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*Full Length Research Paper*

# Rearing the house fly predator *Carcinops pumilio* (Erichson) (Coleoptera: Histeridae) on an artificial diet

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**A number of concentrations of PRO-PLEX™, a protein rich food additive, were incorporated into an artificial diet to determine its effect on the predatory beetle, *Carcinops pumilio* (Erichson). The impact of this host-free artificial diet on developmental time, larval weight, oviposition and mortality was assessed. When *C. pumilio* is fed on a host-free diet, the total development of egg to adult was prolonged in comparison with those reared on natural diet by an average of 3.95 ± 0.17 days. The number of stadia was not affected by the artificial diet. The increase in weight of both 1st and 2nd instar larvae was highly significantly and strongly correlated with the concentration of PRO-PLEX™ in the artificial diet ($P < 0.001$). Adults fed on the artificial diet laid eggs, but significantly less than on the natural diet ($P < 0.05$). *C. pumilio* larvae reared on a natural diet had a significantly faster rate of oviposition, shorter developmental time and lower mortality as compared to those fed on the artificial diet.**

**Key words:** Artificial diet, natural diet, *Carcinops pumilio*, body weight, developmental time, mortality.

## INTRODUCTION

Rearing insects on artificial diets has many advantages over natural food, particularly in the case of parasitoids and predators where the need to provide prey is eliminated. This should reduce the cost of production, one of the problems limiting the use of insect parasitoids and predators in inundative releases (Leppla, 1996; Thompson, 1999). Thus to overcome such problems and meet the demands for large numbers of insects required for fundamental research in the fields of physiology, ecology, genetics and insect control techniques such as male sterilization and integrated pest management (IPM) programs, the use of artificial diets for rearing insects has generated great interest since the 1950's (Singh, 1977). Mass reared insects on an artificial diet could also serve as a cheap source of food for animals in zoos and birds, such as poultry. Endangered insect species could be reared on an artificial diet in the laboratory and later released into their own natural habitat (Singh, 1977). The artificial diet could also be used as a dietary supplement over periods when natural prey is scarce or unavailable for rearing, a suggestion also made by Hattingh (1991).

The first known example of mass rearing an insect on an artificial diet was the larvae of the blowfly, *Cochliomyia hominivorax* (Coquerel) in 1936. This was a hallmark, which facilitated suppression of screwworm by genetic means (Knipling, 1984). Excellent artificial diets are now available for rearing many phytophygous arthropods, but are generally lacking for entomophagous arthropods (House, 1977; Singh, 1977). The house fly is an unsuitable prey source for mass production of its predators such as *Carcinops pumilio* (Erichson), because larvae that escape predation disrupt the substrate and interfere with pupating *C. pumilio* larvae (Geden, 1990). However, alternative prey can be used, such as sphaerocerid flies (Geden, 1984), *Drosophila repleta* (Wallaston) (Fletcher et al., 1991) and *D. melanogaster* (Meigen) (Achiano and Giliomee, unpublished). Geden (1990) stated that cost-effective *C. pumilio* production must await the development of artificial diets and methods of handling that will minimize the effects of cannibalism in culture.

The house fly, *Musca domestica* L. is a serious cosmopolitan pest. Poultry farmers employ various

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techniques to control them but rely heavily on the use of chemicals which constitutes a significant production expense (Lazarus et al., 1989). Such heavy reliance on insecticides for pest suppression has the serious drawback of the target pest becoming resistant (Rutz and Scott, 1990; Howard and Wall, 1996) and the possible destruction of beneficial insects (Theiling and Croft, 1988). This could create new secondary pests and a later resurgence of damaging population levels of the primary pest (Metcalf and Luckmann, 1975). One way of overcoming such problems is to develop an IPM program with emphasis on biological control. In such a programme the availability of an artificial medium for rearing the principal predator, *C. pumilio*, would be of great advantage. However, an economical mass rearing method on an artificial diet is yet to be developed for *C. pumilio.*

In an attempt to find an artificial diet for *C. pumilio*, PRO-PLEX™, a protein rich food additive used by body builders, was supplied. When supplied with PRO-PLEX™ powder, *C. pumilio* attempted to consume it but could not hold on with the mandibles. The powder adhered to the body or it got entangled in the melted powder. It was therefore decided to investigate the possibility of formulating PRO-PLEX™ powder in another form that will enhance the ability of *C. pumilio* to feed on it. The objectives of this study were therefore: (1) to formulate an artificial diet devoid of any prey material using PRO-PLEX™ and (2) to evaluate the nutritional and physical adequacy of the diet formulation using (a) developmental time, (b) mortality rate and (c) ovipositon rate as parameters.

