cabbage. The adults were provided with 10% honey solution soaked in cotton wool and placed inside the cage. The plants were examined twice daily for the presence of *C. pavonana* eggs. First recording were carried out in morning at 08:00 h and second at 20:00 h.

The cabbage leaves were removed from the container and numbers of *C. pavonana* egg masses on the leaves were counted. Fresh leaves were placed inside of the containers. At 8 pm similarly, the leaves were removed and number of egg masses were counted. The steps were repeated until the female death. The data on scotophase and photophase oviposition were collected at 08:00 h and 18:00 h, respectively.

The number of egg masses, size of egg masses, and number of eggs per female were recorded. Egg masses size were measured using a millimeter ruler and graph paper. Eggs in the egg mass were counted under an Olympus stereomicroscope. The egg masses were also observed for colour change and embryonic development under the same microscope. Changes in the colour of egg masses were matched to a royal colour charts. The number of larvae hatched from the egg masses was recorded, and the larvae were reared until pupation. The larvae were monitored for molting and number and duration of each larval instar was recorded. Fresh cabbage leaves were fed to the developing larvae. Fourth instar larvae were transfer to ventilated plastic tubes containing saw dust (5 cm deep). Once pupated, pupae were collected from saw-dust and placed in the plastic vial and observed until adult emergence. Sex ratios of emerged offspring were estimated.

2.3. Data Analysis

Data on life stages of *C. pavonana* such as egg stage, length of different instars (stadium), pupal period and male and female longevity were subjected to Analysis of Variance (ANOVA) and General Linear Model (GLM) analysis. The relationship between female age and oviposition was analysed by regression analysis. All analyses were carried out using Minitab 17 at $\alpha = 0.05$ of level of significance.

3. Results and Discussion

3.1. Oviposition Periodicity and Egg Morphology of *C. pavonana*

Adult *C.* pavonana, both males and females emerged during the scotophase (dark period). Females underwent mating after 1-2 days of emergence and oviposited viable eggs after one day of mating. Female oviposited larger egg masses in their early life, and the number of egg masses decreased with the age of the female. The mean (SE) of egg mass area was 9.0 ± 0.48 mm^2. The egg mass of Samoan *C. pavonana* was much smaller (36 eggs/egg mass) compared to Indian (47 eggs/egg mass) and Indonesian (48 eggs/egg mass) populations (Kannan *et al.*, 2011; Sastrosiswojo and Setiawati 1992). The mean number of egg masses oviposited per female increased initially until the 5^{th} day and then started decreasing until day 20 when it reached a plateau (Figure 1). This suggests that females need some time to develop eggs after emergence and lose their ability to produce eggs after 15-20 days. Another study found that the number of eggs parasitized per egg mass was greater when egg masses were smaller in size (Kant unpublished data). This finding is significant because it suggests that pest management using *T. chilonis* in Samoa could be effective given the smaller *C. pavonana* egg mass sizes.

The mean (± SE) length and width of the egg mass were measured 3.5 ± 0.14 and 2.5 ± 0.10 mm, respectively. Female oviposited pale-green egg masses that eventually turned into light yellow/orange colour, and finally black (Figure 2). Changes in egg colour are due to development of larvae in the egg. The black head of the larvae appeared before hatching. The egg colour and incubation period are in agreement with observations reported in Ooi and Kelderman (1979) and Sastrosiswojo and Setiawati (1992).

The number of eggs oviposited per egg mass varied from 4 to 78. Linear regression analysis between age of female and number of eggs laid showed strong association ($F_{1, 306}$ = 104.69; p = 0.001; Figure 3). The regressing the number of eggs oviposited per female and age of females suggest that ageing negatively affects oviposition in this species (Figure 3).

When the periodicity of the oviposition was analysed, it was determined that the female *C. pavonana* oviposited >90% of their eggs during the scotophase. The mean number of eggs oviposited in scotophase was significantly higher than the number of eggs oviposited in photophase ($F_{1, 24}$ = 152.09; p = 0.001; Figure 4). Females also oviposited larger egg masses (21.32 ± 1.30 eggs/egg mass) in scotophase than in photophase (14.30 ± 2.45 eggs/egg mass) ($F_{1, 24}$ = 107.06; p = 0.001; Figure 4). Very little oviposition was recorded in the daylight.

