Fig. 5. Diel patterns of responses to odours from natural and artificial, sugar and protein, sources by (A) *C. cosyra*, (B) *C. fasciventris* and (C) *C. capitata*. Values represent means ± SE. Results are presented for all age categories combined.

5.3.2 Age differences in feeding and olfactory responses

For all species tested, flies of all age categories fed more frequently on sugar sources than on protein sources (*C. cosyra*: young immature: $\chi^2 = 12.31$, df $= 4$, P$= 0.02$; young mature: $\chi^2 = 40.82$, df $= 4$, P< 0.01; old mature: $\chi^2 = 36.78$, df $= 4$, P< 0.01 ; *C. fasciventris*: young immature: $\chi^2 = 30.26$, df $= 4$, P< 0.01; young mature: $\chi^2 = 14.53$, df $= 4$, P< 0.01; old mature: $\chi^2 = 12.56$, df $= 4$, P$= 0.01$; *C. capitata*: young immature: $\chi^2 = 50.34$, df $= 4$, P< 0.01; young mature: $\chi^2 = 36.20$, df $= 4$, P< 0.01; old mature: $\chi^2 = 30.32$, df $= 4$, P< 0.01) (Fig. 6). For most age categories of the three species studied, the descending order of feeding preference on sugar sources was as follows:
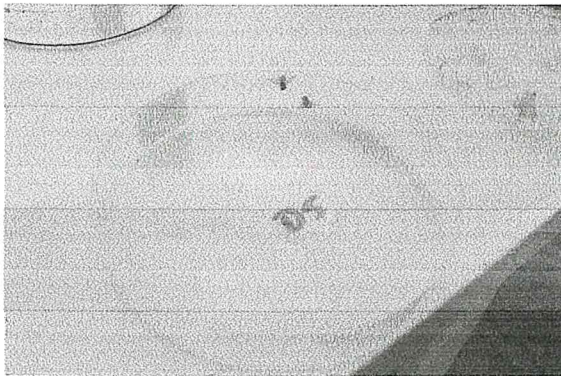
guava juice> honeydew >molasses (Fig 7).

Fig. 7. Fruit flies feeding on Petri dish
containing guava juice. A. Manrakhan, ICIPE.

Among protein sources, except for young immature *C. capitata* flies and old mature *C. cosyra* flies, flies of all species/age categories tested exhibited a higher feeding preference for chicken faeces as compared to ICIPE yeast.
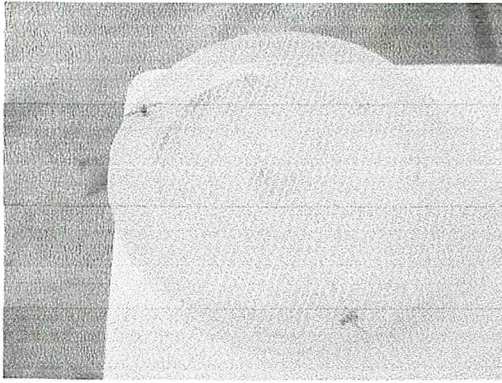
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Fig. 9. *Fruit flies attracted to odours from chicken faeces which was smeared at the bottom of the Petri dish. A. Manrakhan,*

There was a significant effect of food odours (odours from sugar and protein food source minus control) for flies of all species/age categories tested except for young immature *C. capitata* flies and young mature *C. cosyra* flies (*C. cosyra*: young immature: χ^2 =11.65, df =4, P=0.02; young mature: χ^2 =2.08, df =4, P>0.05; old mature: χ^2 =34.74, df =4, P<0.01 ; *C. fasciventris*: young immature: χ^2 = 15.79, df =4, P<0.01; young mature: χ^2 =17.82, df =4, P<0.01; old mature: χ^2 =84.65, df =4, P<0.01 ; *C. capitata*:

young immature: χ^2 =8.90, df =4, P>0.05; young mature: χ^2 =15.49, df =4, P<0.01; old mature: χ^2 =40.75, df =4, P<0.01) (Fig. 8). In general, for three fly species of different age categories, odours from natural food sources were more attractive than odours from artificial food sources (Fig. 9). Young immature *C. cosyra* flies were highly attracted to odours emanating from guava juice. Old mature *C. cosyra* as well as mature (both young and old) *C. capitata* flies were also highly attracted to odours emanating from guava juice. Young immature *C. fasciventris* flies were strongly attracted to odours from honeydew and mature *C. fasciventris* flies, both young and old, were strongly attracted to odours from chicken faeces.

5.3.2.1 Sugar sources

There were no significant differences in feeding responses on the three sugar sources between different age categories for both *C. fasciventris* and *C. capitata*. *C. cosyra*, however, fed preferably on honeydew at 14 – 17 days (χ^2 =19.89, df =2, P<0.01) and on molasses at 4-7 days (χ^2 =15.87, df =2, P<0.01) while there was no significant difference in feeding on guava between different ages (χ^2 =2.82, df =2, P>0.01).

For *C. cosyra*, frequency of responses to odours from guava and molasses culminated at 14 – 17 days (guava: χ^2 =13.07, df =2, P<0.01 and molasses: χ^2 =15.32, df =2, P<0.01) while there was no significant difference in responses to odours from honeydew between different age categories (χ^2 =1.20, df =2, P>0.01). Peak of response to odours from all sugar sources for *C. fasciventris* was noticed at 1-2 days (guava: χ^2 =15.46, df =2, P<0.01; honeydew: χ^2 =11.87, df =2, P<0.01 and molasses: χ^2 =9.91, df =2, P<0.01). For *C. capitata*, similar to

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their feeding responses to sugars, there was no significant difference between age categories in their frequency of responses to odours from the same food sources.

5.3.2.2 Protein sources

Feeding responses of *C. cosyra* on the two protein sources were higher at 4 – 7 days and 14 – 17 days, though this was only significant for feeding on ICIPE yeast (chicken faeces: χ^2 =8.03, df =2, P>0.01 and ICIPE yeast: χ^2 =15.07, df =2, P<0.01). For *C. fasciventris*, feeding responses on both protein sources culminated at 4-7 days (chicken faeces: χ^2 =10.95, df =2, P<0.01 and ICIPE yeast: χ^2 =16.04, df =2, P<0.01). As for *C. capitata*, there was a numerical increase in feeding responses on both protein sources at 4 – 7 days, however this was not significant.

