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Effect of Various Diets on the Development of Scuttle Fly, *Megaselia scalaris* (Loew) (Diptera: Phoridae), Larvae and Pupae and Percent of Adult Emergence and Longevity

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Abstract: Effects of different diets on the development of scuttle flies larvae, Megaselia scalaris (Loew, pupae and adult emergence and longevity were studied. The diets (treatments) used were nutrient agar (NA, control), casein agar (CA), tissue extracts of the round snail, Bradybaena similaris (Fer.), + NA, tissue extracts of the giant African snails, Achatina fulica (Fer.) + NA, and mixed diets (MD, A. fulica tissue + cabbage leaf powder + NA). A comparison was also made on larval and pupal development when larvae were fed on live snails (A. fulica or B. similaris) versus their tissue extract. The developmental time, mean body weight and body length of *M. scalaris* larvae were significantly different among treatments (p<0.05). Larvae fed on tissue extract diet of B. similaris + NA developed faster than larvae fed on NA or MD. In contrast, mean weight of larvae was highest when reared on the MD, tissue extract of A. fulica + NA and CA. Larvae fed on CA had significantly longer mean body length than larvae fed on other diets. The mean weight of pupa was significantly different among treatments (p<0.05), but not its developmental time (p>0.05). The mean weight of pupa was significantly lower when larvae were fed on NA or tissue extract of B. similaris + NA than on CA, tissue extract of A. fulica + NA or MD. The percent of adult emergence and longevity was also significantly different among treatments (p < 0.05). The percent of emergence and longevity of adults that originated from larvae fed CA or tissue extract of B. similaris + NA were significantly higher than adults originated from larvae fed NA and tissue extract of A. fulica + NA. The developmental time of larvae fed live host B. similaris were significantly longer than those fed its tissue extracts + NA (p < 0.05), indicating that the larvae had possibly encountered defensive systems of the hosts. In contrast, pupal development took significantly shorter time when larvae were fed live B. similaris + NA than fed it tissue extract (p<0.05). There was no significant difference in the developmental time of both larvae and pupae of M. scalaris when fed either live or tissue extract of A. fulica + NA (p>0.05). The possible use of CA and natural diets for laboratory rearing of *M. scalaris* as the potential biological control agent of snails is discussed.

Key words: Megaselia scalaris, Bradybaena similaris, Achatina fulica, Scuttle fly, Biological control, Parasitoid

Introduction

The scuttle fly, *Megasellia scalaris* (Loew) (Diptera: Phoridae), is one of the cosmopolitan fly species of the world (El-Miniawi and Moustafa, 1965). It has been reported to be abundant both in the lowland and highland of tropical countries (Idris and Abdullah, 1997). Many *Megaselia* species are opportunists parasitizing or feeding on a wide range of food including animal caracases and feaces (Lever, 1944; McCrae, 1967; Downie *et al.*, 1995; Idris and Abdullah, 1999). However, *M. scalaris* was recorded to parasitize only certain species of adult insect or larvae, snails (Ahmad and Ho, 1980; Robinson, 1975; Idris and Abdullah, 1997, 1999) and frogs, *Agalyhnis annae* (Duellman) (Downie *et al.*, 1995).

The round snails, Bradybaena similaris (Fer.) (Helicidae), is a terrestrial snail species widely distributed in the tropics and prevalent at the altitudes over 1,000 m above sea level (Nor et al., 1995). It has a potential to become a serious pest of vegetable crops in particular the cabbages (Murali, 1991; Ahmad and Ho, 1980). This is because of the damages it caused to cabbage crops, which in some cases, was more severe than damages inflicted by the diamondback moth, *Plutella xylostella* L., a major insect pest of cabbage crops worldwide (Ooi, 1992; Idris and Grafius, 1995). In an effort to reduce insect pest infestation and pesticide usage many vegetable crops and flowers are grown under netting structure system. commercially However, high humidity under netting structure has been said to cause rapid multiplication of B. similaris (Department of Agriculture-personal communication).