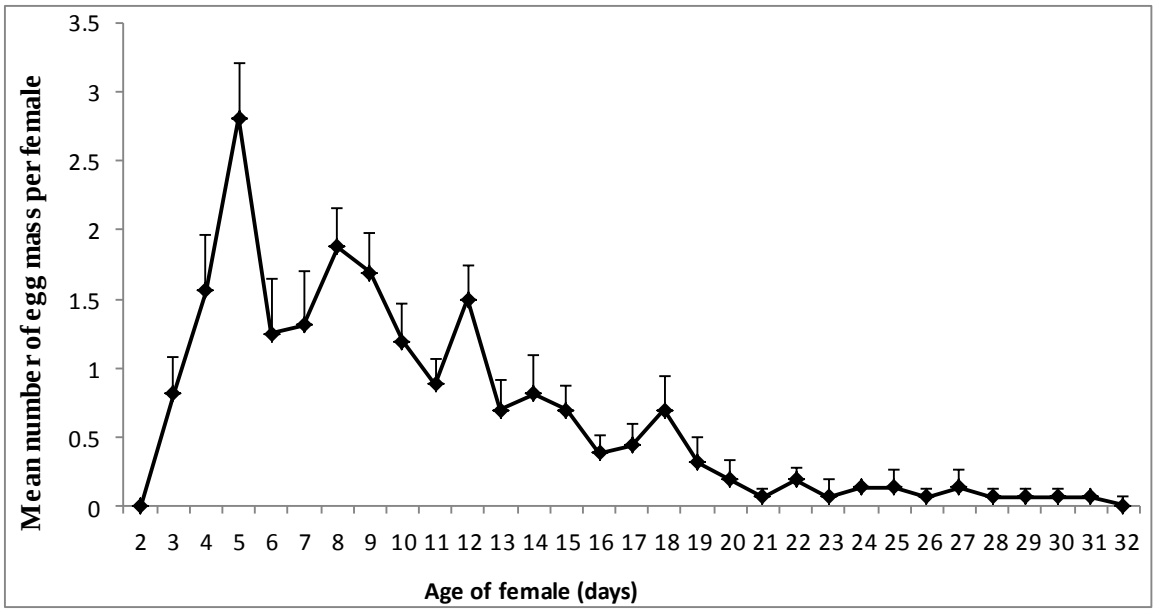

Figure 1. Fluctuation in the number of egg masses oviposited by *Crocidolomia pavonana* with the age of the female. Error bars represents respective standard errors.

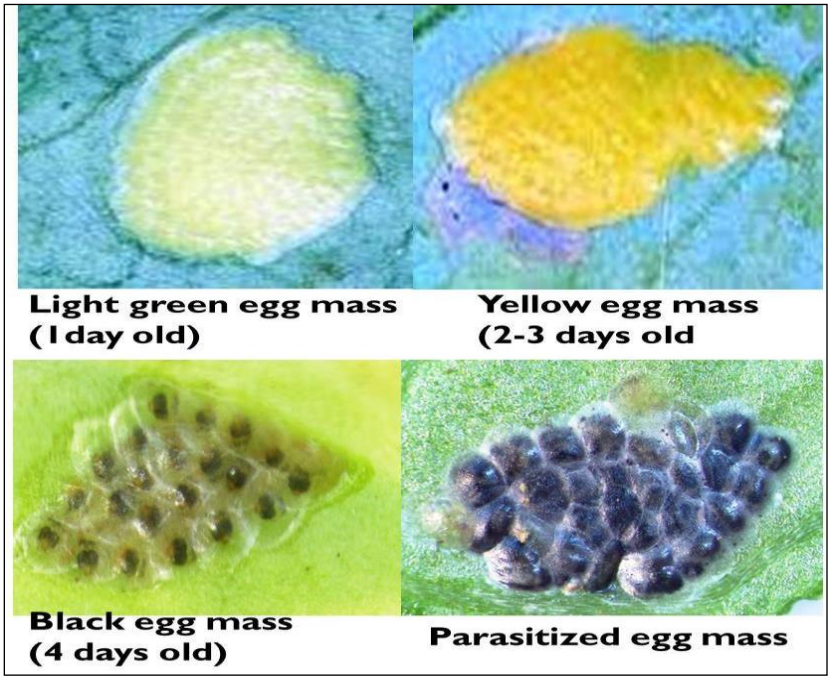

Figure 2. Colour changes in *Crocidolomia pavonana* egg mass. Unparasitised egg masses turn from light green to black while the parasitized egg masses turn to shiny black.

