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Biology of *Macrolophus caliginosus* (Heteroptera: Miridae) Predator of *Trialeurodes vaporariorum* (Homoptera: Aleyrodidae)

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Abstract

Macrolophus caliginosus Wagner (Heteroptera: Miridae) is a highly polyphagous predatory bug, which has proven to be effective in controlling many insect pests of greenhouse vegetables (eggplant, tomato, and cucumber) especially whiteflies, aphids, and thrip. It is mainly used as a biological control auxiliary against *T. vaporariorum* Westwood (Homoptera: Aleyrodidae). The greenhouse whitefly, *Trialeurodes vaporariorum* is particularly harmful to tomato plants grown under the greenhouse. It has become prevalent whenever crops are frequently sprayed with insecticides. Biological control is becoming important for controlling this insect pest. A mirid bug management programme has been developed for an Integrated Pest Management (IPM) in tomato. The objective of the programme was to keep the predator population densities high enough in order to maintain *T. vaporariorum* and other insect pest populations below the economic threshold. In this study, it was very important to determine the biology of predator in term of its life cycle, behaviour at different stages, fecundity, longevity and searching abilities, in order to provide detail data for formulating the means of control against whitefly. Results of this study indicated that *Macrolophus caliginosus* adults fed on whitefly larvae of all stages from the first larval stage to the pupal stage. The predator consumed the preys at almost similar daily rates (average of 5.94 per day). The study implies that *M. caliginosus,* with its life cycle, predation, longevity and fecundity and host preference, is a beneficial insect to combat against whitefly.

Keywords: *Macrolophus caliginosus,* Predator, Life cycle, Fecundity, Longevity

1. Introduction

Macrolophus caliginosus Wagner (Heteroptera: Miridae) is a highly polyphagous predatory bug (Carapezza, A., 1995), which has proven to be effective in controlling many insect pests of greenhouse vegetables (eggplant, tomato, and cucumber) especially whiteflies (Malausa, J.C., 1996), aphids, and thrips (Berengere, C., 1996). It is mainly used as a biological control auxiliary against *T. vaporariorum* Westwood (Homoptera: Aleyrodidae) (Malausa, J.C., 1987). The greenhouse whitefly, *Trialeurodes vaporariorum* is particularly harmful to tomato plants grown under the greenhouse. It has become prevalent whenever crops are frequently sprayed with insecticides. Biological control is becoming important for controlling this insect pest. A mirid bug management programme has been developed for an Integrated Pest Management (IPM) in tomato. The objective of the programme was to keep the predator population densities high enough in order to maintain *T. vaporariorum* and other insect pest populations below the economic threshold (Alomar, O., 1996 and Albajes, R., 1999). In this study, it was very important to determine the biology of predator in term of its life cycle, behaviour at different stages, fecundity, longevity and searching abilities, in order to provide detail data for formulating the means of control against whitefly. In Malaysia, the predator *M. caliginosus* was first reported by Syed Abdul Rahman et al. (2000) (Albajes, R., 1999) on tomatoes in Cameron Highlands. Studies include biology (life cycle, predation, longevity and fecundity) and host preference of *M. caliginosus*.

2. Methods and materials

2.1 Life cycle of predator, Macrolophus caliginosus

This experiment was carried out at the Crop Protection Laboratory, Malaysian Agricultural Research and Development Institute (MARDI), the Cameron Highlands at Tanah Rata, Pahang, starting from February until August 2003. The predator, *M. caliginosus* were collected from brinjal (*Solanum melongena*) and tomato (*Lycopersicum esculentum*) plants grown around MARDI station. From the stock culture, a pair of predator adults were selected and placed in an oviposition container (15 cm x 8 cm) filled with fresh single brinjal leaf. A healthy, 14-day-old brinjal plant was selected and placed together with the male and female predators in the container. Female and male adults were allowed to mate in 24 hours period. The female predators laid the eggs in the stem of leaf. After five days, all the adults were removed from the oviposition container and placed back into the stock culture (50 cm x 45 cm x 60 cm). A total of 10 oviposition containers were prepared for this experiment. Some of the eggs of predators were deeply embedded in the ribs or stems of brinjal plants. No observation was carried out during the egg stage. The development of different stages from nymph to adult was observed daily. The data were recorded at different stages of life cycle and analysed with calculated means of days. The data on body length (mm), antennae, budding of different stages were also recorded and analysed.