## MATERIALS AND METHODS

### Insects

The adults of *C. pumilio* used in this study were from a population maintained in the laboratory, originating from samples obtained from Stellenbosch University Experimental Poultry Farm, Elsenburg, Western Cape Province, South Africa (33° 51´S; 18° 50´E). All experiments were carried out in an incubator set at 30 ± 1°C at a Stellenbosch University Laboratory (33° 54´S; 18° 57´E). Beetles used in the experiment were $F_6$ progeny.

### Diet preparation

The dietary composition of the PRO-PLEX™, designated as "protein" in this study is listed in Table 1. Diets were prepared using protein and agar in various ratios (w/w) so that diets with 30, 40, 50, 60, and 70% protein were obtained. Each mixture was blended for 60 seconds and brought to boil for 5 to 10 min whilst continuously stirring. After it had boiled for 5 minu, 1.5 g Na-methylhydroxy benzoate dissolved in 2 ml alcohol was added to each mixture to prevent fungal growth in the medium. After preparation, while the diet which had a rather smooth texture was still hot, it was poured into 1 litre plastic containers. The containers were left open for 10 min to allow some moisture to evaporate, to cool down and solidify. The top of the solidified medium was then covered with Parafilm®.

The open portion of the container was later covered with organdy held in place with a rubber band. It was then placed in a cool room at 5 ± 1°C until use. On the day of use, several pieces of about 10 g of diet were transferred into each rearing container. The shelf life of the diet was not determined but used diet stored for 30 days in similar experiments gave similar results to those of fresh diet.

### Preliminary experiments

In order to reduce the disturbance of the different stages during the final observations on development, preliminary development times were determined for the stages from egg to hatch, 1st instar larva to 2nd instar larva and 2nd instar larva to adult emergence from the pupa, at each protein concentration level from 30 to 70%. To obtain eggs, freshly emerged adult beetles were placed on the artificial diet for 10 days at each of the different concentration levels. Several pieces of the artificial diet at each protein concentration level were mixed with 1 litre of moistened bran. The bran to water ratio was 1: 2.9 (w/w) which produced about 60-65% moisture content. The mixture was then transferred into 2 litre milk containers. The newly emerged adult beetles that were kept on the artificial diet were placed on the bran and protein mixture for 24 h. They were then removed to prevent them from eating their eggs. Five replicates were used. The 2 litre container with the top removed was covered with organdy held in place with a rubber band. The preliminary tests were observed daily to obtain estimates of the above developmental periods for each concentration level. Similar procedures were also followed for *C. pumilio* fed on *Drosophila melanogaster* as a source of natural diet.

### Developmental times of stages

The developmental times of egg to 1st instar larva, 1st instar to 2nd instar larva and 2nd instar larva to adult emergence from the pupa were determined. The media and the procedure of rearing described above were used in 5 replicates for each protein concentration. After 24 h, the adults were removed and the media of each replicate was divided into 20 test tubes to facilitate easier counting of the larvae. The test tubes, covered with a plastic lid with small holes pierced into it using an office pin, were placed in an incubator at 30 ± 1°C. From two days before the estimated time of egg hatch, based on the preliminary experiment described above, the media were examined for the presence of 1st instar larvae.

The 1st instar larvae removed from each test tube were introduced into new medium with the appropriate protein concentration. Two days before the appearance of the 2nd instar larvae was expected, the test tubes were examined at 24 h intervals to detect their presence.

The developmental time of the 2nd instar larvae to adults was determined by observing the same individuals used in the 1st instar larvae studies. All test tubes were observed for adults as described above, except that a rotational method was used to reduce the disturbance of the pupae.