Because vegetables such as cabbages are an important high value crop for local use and export purposes, controlling of *B. similaris* is obviously critical. Molluscicides such as Siputox and baits containing metaldehyde were widely used for controlling *B. similaris* (Salmijah *et al.*, 1996). However, evidences o f *B. similaris* developing resistance to these

chemicals have been reported (Salmijah et al., 1996; Noran et al., 1 992). Interestingly, this snail was not affected by bioinsecticide developed from Azadirachta indica leaf extract that killed an aquatic snail, Indoplanorbis exustus. Result of a laboratory studies have indicated that M. scalaris has a potential to become a biocontrol agent of B. similaris (Idris and Abdullah, 1997). They reported that 55% of B. similaris exposed to M. scalaris was parasitized. However, percent parasitism of the snail in the field is ranged between 10 and 35%. Lower parasitism rate was probably due to low parasitoid population in the field as a result of heavy use of pesticides to control DBM and other insect pest of vegetables. There is also a possibility that the parasitoid has many alternate hosts in the field (Idris and Abdullah, 1999; Ahmad and Ho, 1980). Disney (1994) reported that the development of *M. scalaris* larva and its parasitism rate is quite dependent on host species, temperature and nutrient provided by the host.

The objectives of our study were to investigate the effects of various diets on the development of M. scalaris larvae and pupae, percent emergence and longevity of its adults. Results of this study could provide information on the type of diet best suited for use in mass rearing of this parasitoid in the laboratory. The reared parasitoid can be field released when needed or at the time when no pesticides spraying was conducted. This will increase its population and role in the field for controlling *B. similaris*.

Materials and Methods

Source of Insect and snails: The giant African snails (*Achatina fulica*) and round snails (*Bradybaena similaris*) were collected around Bangi housing estate and vegetable growing areas in Cameron Highlands, Malaysia, respectively. The snails were placed separately in a plastic container, $19 \times 27 \times 36$ cm with 0.5 cm diameter holes (10) on the lid to allow fly adults to

enter. Some 10-15 snails were housed per container, fed fresh cabbage leaves collected from Cameron Highland. Several snails of each species were crushed and placed two per container to attract the M. scalaris to the live hosts in the container (Brown and Feener, 1991). The containers were placed outside the laboratory for 2 days to attract at least three fly females into it. The container was then covered with screen mesh (300 holes per cm²) tied to its top using rubber band and brought back into the laboratory. The flies were allowed to lay eggs on the snails for 36 hours before being taken out. The fly eggs were allowed to hatch and after 2 days the presence of larvae were checked every B hours and transferred to respective diets prepared as explained below. The fly eggs were also extracted from several snails to be used in the study for comparing the development of larvae and pupae on live and tissue extracts of *B. similaris* and *A. fulica*.

Sources of Diets: Five different diets were used in the study, viz; nutrient agar (NA), casein agar (CA), NA + tissue extract of *B. similaris*, NA + tissue extract of *A. fulica*, and NA + tissue extract of *A. fulica* + cabbage leaves.

Nutrient agar (NA): The NA used was a Gelose nutritive (Diagnostic Pasture, France). A total of 3 g agar was boiled in 75 ml distilled water for 30 minutes after which it was cooled under laboratory environment for about an hour. It was then poured into 1.8 cm diameter test tube, autoclaved for 20 minutes at 15 psi. The test tubes were kept in the autoclave for 12 hours to allow gradual cooling process before taken out and kept in refrigerator at 4°C for used as a control treatment (NA) or to be mixed with other diets.

Casein agar (CA): The CA used was a modification of Brewer's Yeast recipes for culturing Diptera (Zucoloto, 1993). It contain 5.0 glucose (Hamburg Chemical, Germany), 3.0 g starch (BDH Chemicals Ltd., England), 3.2 g yeast (Oxoid, England), 3.0 g empty agar 16DH Laboratory Reagents, England), 6.0 g casein (Sigma Chemicals Ltd., England), 0.04 g multivitamin (Squibb, England). The materials were mixed in 75 ml distilled water to make CA diet that was prepared as above.

NA + **tissue extract of** *B. similaris*: Snail tissue was obtained by crushing the shell and extracting out then cut into small pieces, and dried in an oven at 60°C for 24 hours. The dried tissue was grounded to form tissue powder. A total of 7 g of tissue powder was mixed with 3 g NA prepared as above in a 75 ml distilled water. A 5 ml of agar and tissue mixture was poured into 1.8 cm diameter test tube, in slanting manner, plugged with cotton wool and autoclaved at 15 psi for 15 minutes. Test tubes were left in the autoclave for 12 hours to allow cooling and to avoid the formation of condensed water vapor in it. The NA and tissue mixture was temporarily kept in a refrigerator at 4°C before being used in the experiments.