2.2 Predation of Macrolophus caliginosus on Whitefly immatures

A total of 50 *T. vaporariorum* larvae of various stages on the under surface of a brinjal leaf were selected and marked. They were taken from whitefly stock culture and placed in a plastic container. Then an adult predator of *M. caliginosus* was randomly taken from the culture cage and introduced into the plastic container with the whitefly larvae. The experiment was replicated ten times (PC_1 - PC_{10}). The whitefly larvae killed by predators which had an opening at the centre of its empty body were counted and recorded. A total of 50 whitefly larvae were replaced at every two days until 10 days. Exuviae from successful emergence of whiteflies were not counted. The numbers of larvae killed by the predator were counted daily under microscope for ten days. The daily mortality of preys was regressed to generate the consumption rate of the predator. The experimental design (plastic containers) was arranged in CRD (Complete Randomised Design).

2.3 Adult longevity of Macrolophus caliginosus

Five plastic containers (PC1, PC2, PC3, PC4, and PC5) were prepared in this experiment. Five different treatments were used namely T0 (no treatment - no food provided), T1 (only single leaf plant), T2 (honey), T3 (single leaf plant and infested leaf with whitefly larvae), and T4 (moisturised cotton wool). No food was provided in T0 in order to determine the predator that could survive without food. Treatment T1 was to determine duration of the predator could survive on the plant without larvae or any source of food. Treatment T2 was to determine how long the predator could survive with honey, while treatment T3 consisted of predator which was provided with single leaf plant and larvae of whitefly to determine how long it could survive when it was served with sufficient food. Finally, treatment T4 consisted of predator which was served with water only. The whitefly larvae were placed in T3 for every two days. The adult predator was placed individually in the plastic container. The experiment was replicated three times and the plastic containers were arranged in CRD (Complete Randomised Design). Due to limitation of adults, these experiments randomly used adult male or female predators. In this experiment, the adults were reared by feeding them with various foods to observe how long they could survive by given those foods. One treatment was used as a control whereby no food was given to the predator.

2.4 Adult fecundity of Macrolophus caliginosus

Prior to the experiment, six *M. caliginosus* adults from the stock culture were randomly chosen, anaesthetised and sexed under a dissecting microscope. Following that, a pair of predators was placed in a cylindrical cage provided with a brinjal plant for oviposition. They were fed with new batch of 20 to 30 whitefly larvae of various stages every two days until the end of the experiment. The experiment was repeated three times and the plastic containers were arranged in CRD (Complete Randomised Design). The observation was carried out at the end of 30 days during adult life span, due to the longevity of predator (previously ranging between 32 to 35 days) and to avoid the second generation emergence of predator.

2.5 Data analysis

The data were recorded at different stages of life cycle and analysed with calculated means of days. The data on body length (mm), antennae, budding of different stages were also recorded and analysed. The daily mortality of preys was regressed to generate the consumption rate of the predator, number of days in the longevity of predator adult, number of predator's progeny of on fecundity of predator. Data collected was subjected to Analysis of Variance (One way ANOVA) in order to determine the significance differences among treatment means and Regression analysis using SAS Programme (Syed Abdul Rahman, 2000).

3. Results and discussion

3.1 Life cycle of predator, Macrolophus caliginosus

Observations on the life cycle showed that the females started to lay eggs approximately two days after mating. The eggs were embedded deeply into the stem making them unavailable for observation. Table 1 summarizes the results of the developmental stages in the predator's life cycle. After 8.6 days the eggs hatched and the first instar nymph lasted for 3.3 days before it moulted into the second instar. A total of four nymphal instars were observed in this study and the duration for third and fourth instars were 4.4 and 7.3 days, respectively. The total mean of developmental period for predators from egg to adult was 27.6 days.