The data for developmental time (days) of various stages was analyzed using ANOVA. The means were separated at $p < 0.05$ level by Fisher's LSD.

### Weight measurement

The wet weight of ten 1st and 2nd instar larvae from each protein concentration level of individuals obtained was measured using a Sartorius microbalance which weighed to the nearest 0.01 mg. The larvae were weighed at the beginning of each stage. The effects of diet concentration on weight of 1st and 2nd instar larvae were analyzed by regression.

**Table 1.** Components of PRO-PLEX™ in mg/100 g.

| Nutritional information* | unit |
|---|---|
| Vitamin A, B & C | 0.06 |
| Nicotinamide | 0.71 |
| Calcium pantothenate | 1.90 |
| Phosphorus | 171.00 |
| Calcium | 78.00 |
| Magnesium | 84.00 |
| Copper sulphate | 0.16 |
| Sodium | 1279.00 |
| Pottasium | 1183.00 |
| Protein (Albumin) | 83000.00 |
| Aspartame | 400.00 |
| Essential amino acids | unit |
| L - Histidine | 2403 |
| L - Isoleucine | 2929 |
| L - Leucine | 6833 |
| L - Lysine | 5869 |
| L - Methionine | 3672 |
| L - Phenylalanine | 5009 |
| L - Threonine | 3927 |
| L - Tryptophan | 1196 |
| L - Valine | 4142 |

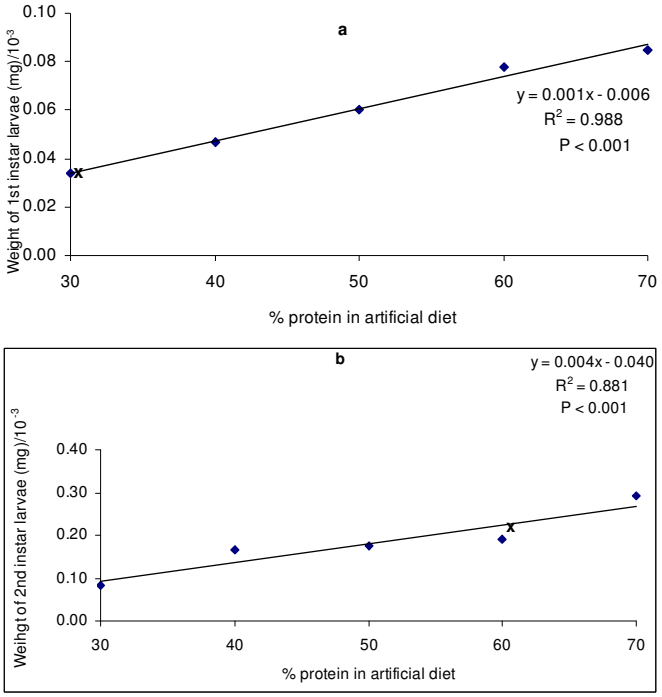


**Figure 1.** Mean weight of **a =** 1st instars; **b =** 2nd instars reared on five different protein concentration levels of artificial diet. **X =** weight of larvae fed on natural diet.

### Mortality rates

To determine the mortality of 1st instar larvae, five newly hatched larvae were transferred onto the medium in test tubes using a fine wet camel's hair brush. There were 20 replicates for each protein concentration of 30, 40, 50, 60 and 70%. Individual 2nd instar larvae obtained from surviving 1st instar larvae were used to determine the mortality rate from 2nd instar to adult emergence. Five 2nd instar larvae per test tube and ten replicates for each concentration level were used. The percentage mortality from 1st to 2nd instar larva and 2nd instar larva to adult emergence was calculated as [ $\bar{x}$ number dead / number of larvae per container] x 100.

### Oviposition

The adults reared on the artificial diet at different concentrations described above were used to determine ovipositon rate. Two pairs of adult males and females from each protein concentration level of 30, 40, 50, 60 and 70% were selected immediately after emergence and were placed in a small plastic container of 5 cm in height and 3 cm in diameter. The lid had a hole of 1.5 cm in diameter and this was covered with organdy, held in place with glue. This was replicated five times for each concentration. The adults were supplied with artificial diet *ad libitum*, with a small amount of moistened bran to serve as an oviposition site. After 24 h, they were removed and placed in a new container with fresh medium and source of food. This routine was followed for ten days. Six days after the first introduction of the adults the media were examined for the presence of larvae and those found were counted. In this study, it was assumed that the number of larvae hatched was equal to the eggs oviposited and that no mortality occurred in the egg stage.