With respect to odours from protein sources, responses of *C. cosyra* culminated at 14 - 17 days (chicken faeces: χ^2 =14.75, df =2, P<0.01; ICIPE yeast: χ^2 =7.87, df =2, P>0.01). There was no significant difference in responses to odours from protein sources between age cagetories for both C. *fasciventris* and C. *capitata*.
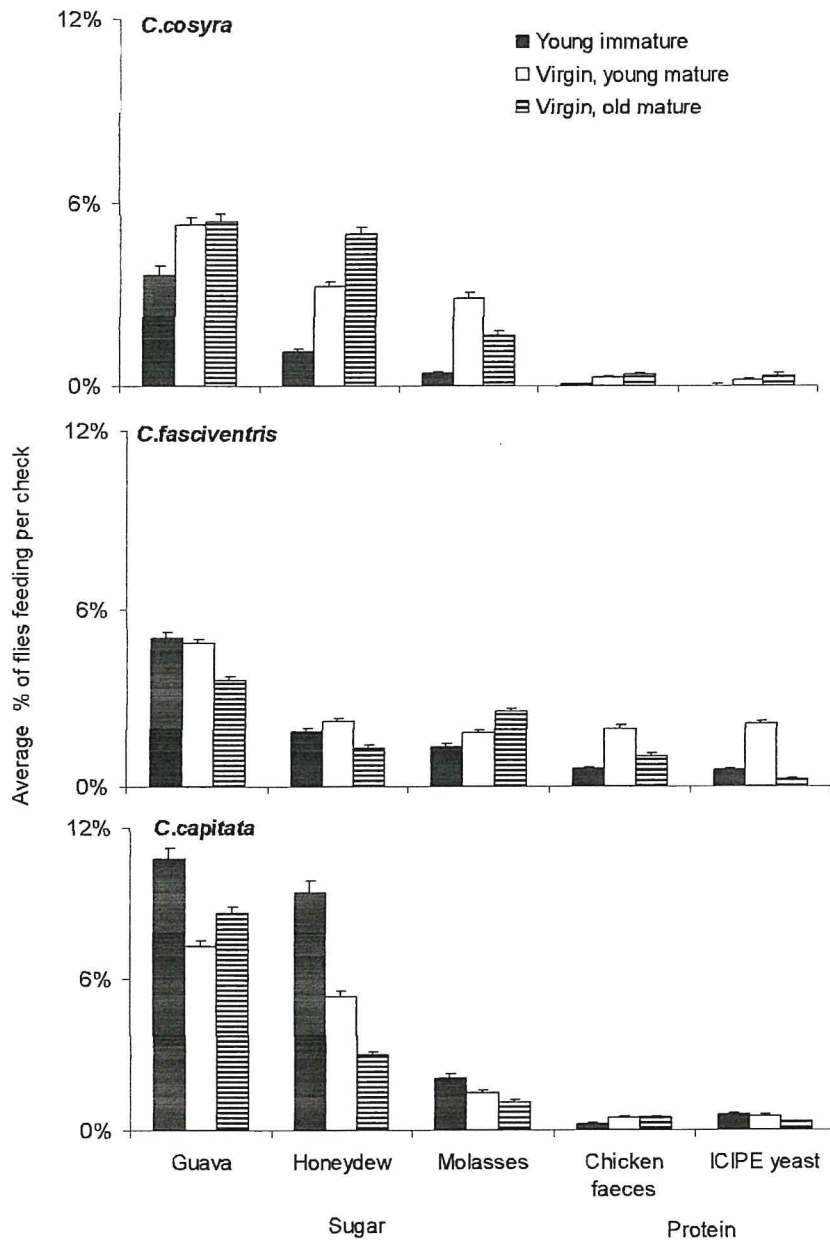
Fig. 6. Age-dependent feeding frequencies on various food sources by unmated flies of (A) *C. cosyra*, (B) *C. fasciventris* and (C) *C. capitata*. Values represent means ± SE.

Fig. 8. Age-dependent responses to odours from various food sources by unmated flies (A) *C. cosyra*, (B) *C. fasciventris* and (C) *C. capitata*. Values represent means ± SE.

5.3.3 Effects of mating status in feeding and olfactory responses

Feeding responses were not significantly influenced by mating status of mature flies irrespective of species and food sources (Fig. 10). Responses to food odours were similarly not influenced by mating status of mature flies the three fruit fly species (Fig. 11).