<<Table 1: The mean number of days (mean ± S.E) for each life cycle stage of the predator *Macrolophus caliginosus*>>

Table 2 and show the size of body length from head to end of abdomen measured at every stages of life cycle. The mean body length of first instar was 0.504 ±0.0002 mm, second instar was 1.249 ± 0.0016 mm, third instar, was 2.12 ± 0.0057 mm, and fourth instar, was 3.021 ±0.0023 mm, and adult, was 3.482 ± 0.0002 mm.

Table 2 is also summarizes the results of antennae lengths which were recorded at every stages in the predator's life cycle. For the first instar, the length of antennae was 0.34 mm, second instar was 0.71 mm, third instar was 1.27 mm, fourth instar was 1.79 mm, and adult was 2.31 mm. The development of antennae seemed to be almost constantly linear at every stage in the predator's life cycle. Table 3 and also show the length of predator's wings measured at every developmental stage of life cycle. There was no wing bud observed during the first instar stage. The bud started to develop in the second instar with a mean length of 0.304 mm. In the third instar, the wing bud increased to 1.035 mm, and in the adult, it was 2.52 mm. Results from the study demonstrated that the morphological characteristics and the immature stage of four nymphal instars were clearly noticeable.

<<Table 2: The length (mean ± S.E.) of body, antennae and wing bud of various life cycle stages of *Macrolophus caliginosus*>>

The developmental period of oligophagus predators varies due to factors such as host plants, temperature, relative humidity (RH), habitat and most importantly the predators' species. The temperature for example, could influence the growth rate of an insect significantly. In this study, *M. caliginosus* spent 27.6 days at temperature of 24 ± ^{o}C to complete its life cycle. At 22 ^{o}C, Berengere et al., (1996) found that *M. caliginosus* completed its life cycle (tomato) within 35-40 days with a preovipositon period of about 5 days. Although the temperature difference was only 2 ^{o}C, the life cycle of *M. caliginosus* showed a variation of 8-13 days. The optimum temperature for species development is extremely important as insect shows maximum growth rate at such temperature. Besides temperatures, host plants influence insects' growth and life cycles of oligophagus heteropteran predator quite tremendously. Nutrients provided by the host plants are important for the egg and nymphal developments before predatory activity is intensified in the adult stage. The turgidity of the leaf ribs and midribs was extremely critical during embryonic development especially for *M. caliginosus* as it affected the hatchability of their eggs (Berengere, C., 1996). Taksdal (1963) (Sandra, D.S., 1987), Evans (1976) and Fauvel et al., (1987) had proven some plants were better hosts for certain predators. Brinjal is one of the good hosts for *M. caliginosus* as its development is relatively short in this study. Nutrients in various host plants also influence insects' mortality. *Macrolophus caliginosus* had shown variable mortality rates when fed on different host plants (Fauvel, G., 1987). Other studies showed significant difference in developmental period of *M.*

caliginosus on *Nicotiana tabacum* and *Pelargonium peltatum* which indicate that some plants are better hosts, as has been reported for several Heteroptera (Sandra, D.S., 1987; Taksdal, G. 1963 and Evans, 1976).

3.2 Predation of Macrolophus caliginosus on Whitefly immature

Macrolophus caliginosus ate whitefly larvae by inserting its proboscis into their bodies. Daily predation rate of *M. caliginosus* has been enumerated from the number of empty larvae with mean ranges of 5.5 to 6.8 larvae per day. The predators were found to attack the larvae of whitefly randomly regardless of larval stages and larval distribution on the leaves. Result from the studies demonstrated that the total mean of whitefly larvae killed by predators' adults was 5.94 per day. However, from the linear regression, it was found that the number of whitefly consumed by *M. caliginosus* was not significantly influenced by the number of days.