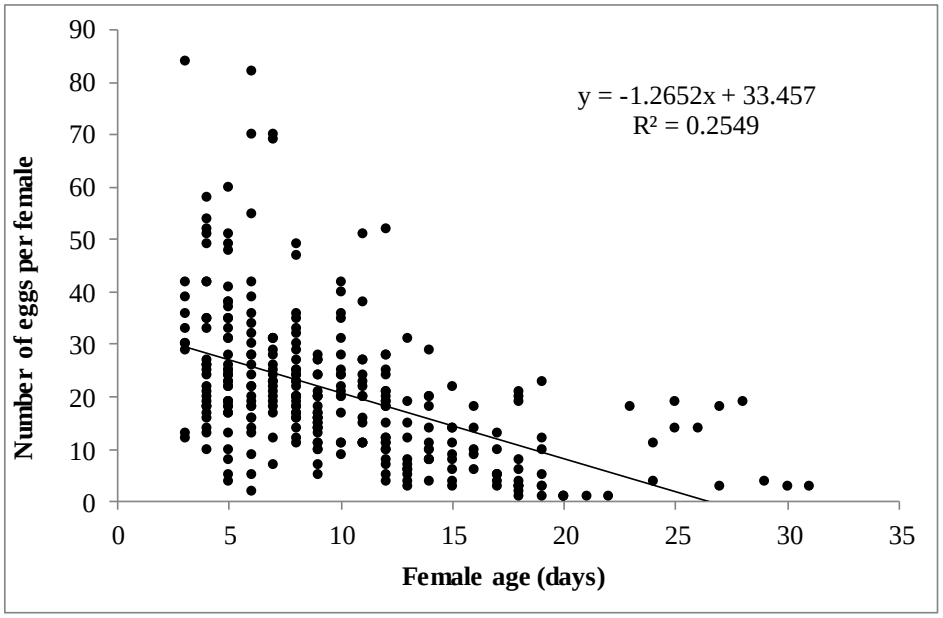

Figure 3. Relationship between the mean numbers of eggs oviposited by female and age over their life time.