Fig. 10. Feeding patterns on various food sources by mated and unmated, mature flies of (A) *C. cosyra*, (B) *C. fasciventris* and (C) *C. capitata*. Values represent means ± SE.
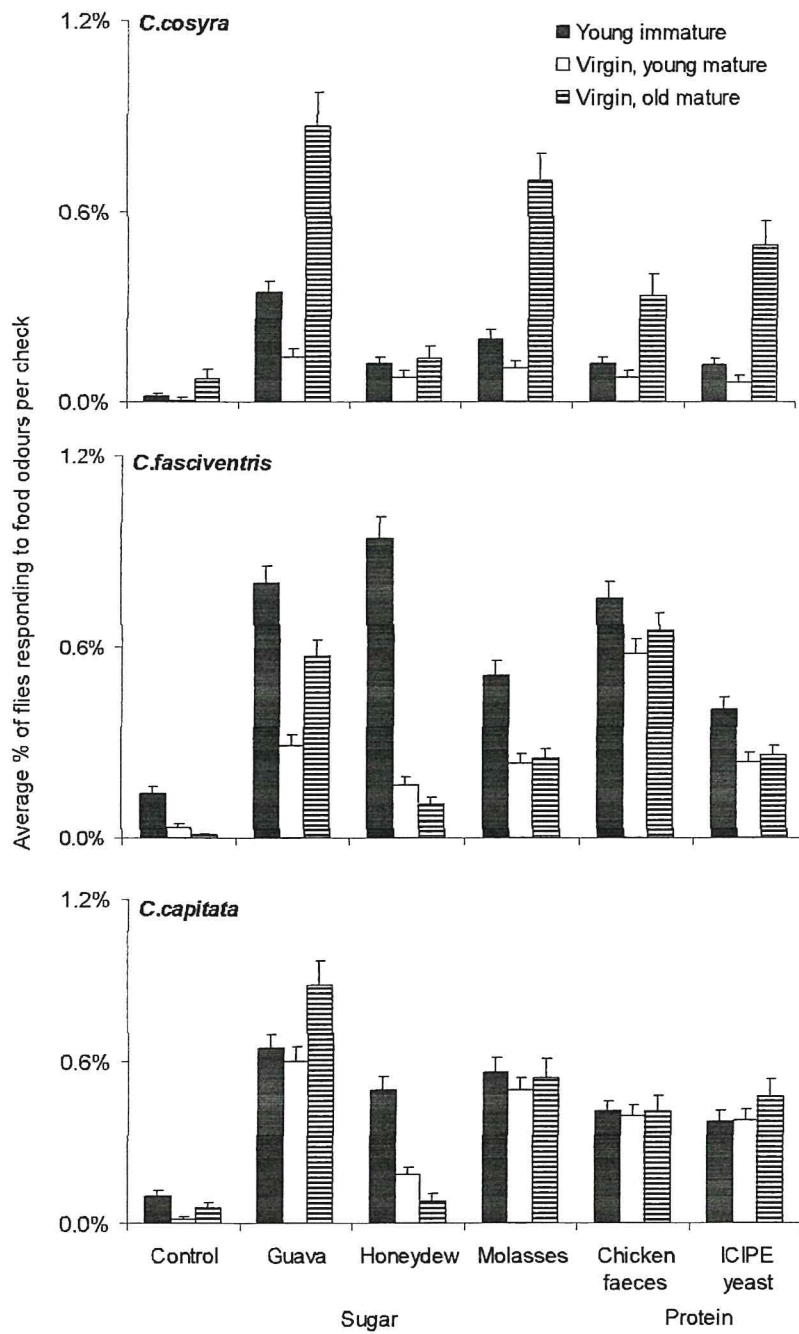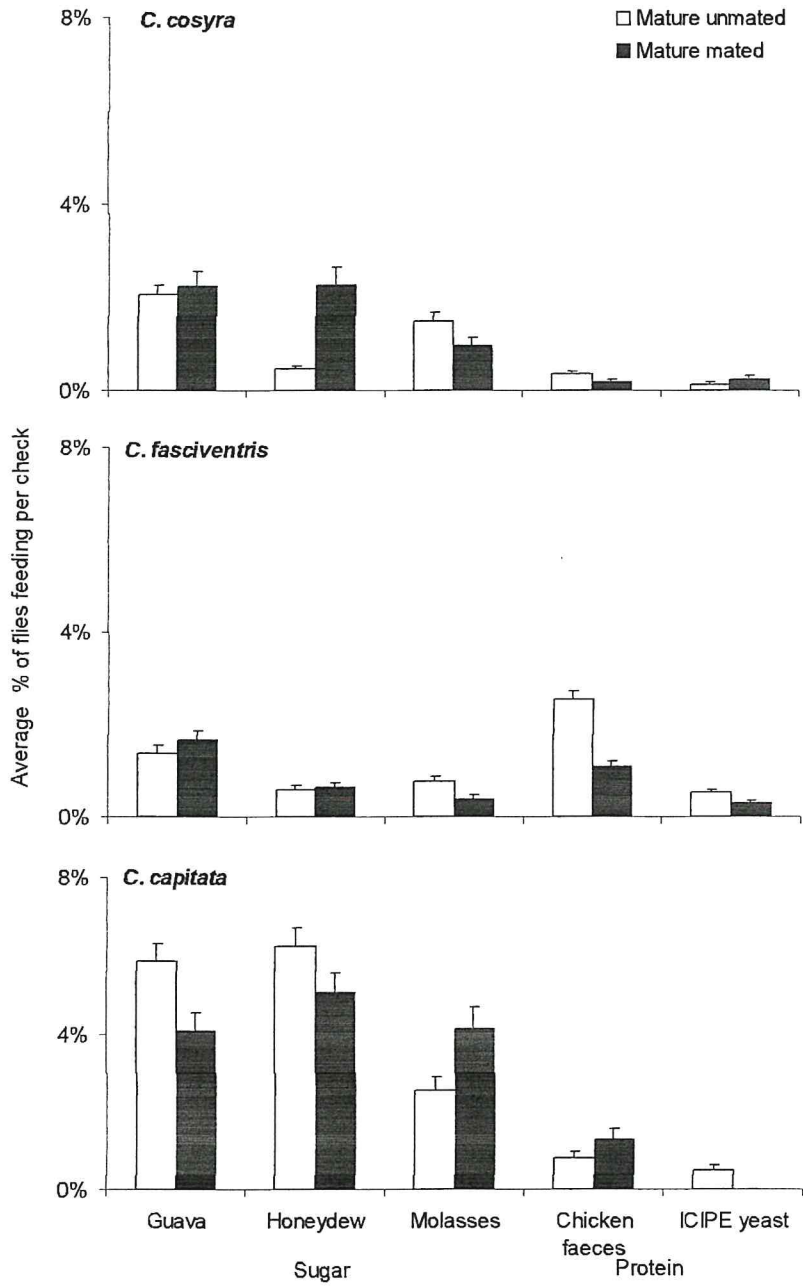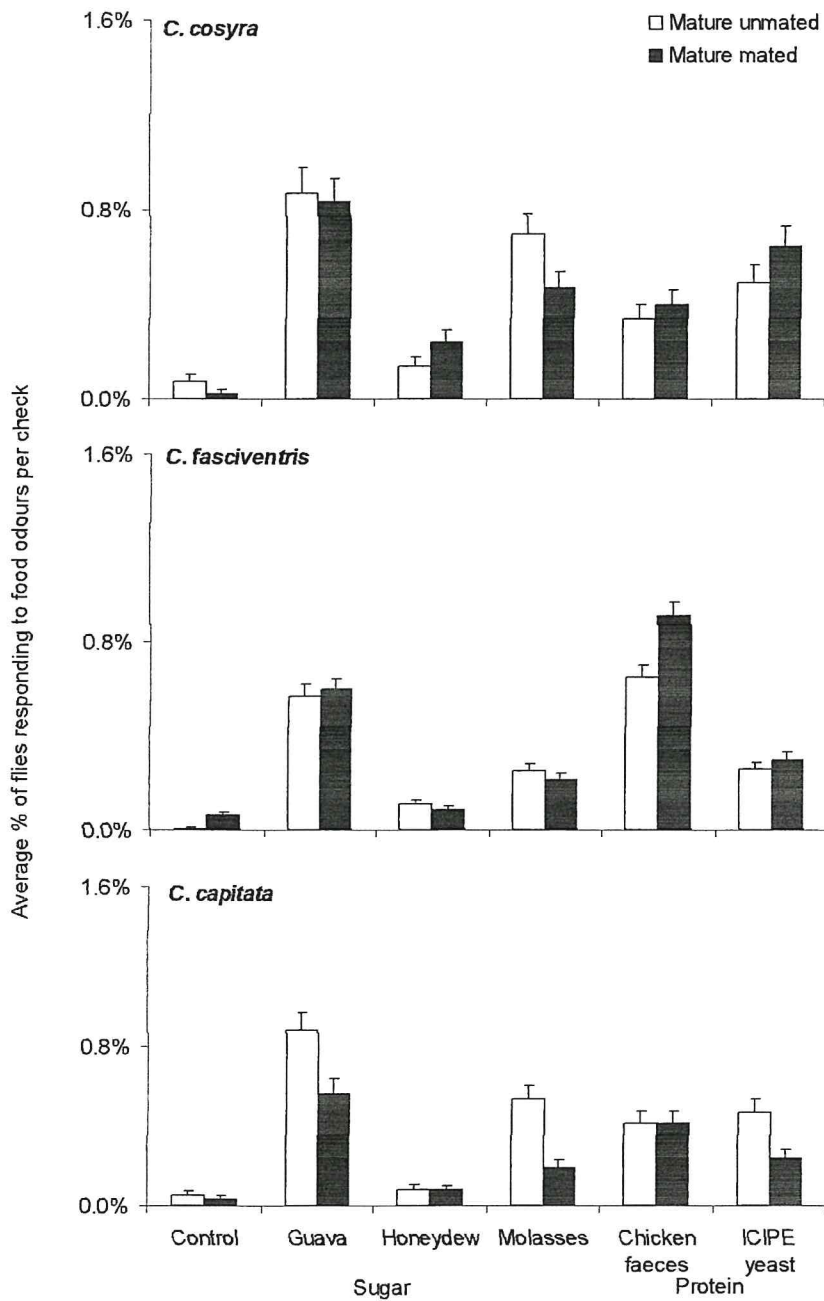
Fig. 11. Patterns of responses to odours from various food sources by mated and unmated, mature males flies of (A) *C. cosyra*, (B) *C. fasciventris* and (C) *C. capitata*. Values represent means ± SE.

5.4 Discussion

The present study shows that sugar based food sources were the most preferred feeding sites for flies of all ages for the three species studied. These results are in agreement with results presented for the same fly species in Chapter 4 with regards to their higher consumption of sugar compared to protein throughout adult life. Sugar, as has been discussed in Chapter 3 and 4, is both essential for fly survival and reproduction and also is highly phagostimulatory.