Macrolophus caliginosus adults fed on whitefly larvae of all stages from the first larval stage to the pupal stage. Within a 10-day observation, the predatory habit did not show any feeding trend. The predator consumed the preys at almost similar daily rates (average of 5.94 per day). Therefore, one predator was capable of decimating approximately 60 larvae in a period of 10 days. This capacity was relatively similar to an earlier study whereby Lucas and Alomar (2002) recorded a predation rate of 5 to 5.3 larvae per day. However when only the second and fourth larval stages whiteflies were offered to the predator, the predation rate dropped to only 2.7 larvae per day (Lucas, E., 2002). Coincidently these larvae were reared on tomatoes. The host plant of the prey could also be a determining factor. The plant nutrient might affect the taste of the preys making them either more tasteful or less desirable to the predators. In this case brinjal might produce better tasting preys as compared to tomatoes. The feeding of *M. caliginosus* was obviously more active in the presence of various prey life stages although all individuals were randomly consumed. They were probably more alerted by different sizes and movements of the preys. *Macrolophus caliginosus* usually attacked the preys nearest to them. Alomar et al., (2002) found that this predator was more abundant in outer crop rows closer to the source of the predator. They also concentrated on plants with higher whitefly densities. Therefore in the field situation the density and location of the whiteflies on the crop plants as well as the direction and distance of predator source or reservoir influence the predation rate of *M. caliginosus* on their preys. Previous study (Barnadas, I., 1998), in the greenhouse, predators attacked the whitefly nymphs as their food source. *Macrolophus caliginosus* was more abundant in outer rows, particularly in fields close to predator sources, which were concentrated on plants with higher whitefly densities (Alomar, O., 2002). Furthermore, Castane et al. (2004) found that *M. caliginosus* was an important source of pupal mortality since no other predators were consistently found in the surveys. The role of the surrounding vegetation in the colonisation is poorly known. Although, the predator *M. caliginosus* was a good biological control agent but the predatory effect it was better when it combined with other natural enemies such as *Encarsia formosa*. For further study, researchers should take advantage of the presence of this predatory complex and consider its interactions with other biological control agents in controlling whitefly. It should also assess the movement of the greenhouse whitefly and its predators especially *M. caliginosus* between greenhouses and the surrounding habitats.

3.3 Adult Longevity of Macrolophus caliginosus

Results from the study showed that the predators could survive for 3.67 days without any food source (Table 3). There was a significant ($F=515.6$; $df=4,10$; $P<0.05$) in longevity of predator among the treatments. Longevity of predator was significantly ($P<0.05$) on brinjal plant with the presence of the whitefly preys and brinjal plant alone than on other treatment. There was no significance ($p>0.05$) difference in longevity when the predators lived on water alone as compared to those without eating any food. The predators lived longer when they were provided with preys (whiteflies). However, since they are polyphagous insect, predators survived quite well on brinjal without prey. When *M. caliginosus* were fed with honey, the longevity slightly improved (live longer) as compared to living on water alone. It was also observed that no food treatment gave the lowest mean days (3.67 ± 0.33), followed by supplied with water alone (4.33 ± 0.33), honey (6.33 ± 0.33) and young brinjal single leaf plant alone (25.67 ± 2.33). Finally, treated predators together with brinjal single leaf and whitefly showed the highest mean days (33.67 ± 2.33) of longevity.

The brinjal plant served as an alternative feeding source to predators, *M. caliginosus*. The ability of predators to survive was significantly different ($p<0.05$) with no food treatment. Treatment with cotton wool moisturised with water did not significantly affect the longevity of the predator. However, they could also live for quite a long time on brinjal plants in the absence of the preys. Single leaf brinjal plant and whitefly larvae show significant ($p<0.05$) influence on the longevity of predators. This was evident that the presence of whitefly larvae in the plastic containers as predators' food supply could increase the survival rate or life span of the predators. *Macrolophus caliginosus* is a relatively hardy insect that survived without food for almost 4 days. In the presence of water, the predator could live slightly longer. Water from moisturised cotton wool did not seem to directly contribute to the longevity of predators. They depend on the plant sap and their preys. Furthermore, *M. caliginosus* has piercing sucking mouthparts and need the food from the plant or their preys based on their acceptance or rejection phenomenon. Although incomparable to host plant and prey, honey prolonged the predator's life even longer (6 days) than living on water alone. This information is useful for emergency cases such as during an experiment whereby the food for this insect is not available. They can be starved up to 3 days

without fearing them dead. In situation where the food supply in the field is cut off due to harvesting of crops or destruction of habitats as well as its preys, the predator could live for a few days before supplementary food is critically required.