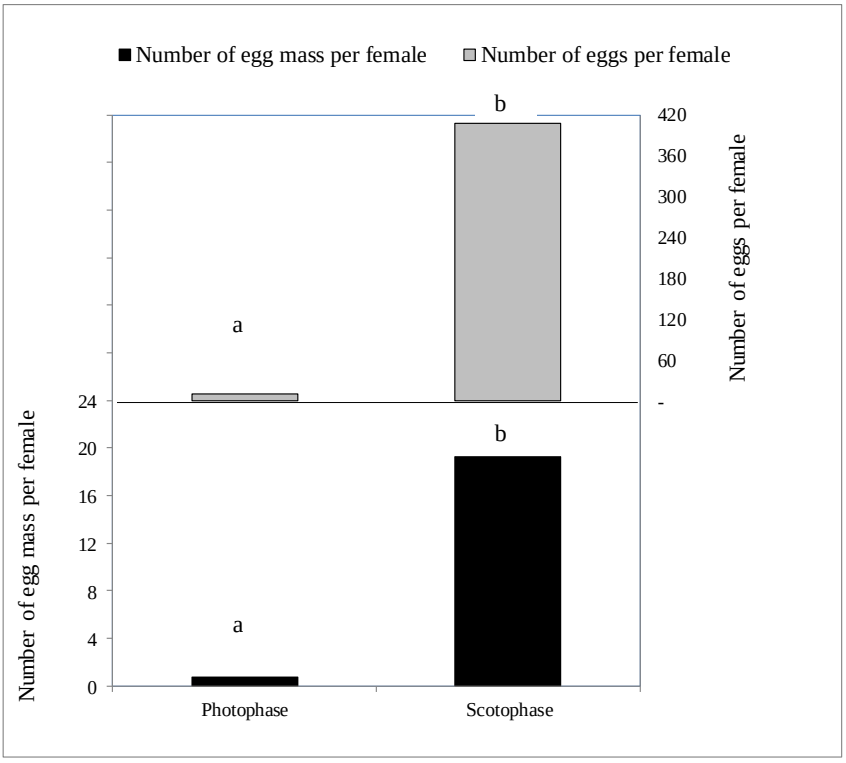

Figure 4. Mean number of eggs oviposited by Crocidolomia pavonana during photophase and scotophase. Values marked with different letters were differ significantly at P<0.05.

3.2. Life History of *C. pavonana*

The Samoan population of *Crocidolomia pavonana* completes its life cycle in 29 ± 0.28 days at 27° C. In contrast, the population of *C. pavonana* from Mauritius takes almost 60 days at 24° C to complete its life cycle. This is thought to be because of the low temperatures in Mauritius. The shortest stage in the life cycle of the Samoan population is the egg stage where larvae hatched out after 4 ± 0.8 days of oviposition (Fagoonee, 1980). The short development period (3-4 days) of this population reduces parasitism opportunities of *T. chilonis*. *T. chilonis* prefers younger eggs to parasitize because development of the host larvae appears to interfere with development of parasitoid larvae when parasitized at a late stage. In the Samoan population of *C. pavonana* larvae went through four instars before they underwent pupation. The first instar (stadium) was of shortest duration while the fourth instar had the longest stadium ($F_{3,\ 108}$ = 7.23; P = 0.01, Table 2). Chinese and Indonesian populations of *C. pavonana* also undergo four instars (Gao *et al.*, 2007; Sastrosiswojo and Setiawati 1992) while the Malaysian and India populations undergo five instars before pupation (Ooi and Kelderman, 1979; Singh and Rawat 1980). Studies on the Mauritius population suggest that this population of the moth undergoes six instars (Fagoonee, 1980). The duration of different stages also depends on the host plant (Gao *et al.*, 2007; Hsiao, 1984). In our studies, freshly formed pupae of *C. pavonana* from Samoa were yellowish-brown, which later became dark brown in colour.

Table 2: Mean number of days *Crocidolomia pavonana* pass through different stages during its life.

Life stages of *C. pavonana*	Mean ± SE (Days)
Egg period	4.0 ± 0.08
Laval period	15.3 ± 0.06
Instar 1	3.5 ± 0.05
Instar II	3.7 ± 0.04
Instar II	3.8 ± 0.03
Instar IV	4.1 ± 0.02
Pupa period	9.7 ± 0.14
Adult male emerge period	10.19 ± 0.22
Adult female emerge period	9.52 ± 0.17
Male longevity	27.0 ± 1.01
Female longevity	19.8 ± 1.14

Pupae to adult emergence was slightly longer in males 10.19 ± 0.22 than in females 9.52 ± 0.17 ($F_{1, 148}$ = 6.35; p = 0.013; Table 2). Thus, adult female moths emerged one day before the males; which is similar to the findings of Kannan *et al.* (2011) and Van Den Oever (1973). Little difference was observed in the pupal period of Samoan *C. pavonana* and *C. pavonana* from other parts of the world (Sastrosiswojo and Setiawati, 1992; Singh and Rawat 1980). *C. pavonana* males lived significantly longer than the females ($F_{1, 31}$ = 21.84; p = 0.001; Table 2).

4. Conclusion

The Samoan population of *Crocidolomia pavonana* completes its life cycle in 29 ± 0.28 days at 27° C which is longer than Chinese and Malaysian populations but shorter than Indonesian populations. The Samoan *C. pavonana* female oviposit smaller egg masses than *C. pavonana* females from India. The Indonesian *C. pavonana* population has a similar life-history to the Samoan population. Younger female produces large egg masses which could be used for the mass rearing of *T. chilonis*. *T. chilonis* is a potential candidate for the management *C. pavonana* in Samoa. However, a detailed study on egg patch exploitation by *T. chilonis* is required.

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