This study also shows an increase in frequency of feeding on protein by all three species as they matured. In all frugivorous species, females are anautogenous requiring protein to realize their reproductive potential. A peak in protein feeding was found to coincide with period of rapid ovarian development for several fruit fly species (Webster et al. 1979; Landolt & Davis-Hernandez 1993; Cangussu & Zucoloto 1992). In some tephritid species protein feeding is essential for males as well (Drew & Yuval 2001). In studies looking at effect of nutrition on the reproductive behaviour of *C. cosyra*, *C. fasciventris* and *C. capitata* (Chapter 3), it was clearly shown that males of these three species had lower frequencies of calling when fed on a diet lacking in protein as compared to when fed on a diet rich in protein.

Differences in diel feeding patterns on sugars and on proteins for all three species were found in this study. Feeding on sugars were constant at different times of the day for *C. cosyra* and *C. fasciventris* while for *C. capitata* feeding occurred preferably in the afternoon hours. The results on feeding on *C. capitata* in this study are consistent with the results which were presented in Chapter 2 in the course of a study of the diel feeding patterns of *C. capitata* in various semi field set-ups.

Feeding on protein sources occurred preferably in the morning for the three fruit fly species studied, more so for the natural protein source, chicken faeces, than for the local protein source, ICIPE yeast. The question that might therefore be formulated from this is whether or not sugar and protein feeding are discrete behaviours, occurring at different times of the day. This is however most likely not the case. Ammonia is the main factor for attraction of flies to protein sources including avian faecal material (Mazor et al. 1987; Roessler 1989; Epsky et al. 1997). In the first experiment on feeding, fresh food sources were used in the

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morning and the same food sources were used throughout the day. It is therefore highly likely that the attractiveness to the protein sources decreased, as a result of a decrease in ammonia release from the protein sources as they dried up, resulting therefore in a decrease in feeding responses. Besides, in the second experiment in this study, where fresh food sources were used at all time periods for the evaluation of responses of flies to food odours , flies of all three species were found to be highly attracted to odours from protein souces, both chicken faeces and ICIPE yeast, in the afternoon hours. Prokopy et al. (1993) found that dried bird droppings were less attractive than freshly collected ones to *C. capitata* flies. Moreover, Epsky et al. (1997) found that ammonia release from crude avian faeces dropped within 24 hours of exposure to *A. suspensa* females and this was directly correlated to the capture of these flies.

Diel patterns of attraction to food odours were not always similar to diel patterns of feeding on the same food sources. In general, flies of the three species were highly attracted to odours from most of the food sources in the afternoon hours. For *C. capitata*, the high attraction to food odours in the afternoon hours reflected their higher feeding responses at that time of the day. Whilst for the two other species, this higher attraction to food odours might have been motivated by hunger since the flies had no access to food during the whole test day in the second experiment unlike in the first one when they had free access to food throughout the day. Moreover, odours from guava juice, which were highly attractive to flies of all three species in the afternoon, might have elicited two types of resource-oriented behaviour, the search for food, as well as oviposition sites for female fruit flies (Cornelius et al. 2000).

Age related patterns of responses to food odours were not always similar to age related patterns of feeding for both sugars and protein. The increase in protein feeding as the flies matured did not reflect in an increase in responses of *C. fasciventris* and *C. capitata* to odours from protein sources at the mature stages. Immature flies of *C. fasciventris* and *C. capitata* flies were as equally attracted to odours from protein as mature flies. In this study, all flies used were protein fed before release. This might explain the reason why mature flies were not highly motivated to search for protein odours despite that when they had access to the protein sources they fed more. Protein deprivation was found to increase attractiveness of mature *A. ludens* to odours from protein sources (Robacker 1991).

This study demonstrated that odours from artificial food sources were less attractive than odours from natural food sources. Host fruit odours have been found to be attractive to

Mediterranean fruit fly (Light et al. 1988; Vargas et al. 1997), the Mexican fruit fly (Robacker 1991), the Carribean fruit fly (Nigg et al. 1994) and to the Oriental fruit fly (Cornelius et al. 2000). Odours from bird's droppings were found to be highly attractive to medflies and to Carribean fruit flies and when even more attractive than proteinaceous food baits used in control and monitoring of flies (Prokopy et al. 1993; Prokopy et al. 1992; Epsky et al. 1997).

Practical Implications of Findings. The strong dependence of fruit flies on natural sources of sugars could have implications for fly control. Natural sugar sources such as fruit juices from fallen or overripe fruits and honeydew from aphid-infested trees might sustain fly populations as well as reduce efficiency of artificial baits used in control of fruit flies. Measures such as maintaining fruit orchards free of natural sugar sources through sanitation (removal of fallen fruits) and through treatment of aphid-infested trees must therefore be included in a fruit fly control programme using artificial food baits.

Baiting stations containing artificial food sources especially when integrated with other tools such as pathogens have been discussed to be the most promising and appropriate method for fruit fly management in small-scale, fragmented horticulture in sub-Saharan Africa. Protein baiting stations equipped with pathogens were found to be highly effective in reducing fruit fly populations in small scale mango orchards in Kenya (Lux et al. 2003). These stations were auto-inoculative devices which housed fungal spores. In order to increase the efficiency of these baiting stations, a phagostimulatory food source such as a sugar source as found in this study could be incorporated into these stations which would thus allow attracted flies to feed longer on the food sources, and this would in turn increase their probability of picking up the pathogens.

Data from this study indicate that protein fed flies in particular *C. fasciventris* and *C. capitata*, both immature and mature, are equally attracted to protein sources. This can have implications regarding information obtained from population monitoring programmes that utilise protein baits since the latter might target not only mature flies, as expected, but also immature flies.

Finally the stronger attraction of flies to odours from natural food sources has serious implications as discussed by Prokopy et al. (1992) in adjusting spatial and temporal patterns of baiting stations or food bait spray applications according to the estimates of the composition and abundance of natural fly food in infested regions.

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6. Effect of food deprivation on attractiveness of natural and artificial, sugar and protein, food sources to three African fruit flies: *Ceratitis cosyra*, *C. fasciventris* and *C. capitata* (Diptera: Tephritidae)

6.1 Introduction

Food foraging behaviour, as for searching behaviour in general, represents the confluence of three kinds of factors: (i) biological characteristics of an insect, (ii) external environmental factors and (iii) internal factors such as nutritional state (deprivation) or sexual receptivity (Bell, 1990). Nutritional state influences the readiness of an insect to engage in behaviour directed towards the acquisition of any kind of nutrient and affects food preference (Browne, 1993). A range of neural and hormonal mechanisms involving volumetric and nutritional feedback have been identified for some insects as being the physiological basis for the effects of nutritional state on insects on their food oriented behaviour (Dethier, 1976; Simpson et al., 1995).