Nevertheless, in field conditions, there may be different results in longevity due to their behaviour in searching for food which are also affected by the environmental factors such as photo period, humidity, and temperature (Castane, C., 2004). *Macrolophus caliginosus* thrived better and live longer on host plants with a lot of whiteflies. This longevity was significantly longer than when the predator was denied of preys. As an oligophagus feeder it enjoyed food from plant and animal sources for a longer life. Feeding on mixed diet was found to improve the performance of the predator as compared to living on single-prey diets [19]. In the presence of more than one prey species, *M. caliginosus* often exhibited the tendency to choose varieties of them. When preys, *Trialeurodes vaporariorum* and *Bemisia tabaci* were available, the predator fed on both species, shifting from one to the next as availability changes (Dean, 1995). This behavioural plasticity enabled the predator to exploit alternative food when prey populations are low or temporarily absent.

<<Table 3: The survival period (days) of the predators, *Macrolophus caliginosus* with various food>>

3.4 Adult Fecundity of Macrolophus caliginosus

The reproduction of the predators was observed for a month. The total mean number of predators in each cage was 51, indicating that each female could produce an average of 51 progenies within 30 days. The eggs of the predators were not clearly seen. At the end of 30 days, some of the eggs had developed into adults. There were four nymphal instars with the mean period of 11.7, 14.7, 16 and 5.7 days for the first, second, third and fourth instars respectively. Therefore, the life cycle of *M. caliginosus* was completed within slightly less than a month (Table 4).

<<Table 4: Total number of predators, *Macrolophus caliginosus* after 30 days>>

In this study, it was found that a female *M. caliginosus* fed with whitefly immatures produced 51 nymphs in 30 days on brinjal plant. Unfortunately the eggs of the predators could not be observed because they were deeply embedded in the ribs of the leaves. Assuming that eggs hatchability was 100%, the hatching rate was at an average of 1.7 eggs per day. The difference in the number of progenies produced by a female was very much determined by the host plant characteristics (Gerling, D., 2001) such as hardiness of the leaf petioles and midribs. Berengere et al., (1996), recorded hatching rates of *M. caliginosus* at 3 and 1.6 eggs per female per day on tobacco and a medium with *Inula viscose* extract respectively. Should hatching be approximately 100% in their study, hatchability would be of the same rates on each medium. Comparing the present result to these two records, the oviposition and eventually the hatchability rates of *M. caliginosus* eggs were closer to that of artificial substrate. Structurally, brinjal leaves are harder than tobacco leaf although both of them have a lot of hairs. It is obvious that tobacco was a much preferred host for oviposition. Therefore, the hardiness, thickness and possibly the moisture content of the host plant influence the number of eggs laid by females on specific hosts. Based on previous study (Evans, H. F., 1976), the diet of the predator was very important for the fecundity of *M. caliginosus.* When the predator was fed with the eggs of *Ephestia kuehniella* (Zeller) (Lepidoptera: Pyralidae) and allowed to oviposit on *Pelagonium peltatum*, a female predator produced 268 eggs at 20^{O}C in 35 days. The hatchability rate increased from 3 to 5.67 nymphs per day after 10 days. It peaked on day 15th to 16 nymphs per day before dropping 11.7 nymphs per day on day 25th. Feeding on whitefly larvae such as in this study, only 51 progenies were produced by a female *M. caliginosus* over a period of one month. However, if this study were prolonged until the female died, the number of eggs and offspring could be different or higher. In the life cycle structure recorded after 30 days, there were approximately 13% (11.7 nymphs) of first instar larvae. The tendency was that more eggs were hatching and more young larvae would add to the total number of the offsprings should the period of study be extended. The comparison to the previous data would then be more meaningful. Nevertheless, the aspect of environment should be taken into account as it might affect the fecundity of a female predator. In the laboratory study, caging might affect the fecundity of predators. Fecundity of *M. caliginosus* was also studied by Van Schelt et al. (1995), who placed one female and one male in a Petri dish (8 cm diam. X 3 cm) with *T. vaporariorum*. Result of the study were not comparable to the present study as the types of cages used was a cyclinder cage (15 cm diam. X 30 cm) which were less suitable for fecundity of *M. caliginosus*. However, the study of *M. caliginosus* fecundity in Petri dishes does not seem to be the best approach as females can easily escape from the dishes (Van Schelt, 1995). Interestingly, *M. caliginosus* was commercialized in Europe a biological control agent for greenhouse pests such as whiteflies. At the moment, egg laying and subsequent developments to the adult stage have been initiated on artificial substrates (Berenger et al., 1996). The predator can be mass produced to meet the need of greenhouse farmers. Nevertheless, more researches are required for cheaper and more efficient production of this predator.