NA + **tissue extract of** *A. fulica*: Tissue powder of *A. fulica* was prepared similarly to the tissue powder of *B. similaris.* The mixture of nutrient agar and tissue extract of *A. fulica* was also prepared as above.

NA + tissue extract of *A. fulica* + cabbage leaf powder (mixed diet, MD): Tissue powder of *A. fulica* was prepared as above. To prepare cabbage leaf powder, we cut cabbage leaves into small pieces and dried in an oven at 60°C for 24 hours. Dry cabbage leaves were grounded into powder. Some 3.0 g agar, 3.5 g cabbage leaf powder, 3.5 snail tissue powder were mixed in 75 ml distilled water to get the diet mixture, which was then prepared as above.

Experiment 1

Developmental time of *M. scalaris* larvae and pupae. percent

of adult emergence and longevity: Six M. scalaris larvae were released per test tube per diet (four tubes per diet or treatment) using a soft paintbrush. Test tubes were covered with muslin cloth and kept in laboratory environment. Larvae were checked every day to measure their body lengths and weights. Body length was measured from anterior to posterior portion using digimatic caliper (Tokyo, Japan), while body weights were obtained by using electronic balance (A and D Company Limited, Tokyo, Japan). The measurements were done daily and continued until the larvae became pupae. Pupae were transferred to 10 cm diameter petri dish, one pupae per dish labeled with respective treatments and kept in laboratory environment ($33 \pm 2^{\circ}C$, 12L:12D, 70 ± 5% for temperature, photoperiod and relative humidity, respectively) until adult emergence. We recorded the weight of the pupae 12 days after pupation) and its developmental time (from pupa formed until adult emergence). The number of emerged adult was recorded daily. Each emerged adult female was transferred into 8.0 cm plastic cup labeled with type of diet, where it was raised, and lid covered with muslin cloth. Longevity of each adult female (without food) was measured by recording it mortality every 24 hours.

Experiment 2

Development of *M. scalaris* larvae and pupae on living host versus tissue extract of the host: Five live *B. similaris* and *A. fulica* and their tissue extracts (prepared as above) were artificially infested with 10 *M. scalaris* eggs using a soft paintbrush. The treatments were placed in a plastic container as described above and kept in laboratory environment as mentioned in experiment one. To record the development time of larvae and pupae the treatments were checked 3 days (egg hatch is about 2 days after laid) (Idris and Abdullah, 1997) after being infested by the flies. This was done to avoid damaging the fragile shells of the live snails.

Data Analysis: The body length and weight of larva or pupa were averaged by adding the daily body length and weight and then divided by the numbers of day data collected. Data of the average body length and weight, the developmental time of larvae and pupae, and percent adult emerged per treatment and adult longevity were analyzed by one-way ANOVA using statistical program (SAS, 1990). Duncan multiple range test was used to separate the means between treatments. The relationship between larvae or pupae developmental time and its mean weight were analyzed by simple correlation analysis using SuperAnova program (Abacus Concept, 1991). The developmental time of larvae and pupae on live and tissue extract of both snails were analyzed by unpaired t-test.

Results and Discussion

Experiment 1: Developmental time of *M. scalaris* larvae and pupae, percent of adult emergence and longevity

Larvae: There was a significant difference in the developmental time of *M. scalaris* larvae fed on different diets (F = 11.27; df = 4, 73; p < 0.05) (Fig. 1A), suggesting that the diets had differential effects on the development of fly larvae. The larval developmental time was significantly longer (13.5 days) when fed on a NA than on other diets (p < 0.05). The shortest developmental time was on tissue extract of B. similaris + NA 14.2 days) and CA 14.6 days), indicating that the two diets are the most suitable for the larval development. There was no significant difference in developmental time of larva fed on tissue extract of *B. similaris* (4.2 days) or A. fulica + NA (5.2 days). However, Idris and Abdullah (1997) reported that larval stage took 5.5 days when larvae were fed on live B. similaris. This suggests that the fly encountered less defensive systems in host tissue extract than in life host tissue and was able to use nutrients provided by the host

tissue for its development. The development of fly larvae is largely dependent on nutrient availability within their host or food sources (Vison and Barbosa, 1987). The larva of *Exeristes roborator* needs 10 types of amino acids (arginine, histidine, isoleusine, leusine, lycine, methionine, fenilalanine, thereonine, thryptophan and valise) for maximum growth. Result also showed that *B. similaris* is likely to be the better host of the *M. scalar's* larvae than that of *A. fulica.*