In previous studies, responses to food sources for several fruit fly species were found to be attenuated by feeding history and age. Robacker (1991) found that fruit derived attractant appealed highly to immature adult flies deprived of all food except water and to mature flies deprived of sugar for one day. Protein deprivation was found to increase attractiveness of mature flies to protein baits (Robacker, 1991; Prokopy et al., 1992; Robacker & Moreno, 1995; Prokopy et al., 1996; Cornelius et al., 2000; Cohen & Voet, 2002; Barry et al., 2003). Moreover, attraction to protein baits was found to increase with age of protein-deprived flies (Prokopy et al., 1992; Prokopy et al., 1996). Prokopy et al. (1996) have shown that physiological state of *C. capitata*, with respect to the amount of protein consumed in life and to a lesser extent fly age, can have a significant impact on the propensity of flies attracted to protein-odour baited trap to enter the trap.

Studies on effects of nutritional state on responses of flies to food odours, both natural and artificial, can have practical impact on strategies for managing fruit fly pests using food baits. Flies may adjust their foraging behaviour under different conditions of their natural setting: orchards with abundant natural fruit fly foods and orchards with scarce natural fruit

fly foods. Natural conditions in the orchard determines the nutritional state of flies and therefore flies might respond differently to food baits under different natural conditions.

The aim of this study was to determine the effects of food deprivation on attractiveness of natural and artificial food sources to the three mango infesting flies in sub-Saharan Africa: *Ceratitis cosyra* (Walker), *C. fasciventris* (Bezzi) and *C. capitata* (Wiedemann). Chicken faeces and guava juice were evaluated as natural food sources. Two artificial food sources were evaluated: (1) a bait developed from locally available brewery waste and (2) molasses (local sugar factory). The specific objectives of this work were to address the following: (1) effects of different feeding history on attractiveness of immature and mature adult flies to odours from natural and artificial sugar and protein sources, (2) relative food preference (sugar vs protein or natural vs artificial) for flies under different feeding history (2) relative influence of food deprivation, age and mating status in responses of flies to food odours.

6.2 Materials and Methods

6.2.1 Study area

This study was carried out at Mbita Point Field Station of the International Centre of Insect Physiology and Ecology (ICIPE), at Lake Victoria, Western Kenya (altitude of 1240 meters, latitudes between $0°$ 25 S and $0°$ 30 S and longitudes $34°$ 10 E and $34°$ 15 E). Tests were conducted from October 2002 to June 2003 in the afternoon hours from 15 00 hours to 17 00 hours, which were hours of no or very low reproductive activities for all species tested. Field cage conditions were $26.85 \pm 0.14°C$ and $57.00 \pm 0.80\%$ Relative Humidity.

6.2.2 Insect material

Flies were obtained as puparia from colonies of *C. cosyra*, *C. fasciventris* and *C. capitata* maintained at ICIPE headquarters in Nairobi, Kenya. The following categories of flies were used in the tests: (a) immature, 1-2 days old and (b) mature, virgin and mated, 14-17 days old. Flies were kept under laboratory conditions at ambient temperatures ranging from 23 $°C$ to 30 $°C$, relative humidity between 45-57 % and on a photo phase from 0600 to 1900 hours.

the dusk mating species, *C. cosyra* and *C. fasciventris* across the weeks. Mating activities of all species were found to be significantly higher in the second and third week after emergence for all species. Similar observations were made on calling and mating activities of *C. capitata* studied in the wild in Egypt (Hendrichs & Hendrichs, 1990). First week after adult emergence, nearly all or all of the flies were mature and this might therefore explain the increase in sexual activities as from the second week.

Finally, oviposition activities of all species were also found to peak in the second week after emergence. In female tephritids, more particularly in medflies, mating is known to trigger profound physiological changes resulting in a switch in preference by females to host-fruit odours (Jang, 2002). The author also found that females which were naturally mated laid significantly more eggs than virgin females.

2.4.4 Studies on behavioural ecology- influence of set-ups

Herein, results from the three different set-ups were complementary. Plexiglass cages offered a higher protection of flies from predators and extremes in temperature and therefore enabled a higher survival and a longer follow up of their behavioural activities as they aged. However, studies in field cages and greenhouse allowed for a more natural environment for the flies and therefore enabled the distribution of activities over host and non-host trees to be determined.

Moreover, certain aspects of reproductive activities, in particular calling activities of *C. cosyra* which were not observed in field cages and in the greenhouse, could be seen in plexiglass cages. *C. cosyra* males, during calling activities, were very sensitive to any movement and since their calling activities were not as elaborate as for *C. fasciventris* and *C. capitata* males, the calling activities in field cages were often overseen. In the greenhouse, observation of calling and even mating activities of *C. cosyra* proved to be even more difficult since at dusk flies engaged in upward flights, up to the ceiling of the greenhouse where sexual activities were presumed to occur.

Differences in diel patterns of activities as a result of set up were mainly found to occur for feeding activities. Patterns of feeding activities were more consistent in plexiglass cages and field cages than in the greenhouse. In the latter, fluctuations in temperature, relative humidity and light intensity were greater, thereby leading to some differences in feeding patterns for *C. cosyra* and *C. fasciventris* females with distinct peaks during the cooler parts

of the day, either morning hours or evening hours. Patterns of reproductive activities were more or less constant in the three set-ups.

Intensities of activities of the three flies during the day differed between set-ups. Feeding events were more frequently recorded in the field cages. Flies in plexiglass cages had the lowest frequency of feeding compared to flies in field cages and in the greenhouse. The easy accessibility of flies in plexiglass cages to protein rich food such as yeast hydrolysate and concentrated sugar such as laboratory sucrose might have possibly appeased their hunger more efficiently and therefore feeding duration was lower in that set-up compared to duration of feeding in greenhouse and field cages. In the latter environments, flies had to (1) incur costs in searching for food that was more dispersed than in plexiglass cages and (2) encountered less protein and sugar rich food, which thereby increased their overall feeding duration. Comparing field cages and the greenhouse, more feeding events were recorded in the former than in the latter. The relatively smaller environment of the field cages made it possible to observe activities of more flies and therefore more feeding events and might be the reason why higher feeding frequency was observed there.

Callling events by *C. fasciventris* and *C. capitata* males were more frequent in the greenhouse, possibly due to better environmental conditions prevailing and also better canopy area for lek formation compared to field cages where plants were smaller. Frequency of mating was higher for *C. cosyra* and *C. fasciventris* in plexiglass cages. In these cages, it was easier for males to "rape" females since fly density was higher and chances to escape therefore lower. For *C. capitata*, however, mating frequency was higher in field cages. This was possibly an effect of a longer duration of mating in this set-up possibly due to the right host and abiotic environment.

Finally frequency of oviposition was highest in plexiglass cages for *C. fasciventris* and *C. capitata* females. Observations on *N. cyanescens* by Brevault & Quilici (2000) showed that when a group of females were placed in small cylindrical cages (20 cm height and 10 cm diameter) together with the oviposition substrate, frequency of oviposition was higher compared to when placed in big field cages as in this study. The authors suggested that this might due to presence at a short distance of the suitable oviposition substrate and therefore causing the females to lay eggs without previous searching activity.

6.2.3 Food sources evaluated

Food consisted of natural and artificial protein and sugar sources.

Protein sources were in the form of:

(a) chicken faeces from caged chickens– used 1 day after collection and storage at 4^0C (natural source)

(b) ICIPE yeast, which is a modified local brewery by-product (artificial source).