4. Conclusion

It can be concluded from this study that *M. caliginosus* contribution in managing whitefly is significant. However, *M. caliginosus* alone is insufficient to restrain whitefly. Good farming practices are needed to complement the effects of

M.caliginosus in controlling whitefly. Future work should be carried out on other beneficial crops, large scale production of solanaceae crops and low land area to determine its effects.

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Table 1. The mean number of days (mean ± S.E) for each life cycle stage of the predator *Macrolophus caliginosus*

Stages	**Means (day) mm ± (S.E.)**	**Range (days)**
Eggs	8.6 ± (0.31)	7 – 10
First Instar	3.3 ± (0.15)	3 – 4
Second Instar	4.0 ± (0.26)	3 – 5
Third Instar	4.4 ± (0.16)	4 – 5
Fourth Instar	7.3 ± (0.3)	6 – 9
Total	27.6 days	-

Table 2. The length (mean ± S.E.) of body, antennae and wing bud of various life cycle stages of *Macrolophus caliginosus*

Stages		**Length mean (mm) ± S.E.**	
	Body	**Antennae**	**Wing Bud**
First Instar	0.50 ± 0.000 (0.48 – 0.52)	0.34 ± 0.016 (0.3 – 0.4)	0
Second Instar	1.25 ± 0.002 (1.20 –1.30)	0.71 ± 0.031 (0.6 – 0.8)	0.30 ± 0.008 (0.27 – 0.34)
Third Instar	2.12 ± 0.006 (2.00 –2.20)	1.27 ± 0.026 (1.2 – 1.4)	0.51 ± 0.008 (0.48 – 0.55)
Fourth Instar	3.02 ± 0.002 (2.96 – 3.10)	1.79 ± 0.023 (1.7 – 1.9)	1.04 ± 0.024 (0.9 – 1.11)
Adult	3.48 ± 0.000 (3.46 – 3.50)	2.31 ± 0.023 (2.2 – 2.4)	2.52 ± 0.020 (2.40 –2.60)

Table 3. The survival period (days) of the predators, *Macrolophus caliginosus* with various food

Treatments	**Mean days survival + S.E.**
No food (T0)	3.67 ± 0.33 d
Young brinjal single leaf plant (T1)	25.67 ± 2.33 b
Honey (T2)	6.33 ± 0.33 c
Brinjal single leaf plant + whitefly larvae (T3)	33.67 ± 2.33 a
Water (T4)	4.33 ± 0.33 d

Means with the same letters are not significantly different at P= 0.05 based on Duncan Multiple Range Test (DMRT).

Table 4. Total number of predators, *Macrolophus caliginosus* after 30 days

Stages\Cylinder Cages	**CC 1**	**CC 2**	**CC3**	**Total**	**Means ± S.E**
Eggs	0	0	0	0	0
First Instar	14	9	12	35	11.7 ± 6.3
Second Instar	16	13	15	44	14.7 ± 2.3
Third Instar	15	14	19	48	16.0 ± 7.0
Fourth Instar	5	6	6	17	5.7 ± 0.3
Adult	2	3	4	9	3.0 ± 1.0
Total Number of Predators	52	45	56	153	51.0 ± 31.0

Note: CC - Cylindrical cage (CC1, CC2, and CC3)