The data for oviposition rates was analyzed using ANOVA. The means were separated at $P < 0.05$ level by Fisher's LSD.

## RESULTS

### Developmental time

Developmental times of the various stages differed significantly between the artificial diet and natural diet ($P < 0.05$) (Table 2). *C. pumilio* reared on the artificial diet developed more slowly than those reared on natural diet. The developmental time from 1st to 2nd instar larva in relation to the protein concentration or natural diet followed no particular pattern. The developmental time from the 2nd instar larvae to adult stage was also significantly shorter on the natural diet as compared to larvae fed on artificial diet. The total developmental time from egg to adult of *C. pumilio* fed on artificial diet was longer than that for *C. pumilio* fed on natural diet (Table 2). The number of stadia was not affected by the artificial diet.

### Effect of artificial diet on larval weight

The 1st instar larvae that emerged from eggs of adult *C. pumilio* fed on the artificial diet at the higher protein concentrations were heavier than those fed on natural diet (Figure 1). However, the 1st instar larvae of adults fed on the natural and artificial diet with a protein

**Table 2.** Developmental time of various stages of *C. pumilio* fed on natural and artificial diets.

| % protein in artificial diet | Developmental time (days) (mean ± SE.)* | | | Total developmental time |
|---|---|---|---|---|
| | Egg - 1st instars larva | 1st instars - 2nd instar larva | 2nd instar - adult | |
| 30 | 5.2 ± 0.09 b | 4.70 ± 0.16 a | 13.50 ± 0.17 b | 23. 40 ± 0.16 b |
| 40 | 5.0 ± 0.01 b | 5.30 ± 0.11 b | 13.85 ± 0.31 b | 24.15 ± 0.21 b |
| 50 | 5.1 ± 0.07 b | 4.55 ± 0.11 a | 15.95 ± 0.44 c | 25.60 ± 0.27 c |
| 60 | 5.0 ± 0.06 b | 5.40 ± 0.11 b | 16.60 ± 0.78 c | 27.00 ± 0.34 c |
| 70 | 5.0 ± 0.09 b | 4.40 ± 0.11 a | 15.70 ± 0.50 c | 25.10 ± 0.30 c |
| Natural diet | 3.8 ± 0.02 a | 5.1 ± 0.10 b | 11.3 ± 0.15 a | 20.10 ± 0.11 a |

*Figures followed by the same letters are not significantly different; P < 0.05

**Table 3.** Mortality rates of *C. pumilio* on an artificial and natural diet.

| % protein | % mortality | |
|---|---|---|
| | 1st to 2nd instar larvae* | 2nd instar larvae to adult emergence** |
| 30 | 95.5 a | 96.0 a |
| 40 | 92.0 a | 94.0 a |
| 50 | 92.5 a | 76.0 a |
| 60 | 89.0 a | 66.0 a |
| 70 | 86.0 a | 70.0 a |
| Natural diet | 22.0 b | 16.0 b |

N = 10, 5 larvae/container*
N = 10, 5 larvae/container**
Figures with the same letters are not significantly different. P < 0.05

**Table 4.** Oviposition rates (eggs/female/day) of *C. pumilio* on an artificial and natural diet.

| % artificial diet* | Oviposition (mean ± SE.)** | Oviposition day$^{-1}$/female |
|---|---|---|
| 30 | 0.9 ± 0.41 a | 0.09 |
| 40 | 2.7 ± 0.50 a | 0.27 |
| 50 | 1.5 ± 0.50 a | 0.15 |
| 60 | 2.0 ± 0.49 a | 0.20 |
| 70 | 1.0 ± 0.30 a | 0.10 |
| Natural diet | 7.5 ± 0.70 b | 1.50 |

*N = 10. **Figures with the same letters are not significantly different; P < 0.05.

concentration of 30% were the same weight. The 2nd in star larvae fed on 70% protein were also heavier than those fed on the natural diet. At the lower protein concentrations the differences were not significant except at the lowest concentration (30%) where the larvae were significantly lighter. The increase in weight of both 1st and 2nd instar larvae were highly significantly and strongly correlated with the protein concentration in the artificial diet (P < 0.001) (Figure 1).