Sugar sources were in the form of:

(c) guava juice (*Psidium guajava*) made from mature fruits collected from trees (natural source)

(d) sugar cane molasses obtained from the Muhoroni sugar factory, Kenya (artificial source).

6.2.4 Experimental set-up

Experiments were conducted in two cylindrical clear-nylon-screened field cages (2 m tall x 3 m diameter). A clear plastic sheet was used to cover the top of each field cage to exclude rain and reduce intensity of sunlight. Four potted non-host banana plants, *Musa* sp, (0.75 cm tall) were placed equidistant around the circumference inside each field cage in order to provide resting sites for fruit flies.

The arena containing food sources to be tested was exposed on a table (68 x 69.5-cm and 48.5-cm high) placed at the center of each cage. In all experiments, all food sources were offered simultaneously in petri dishes of 8.5 cm diameter, arranged in an array with a Latin square design. Petri dishes were covered with a netting material (1 mm mesh size) to prevent access of flies to food sources in order to test only the effect of food odours. A control (an empty petri dish) was included in each test.

During observations, temperature and Relative Humidity in the cages were determined every hour using a hygrothermometer (Cole-Parmer Instrument Company, Chicago, Illinois).

6.2.5 *Effect of food deprivation on attractiveness of natural and artificial, protein and sugar sources*

Responses to food sources were evaluated using male and female, immature and mature, flies on various feeding-starvation regimes (feeding history).

There were four feeding history groups for immature flies. Upon emergence, the latter were fed on one of the following:

(a) water only (deprived of all food)

(b) water and sugar (*ad libitum*)

(c) water and yeast hydrolysate (*ad libitum*)

(d) water, sugar and yeast hydrolysate (*ad libitum*)

There were three feeding history groups for mature, virgin and mated flies. Mature, virgin and mated flies were allowed to feed *ad libitum* on water and one of the following upon emergence:

(e) sugar

(f) sugar and yeast hydrolysate, but deprived of protein hydrolysate during the 2nd week after emergence

(g) sugar and yeast hydrolysate until release.

When presented together to flies in the same cage, sugar and yeast hydrolysate were offered separately, in order to enable flies to self-select their nutrients according to their needs (Waldbauer & Friedman, 1991).

50 flies of mixed sex (1:1), belonging to one of the above age-feeding history group were released in each cage 1 hour before the beginning of each test. There were four replicates for each age-feeding history group tested. In all experiments, the number of flies landing on the different food sources was recorded at 5 minutes intervals. The table containing an array of food sources was rotated clockwise by 90^0 every 30 minutes.

6.2.6 *Data analysis*

Data were analyzed by a parametric analysis of variance using the procedure GENMOD (SAS 2001) which fitted the data to a Poisson regression. The data for the two hour interval were treated and analyzed as repeated measures since flies recorded at one check point of a 5 minute interval, might or might not have been the same flies recorded during the previous interval. A significance level of 0.05 was set for all statistical tests.

6.3 Results

Patterns of responses to food odours differed between species and between males and females. In this respect, results are presented separately for males and females of each species.

6.3.1 Effect of food deprivation on attractiveness of immature adult flies to food odours

For immature adult males and females of all species, sugar feeding attenuated responses to food odours (Fig. 1).

C. cosyra males and females which were deprived of all food (water only) had the highest response to odours from both sugar and protein sources compared to flies fed on the other three diets, protein only, sugar only and sugar and protein (males: χ^2=17.65, df=3, P<0.01; females: χ^2=17.55, df=3, P<0.01) . While for *C. fasciventris*, the highest response to odours from both sugar and protein sources by males and females were from flies deprived of all food except water as well as from those deprived of sugar (protein only) (males: χ^2=32.24, df=3, P<0.01; females: χ^2=22.84, df=3, P<0.01). *C. capitata* males and females fed on protein only since emergence were more attracted to odours from sugar and protein sources compared to flies on a diet of either water only or sugar only or on a full diet of sugar and protein (males: χ^2=26.63, df=3, P<0.01; females: χ^2=23.29, df=3, P<0.01).

6.3.1.1 Food preference

1- 2 day old adult flies of the three species which were either deprived of all food except water or for those which were fed on sugar (either sugar only or sugar and protein), there were no significant differences in attractiveness of flies to odours from different food sources, both sugar and protein.

On the other hand, when immature *C. fasciventris* males and females were fed on only protein only, they were highly attracted to odours from guava, a natural sugar source, followed by chicken faeces, a natural protein source, compared to odours from other food sources (males: χ^2=15.94, df=4, P<0.01; females: χ^2=11.66, df=4, P=0.02). Even though, *C. capitata* immature males and females fed on only protein were attracted more to odours from guava compared to other food odours, the differences were not significant (males:

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χ^2=7.78, df=4, P=0.10; females: χ^2=7.60, df=4, P=0.11). There were also no significant differences in attractiveness towards different food odours for immature *C. cosyra* males and females fed on protein only.
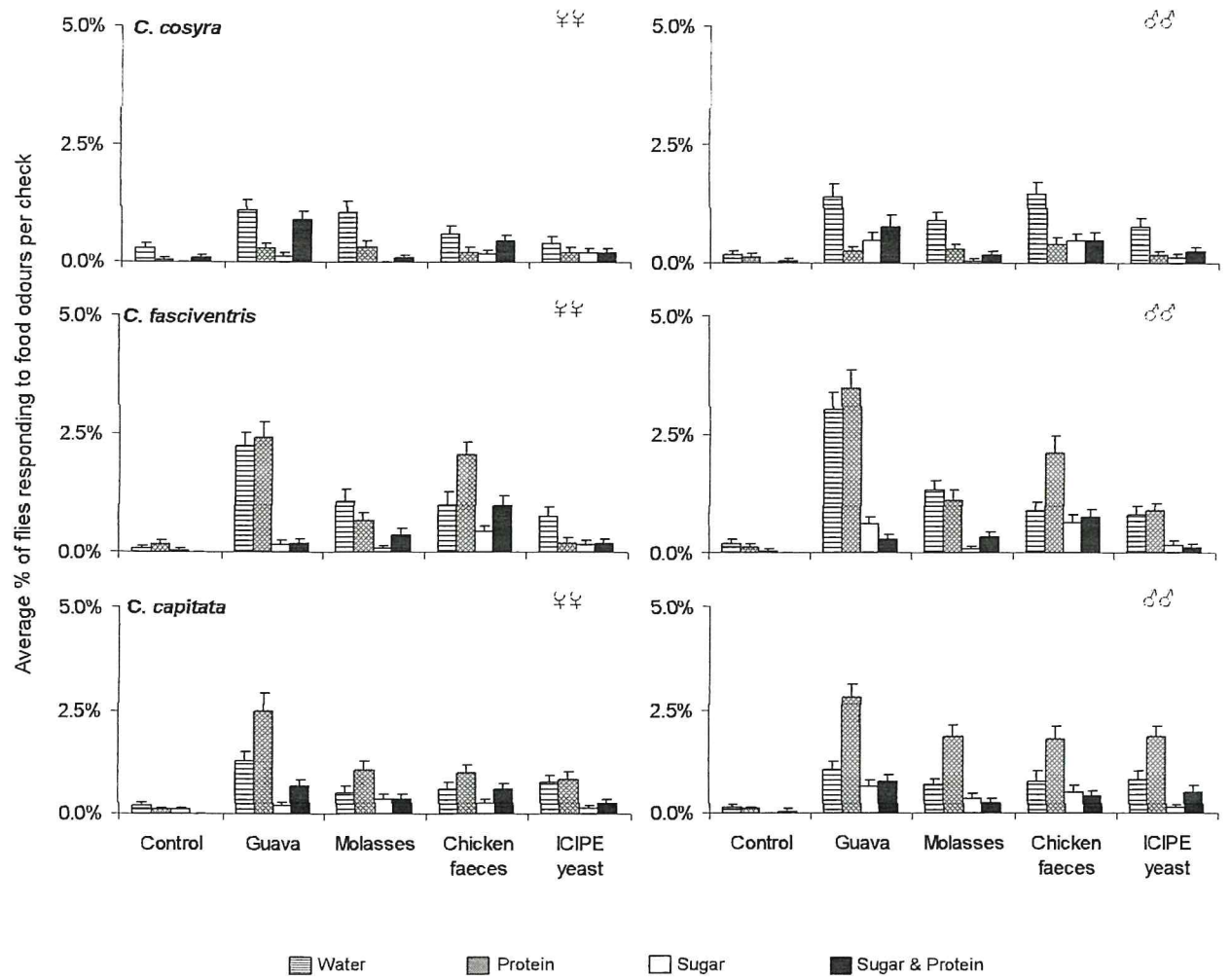
Fig. 1. Attractiveness of natural and artificial, sugar and protein, food sources to immature
(1 – 2 days old) *C. cosyra*, *C. fasciventris* and *C. capitata* flies of different feeding histories
Values represent means ± SE.