The shorter developmental time of larvae on CA (4.25 days) than on NA and MD is probably due to the presence of yeast brewer, multivitamin, glucose and starch in addition to the complete protein content in the CA (Davis, 1972; Bratti and Coulibaly, 1995). Brust and Fraenkel (1955) found that the development of blowfly, *Phormia regina* (Meig.), was significantly faster on diet containing casein, yeast brewer, sterol and B vitamin. In contrast, the developmental time of *M. scalaris* larvae fed on artificial diets without casein was almost twice longer (7.3 days) than on CA in our study (Prawirodisastro and Benjamin, 1979).

As compared to other diets particularly the tissue extract of both snails + NA, the NA a lone may have less complete diet for the development of *M. scalaris* larvae then the other diets (Bratti and Coulibaly, 1995). Cannibalism was also observed among larvae fed on NA, indicating that the diet contain incomplete nutrients required for larval development as compared with CA and those made of natural diets (Zucoloto, 1993). The cannibalism may also due to competition for important nutrients such as casein present in low amounts in the NA (Carroll, 1986; Joyner and Gould, 1987). Larvae of *Ceratitis capitate* (Diptera: Tephritidae) that cannibalized other larvae were found to grow faster than those do not involved in cannibalism (Carroll, 1986).

It was intriguing to see that larval developmental time on a MD was significantly longer (p < 0.05) than on the CA and tissue extract of B. similaris + NA (Fig. 1). There may be some interactions between tissue extract of A. fulica and cabbage leaf that negatively affect the development of the fly larvae. The mean body weight of *M. scalaris* larvae was significantly different among treatments (F = 6,63; df = 4, 10; p < 0.5) (Fig. 1B). The body weight was significantly higher (p<0.05) when larvae were fed on diets containing A. fulica tissue extract + NA or a MD than on diets containing NA alone or tissue extract of *B. similaris* + NA. This suggests that the fly larvae had higher growth rate and accumulated more food reserves for the later stages (pupae and adult) when fed on diet containing tissue extract of A. fulica + NA than on other diets (Disney, 1994). The CA seemed to be a much higher quality food than NA, as the larval body weight was significantly higher when they were fed on CA than on NA. The larva of blow fly (Phormia regina) was reported to have higher body weight when fed on diet containing casein, yeast brewer, sterol and vitamin B than on diet without casein (yeast, sterol and vitamin B) (Brust and Fraenkel, 1955).

There was a significant difference in the mean body length of *M. scalaris* larvae among treatments (F = 9.47; df = 4, 7); p < 0.05) (Fig. 1C). The mean body length of *M. scalar's* larvae was significantly higher when fed on CA than on other diets (p < 0.05), suggesting that CA is an essential nutrient and can be considered as protein-rich meal for growth of fly larvae (Disney, 1994). The mean larval body length was shortest and longest when fed on diet containing tissue extract of *B. similaris* + NA or CA, respectively. Surprisingly, the body lengths were not significantly different when larvae were fed on either NA or tissue extract of *B. similaris* + NA. Probably, *B. simians* provided inadequate amount of nutrients necessary for larval growth than nutrients provided by

A. fulica + NA or CA.

Pupae: There was no significant difference in the developmental time of *M. scalaris* pupa among treatments (F = 0.86; df = 4, 39; p > 0.05) (Table 1), indicating that diets types had no influence on the development of pupae from pupariation to adult emergence (Disney, 1994). The humidity and probably extreme temperature are the main factors that influence the developmental time of *M. scalaris* pupae (Hussey and Gurney, 1963; Benner and Ostermeyer, 1980). In our laboratory these two factors (see materials and methods) seemed to have no significant effect on the results. However, there was a significant difference in the mean pupal weight among treatments (F = 8.07; df = 4, 39; p<0.05) (Table 1), suggesting that the diets consumed by the larvae had influenced the pupal weight. The pupal weight was significantly higher when larvae were fed on CA, tissue extract of A. fulica + NA or MD than on NA or tissue extract of B. similaris + NA. As discussed earlier for larva the CA and diets containing tissue extract of A. fulica + NA may provide more of the protein that are needed to build up food reserves for the pupae, adult and eggs (Zucoloto, 1987). This was showed by a positive and significant correlation between body weight of *M. scalaris* larvae and pupae (r = 0.54; F = 5,22; df = 1, 3: p < 0.05). Probably, this is an explanation for our result that the pupal weight was significantly tower when larvae were fed on *B. similaris* + NA than those larvae fed on the CA, tissue of A. fulica + NA and MD (Table 1).