### Effect of artificial diet on mortality

The mortality of 1st and 2nd instar larvae reared on the artificial diet was extremely high, exceeding 85% and 65%, respectively, whilst the mortality of larvae reared on natural diet was comparatively low at 22% and 16%, respectively. The mortality of both 1st and 2nd instar larvae reared on artificial diet was four times more than those reared on natural diet (Table 3).

### Effect of artificial diet on oviposition

From the oviposition rate as a measure of the reproductive potential of *C. pumilio* reared on the artificial diet, it was clear that adults fed on the natural diet had the highest oviposition rate of 7.5 eggs per day. This differed significantly from the oviposition rate of adults fed on artificial diet at all concentration levels (P < 0.05) (Table 4). There was no significant difference in the rate of oviposition between the various protein concentrations.

## DISCUSSION

The developmental time for an insect reared on an artificial diet is often prolonged, as was the case with *C. pumilio*. This was also found by De Clercq and Degheele (1992) and Wittmeyer and Coudron (2001) for *Podisus maculiventris* (Say), a generalist stinkbug predator, and

Carpenter and Greany (1998) and Gelman et al. (2000) for *Diapetimorpha introita* (Cresson), a parasitoid wasp. The prolongation of the developmental time of *C. pumilio* was most pronounced during the last stadium. This information provides direction for future research on improving this artificial diet to reduce developmental time. Furthermore, there were a small number of individuals that developed faster than others, indicating they adapted more quickly to the diet. From such fast developers a strain could be selected that are more suited to the artificial diet formulated, a suggestion also made by Wittmeyer and Coudron (2001) for *P. muculiventris.*

At certain protein concentrations, the weight of the 1st and 2nd instar larvae was similar or higher than those fed on natural diet, indicating that they developed adequately on the artificial diet. However, adult females that developed from larvae reared on the artificial diet had a significantly lower rate of oviposition than those reared on the natural diet. From the weight alone, one would have expected the females from the artificial diet to produce similar or higher numbers of eggs, as heavier females usually produce greater quantities of eggs as was observed by Lawrence (1990), Trudel et al. (1994) and Hirschberger (1999). However, they had a longer developmental time and higher mortality. Adults of larvae reared on the natural diet had a significantly higher rate of oviposition, shorter developmental time and a lower mortality rate. The protein concentration in the artificial diet had no effect on the rate of oviposition as it remained low at all concentrations. This indicated that the poor performance of *C. pumilio* on the artificial diet might have been due to an inappropriate balance or lack of nutrient(s). Clancy (1992) also reported that the poor performance of an artificial diet for rearing western spruce budworm could be attributed to inappropriate balances of one or more minerals in the diet. Similar observations were made by VanderSar (1978), who found that *Pissodes strobi* Peck appeared to feed normally on an artificial diet, but did not lay the expected number of eggs; this was attributed to feeding and oviposition stimulants being different from the natural diet.

The low oviposition of *C. pumilio* fed on the artificial diet showed the need to improve the diet for better results. It is therefore suggested that chemical analyses of the eggs and/or 1st instar larvae *M. domestica* or *Drosophila melanogaster*, which serve as natural prey, be done. This will help to identify both the qualitative and quantitative nutritional requirements of *C. pumilio*. Brewer and Lindig (1984) and Hattingh (1991) also recommend that the ratios of various chemical constituents obtained from the analysis of the natural prey should be used to help formulate the artificial diet.

The most positive aspects of the results were that *C. pumilio* completed its development on the artificial diet and that both the $F_1$ and $F_2$ generations fed on an artificial diet were able to lay eggs. This could be the first step towards finding an artificial diet that would allow continuous rearing of *C. pumilio*, ensuring its availability at all times for utilization in the biological control of house flies.

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