6.3.2 Effect of food deprivation on attractiveness of mature, virgin and mated, flies to food odours

Protein deprivation, either partially or completely, was influential in attractiveness of mature males and females of all fly species to food odours.

Mature virgin and mated females of all species which were protein deprived (either partially or completely depending on species and their mating status) were more responsive to odours from both sugar and protein sources compared to females fed on a complete diet of sugar and protein (*C. cosyra*: virgin: $\chi^2=7.56$, df=2, P<0.01; mated: $\chi^2=15.10$, df=2, P<0.01; *C. fasciventris*: virgin: $\chi^2=11.39$, df=2, P<0.01; mated: $\chi^2=6.89$, df=2, P=0.03; *C. capitata*: virgin: $\chi^2=18.48$, df=2, P<0.01; mated: $\chi^2=4.26$, df=2, P=0.04) (Fig. 2).

Mature males of all species, except for virgin *C. cosyra* and *C. fasciventris*, which were protein deprived (either partially or completely) were more responsive to odours from sugar and protein sources compared to males fed on a complete diet of sugar and protein (*C. cosyra*: virgin: $\chi^2=1.06$, df=2, P=0.30; mated: $\chi^2=10.98$, df=2, P<0.01; *C. fasciventris*: virgin: $\chi^2=2.24$, df=2, P=0.33; mated: $\chi^2=23.66$, df=2, P<0.01; *C. capitata*: virgin: $\chi^2=15.37$, df=2, P<0.01; mated: $\chi^2=12.80$, df=2, P<0.01) (Fig. 3).

6.3.2.1 Food preference

Both virgin and mated females of all species which were completely deprived of protein since emergence and fed exclusively on sugar preferred odours from protein sources, in particular from chicken faeces, over odours from sugar sources, though this preference was significant only for virgin *C. capitata* females (virgin *C. capitata* females: $\chi^2=11.34$, df=4, P=0.02, mated and virgin females of *C. cosyra* and *C. fasciventris* & mated *C. capitata*: $\chi^2<10$, df=4, P>0.05).

In addition, both virgin and mated females of *C. capitata* and *C. fasciventris* which were partially deprived of protein were highly attracted to odours from chicken faeces, compared to odours from other food sources, though again this was significant for only virgin *C. capitata* females (virgin *C. capitata* females: $\chi^2=10.23$, df=4, P=0.04, mated and virgin females of *C. fasciventris*: $\chi^2<10$, df=4, and df=4, respectively, P>0.05). On the other hand, there were no significant differences between responses to different food odours by both virgin and mated *C. cosyra* females which were partially deprived of protein.

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When mature virgin and mated females of all three species were fed on a complete diet of protein (with sugar), there were no significant differences in responses of flies to different food sources.

For males (virgin and mated) under different feeding regimes except for virgin *C. capitata* which were completely deprived of protein (fed exclusively on sugar), there were no significant differences in attractiveness of flies to different food odours. Virgin *C. capitata* males, which were completely deprived of protein, were highly attracted to odours from protein sources, both chicken faeces and ICIPE yeast (χ^2=10.53, df=4, P=0.03).