Adult: The percent of adult emergence was significantly different among treatments (F = 7.84; df = 4, 39; p < 0.05). (Table 1). Percent adult emergence was higher on CA and tissue extract of *B. similaris* + NA than on the tissue extract of *A. fulica* + NA or NA. This suggests that CA is a better diet for adult development (within pupa) as compared to the NA and tissue extract of *A. fulica* + NA. Although the weight of larvae and pupae were significantly lower (p < 0.05) when larvae were fed on tissue extract of *B. similaris* + NA than on *fulica* + NA, the percent of adult emergence was higher on *similaris* or *A. fulica* + NA (Fig. 1A-B, Table 1). This indicates that substances other than protein-rich meal present in *B. similaris* consumed by the larvae play a part in determining the optimum adult development within the pupa (Disney, 1994).

The longevity of adult female was differed significantly among treatments (F = 5.3; df = 4, 49; p < 0.05) (Table 1). Adult female lived significantly longer when larvae were fed on the CA (3.56 days) than on the NA (1.15 days) and tissue extract of A. fulica + NA (2.53 days 1 or MD (2.88 days) (p<0.05). This indicates that CA is a better quality food than the other diets. Fly larvae fed on CA might have accumulated more food reserves for its pupa and adult development and a longer adult life span than larvae fed other diets. The amount of food reserved for pupa and adult development, and adult fife span after emergence, if there is no food taken or provided, is largely dependent on the food consumed by the larvae (Blum, 1985; Disney, 1994). However, data on the effect of food on adult longevity of M. scalaris adult are largely lacking (Disney, 1994). Many studies were only conducted on the effect of temperature on adult longevity (El-Miniawi and Moustafa, 1965; Prawirodisastro and Benjamin, 1979). However, Idris and Abdullah (1997) reported that longevity of adult female M. scalaris was 24.3 days when fed on diluted honey solution. Longevity of Diadegma insulate (Hymenoptera: ichneum onidae), a parasitoid of P. xylostella, ranged from

Table 1: Developmental time and mean weight of pupae, and percent emergence and longevity of female adults *Megaselia scalaris* (Loew) fed on different diets^{a,b}

Diets	Developmental	Pupa Weight	Percent Adult	Adult Longevity
	Time (days \pm S.E)	$(mg \pm S.E)$	Emerged (\pm S.E)	$(days \pm S.E)$
Nutrient agar	11.25±0.57a	$1.42 \pm 0.56b$	$28.54 \pm 5.73c$	$1.15 \pm 0.52d$
Casein agar	$11.43 \pm 0.55a$	$3.45 \pm 0.65a$	$57.32 \pm 7.62a$	$3.56 \pm 0.46a$
B. similaris	$12.33 \pm 0.62a$	$1.87 \pm 0.53b$	$49.71 \pm 6.54 ab$	$3.27 \pm 0.53ab$
A. fulica	$12.54 \pm 0.67a$	3.51±0.54a	$32.82 \pm 3.44c$	$2.53 \pm 0.42c$
Mixed ^c	$11.23 \pm 0.53a$	$3.23 \pm 0.56a$	$43.52\pm5.65b$	$2.88\pm0.38b$

^aLongevity of adult females was measured after emergence with no food given.

^bMeans in column with the same letters are not significantly different (Fisher's Protected LSD, p>0.05)

^cnutrient agar + tissue extract (powder) of A. fulica + tissue powder of cabbage leaf

Table 2:	Developmental time (days ± S. E.) of <i>Megaselia scalaris</i>			
	(Loew) larvae and pupae (until adult emergence) when larvae			
	were fed on tissue extractS of Bradybaena similaris or			
Achatina fulica and on live snails				

Treatments		Larvae (n = 30)	Pupae (n = 20)		
A. fulica	Live snail	$5.45 \pm 0.64a$	$11.22 \pm 2.31a$		
	Tissue extract	$5.23 \pm 0.45a$	$12.46 \pm 2.55a$		
B. similaris	Live snail	$4.85 \pm 0.57a$	$10.24 \pm 2.43a$		
	Tissue extract	$4.22 \pm 0.53b$	$12.24 \pm 2.14b$		

Means in column with similar letter are not significantly different (unpaired t-test, p > 0.05)

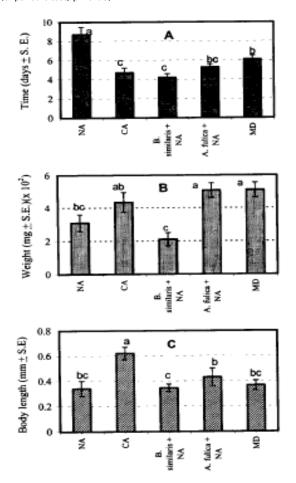


Fig. 1(A-C): (A) Developmental time, (B) mean body weight and (C) body length of *Megaselia scalaris* larvae fed on different diets. (NA, nutrient agar; CA, casein agar; different diets (NA, nutrient agar; CA, casein agar; MD, mixed diets, *B. similaris* + NA, A. Fulica + NA)

1,5 to 2.8 days depending on types of food given (Idris and Grafius, 1995; Idris, 1995). This tends to agree with our result that life span of adult *M. scalaris* is largely dependent on food intake by the larvae (Table 1).

Experiment 2

Development of *M. scalaris* larvae and pupae on living host versus tissue extract of the host: There was a significant difference in the developmental time of larvae (t = 4.3; df = 1; p < 0.05) and pupae (t = 6.4, df = 1, p < 0.05) of M. scalaris when larvae were fed on live or tissue extract of B. similaris + NA (Table 2). However, neither live nor tissue extract of the A. fulica caused differences in the development time of the fly larvae and pupae (p>0.05). The larval developmental time was significantly longer (p < 0.05) when larvae were fed on live *B. similaris* than on its tissue extract. Reports on how phorid larvae suppress or evade the defense reactions of their host are lacking (Disney, 1994). However, the M. scalaris larvae may have encountered a defensive system of the live host as it was reported to have occurred in host of other parasitoid species in the order of Hymenoptera (Vison and Barbosa, 1987). The presence of defense system in the live B. similaris and A. fulica might stimulate the formation of nutrient complexes in host tissue that would render it usable for larvae (Vison and Barbosa, 1987). The trend was reverse for the developmental time of pupae (Table 2). This indicates that once the parasitoids have passed the larval stages they will escape the defensive reactions of their host. Shorter pupa developmental time on live snails suggests that the parasitoid larvae had accumulated food reserve enough for faster pupa development as compared to food accumulated of the snail's tissue extract. The developmental time of M. scalaris larvae was relatively shorter when fed on both live and tissue extract of B. similaris than on the live and tissue extract of A. fulica (Table 2). B. similaris may contain less effective defense system to succumb parasitoid larva attack than that of A. fulica. However, further study needs to be conducted to get more definite causes. Our result also suggests that the fly larvae may have possibly adapted to the immune system of B. similaris and that they might have been in association with this snail longer than with A. fulica.

Conclusion: Our results indicated that diets had differential influence on the development of *M. scalaris* larvae and pupae, and percent adult emerged and longevity. The CA seems to be the most suitable diet for rearing *M. scalaris* even when compared with the tissue extract of host snails. However, 'chemical legacy hypothesis' has predicted that the sympatric speciation and host-mediated conditioning are important factors in influencing adult fly behavior (Corbet, 1985). For example, Hoffman and O'Donnell (1992) reported that there

was a variation in resource fidelity in *Drosophila melanogaster*, in relation to different types of fruit in the field.

Although the *B. similaris* is seemed to be less effective diets and difficult to be prepared commercially than CA, they may be the better choice for use in rearing of *M. scalaris* than the CA. This is because we can be sure that the fly female will more likely to oviposit on *B. similaris*, and that will increase parasitism rate of this snail by the *M. scalaris*. In addition, the fly male will be more likely to choose mates carrying the same chemical legacy as them or seek mates in the vicinity of the same pabulum or habitat as that experienced by their own larvae. However, further study needs to be carried out before any decision can be made regarding suitability of constituted diets for the rearing of *M. scalaris*. Such studies are probably about the effect of diets on parasitism rate, mating behavior and success as well as the sex ratio, These studies should be done in laboratory before field-testing is conducted.

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