Fig. 2. Attractiveness of natural and artificial, sugar and protein, food sources to virgin and mated mature (14 – 17 days old) *C. cosyra*, *C. fasciventris* and *C. capitata* females of different feeding histories Values represent means ± SE.
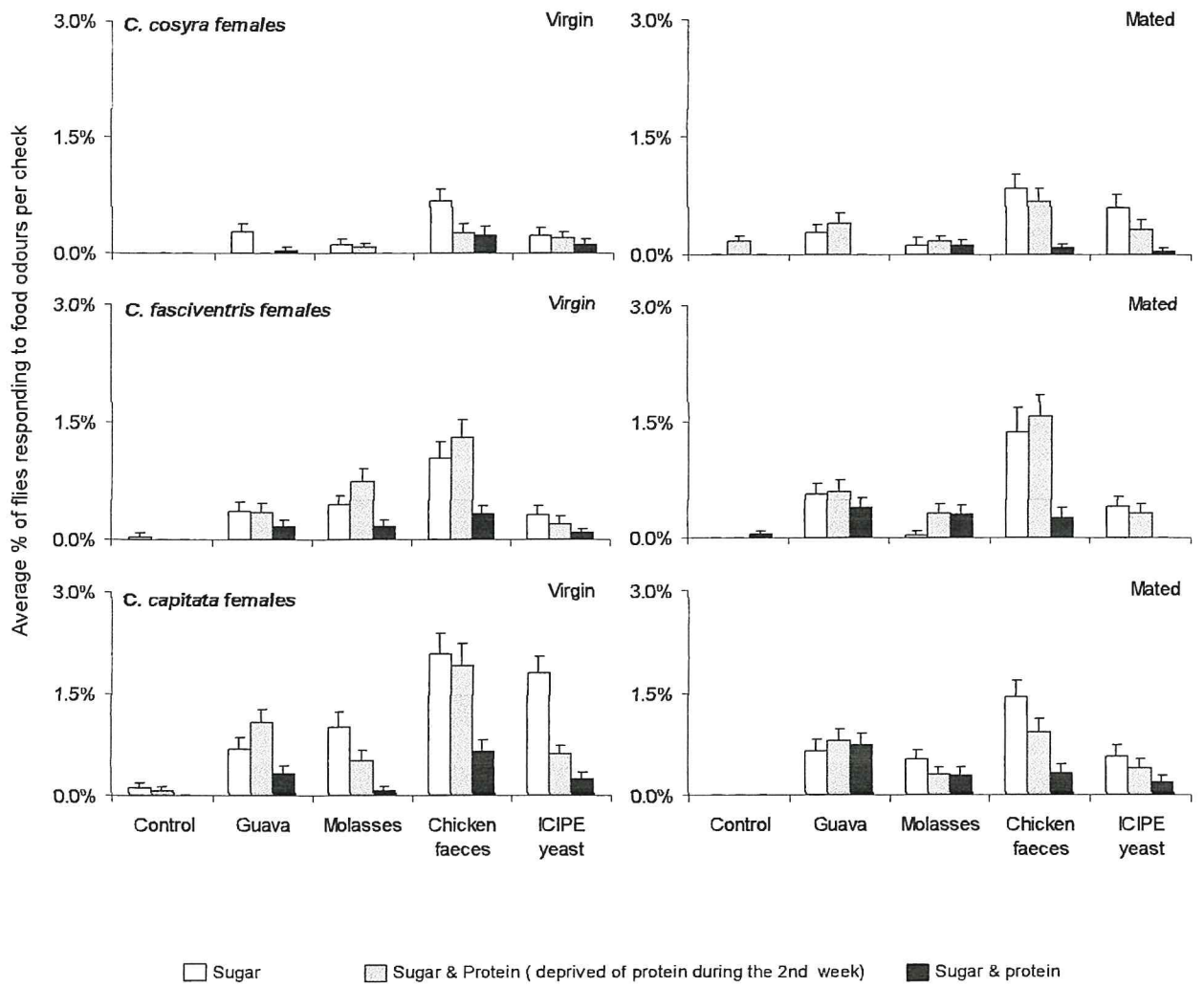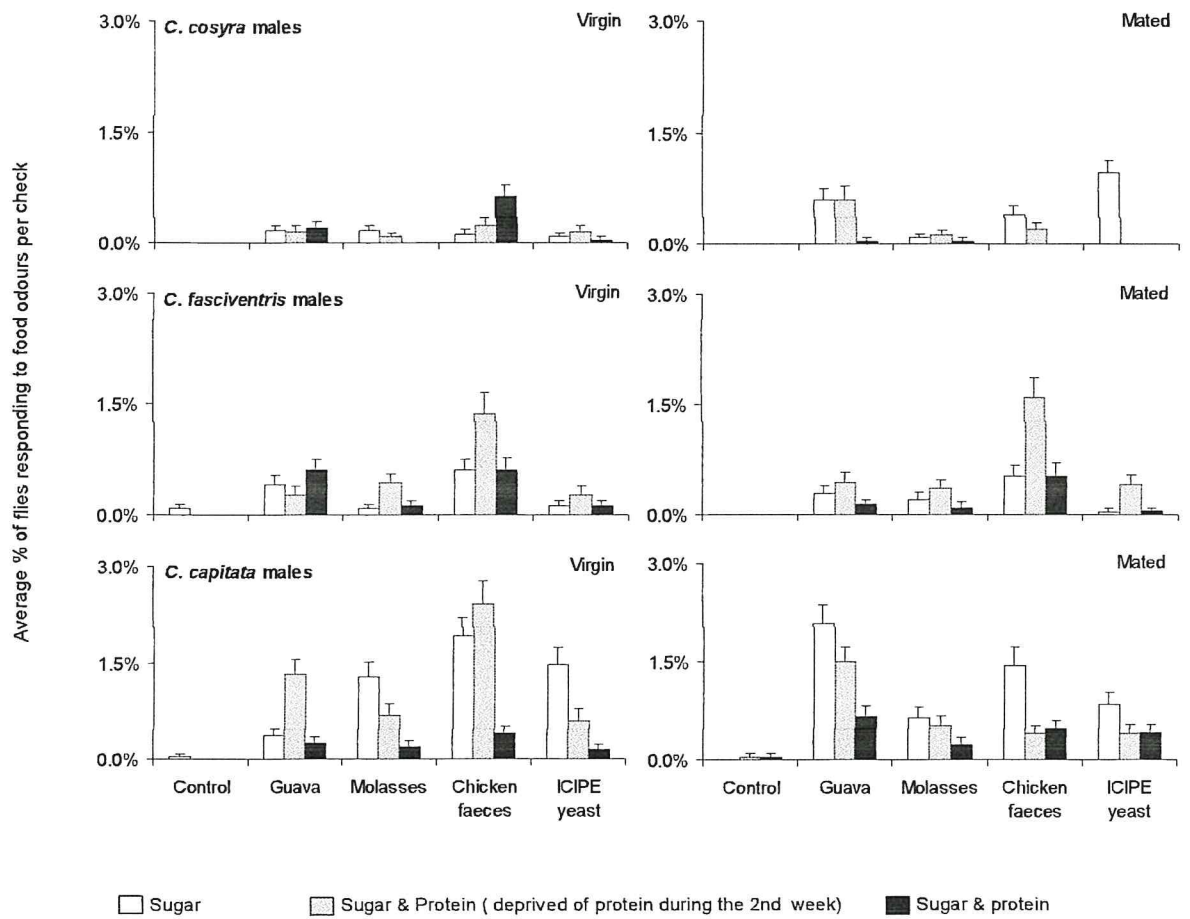
Fig. 3. Attractiveness of natural and artificial, sugar and protein, food sources to virgin and mated mature (14 – 17 days old) *C. cosyra*, *C. fasciventris* and *C. capitata* males of different feeding histories Values represent means ± SE.

6.3.3 Effects of age and protein deprivation on attractiveness of flies to food odours

The two feeding history groups which were considered for an analysis of age-dependent and deprivation-induced effect, in particular protein deprivation, on responsiveness of flies to food odours were virgin flies fed on sugar only (protein deprived) and flies fed on sugar and protein (protein fed) since these groups were tested for both immature and mature flies.

Mature protein deprived females had significantly higher responses to odours from both sugar and protein sources compared to immature protein deprived females (*C. cosyra*: χ^2=5.77, df=1, P=0.02; *C. fasciventris*: χ^2=5.53, df=1, P=0.02; *C. capitata*: χ^2=13.86, df=1, P<0.01) (Fig. 4). When protein fed however, mature *C. cosyra* females had a significantly lower response to food odours, in particular to odours from guava juice, compared to immature flies (*C. cosyra*: χ^2=5.80, df=1, P=0.02). In contrast, there were no significant differences in responses to food odours between protein fed mature and immature females of *C. fasciventris* and *C. capitata* (Fig. 5).

With respect to mature males of *C. cosyra* and *C. fasciventris*, there were no significant differences in attraction to food odours between immature and mature flies which were either protein deprived or protein fed. In contrast, mature protein deprived *C. capitata* males were significantly more attracted to food odours, more so for odours from both protein sources (chicken faeces and ICIPE yeast), compared to immature protein deprived males (χ^2=7.07, df=1, P=0.01). When *C. capitata* males were protein fed, there was no significant difference in attraction to food odours between immature and mature flies.

Fig. 4. Attractiveness of natural and artificial, sugar and protein, food sources to immature (1 − 2 days old) and virgin mature (14 − 17 days old) males and females of *C. cosyra*, *C. fasciventris* and *C. capitata* males which were completely deprived of protein and fed on sugar only since emergence. Values represent means ± SE.
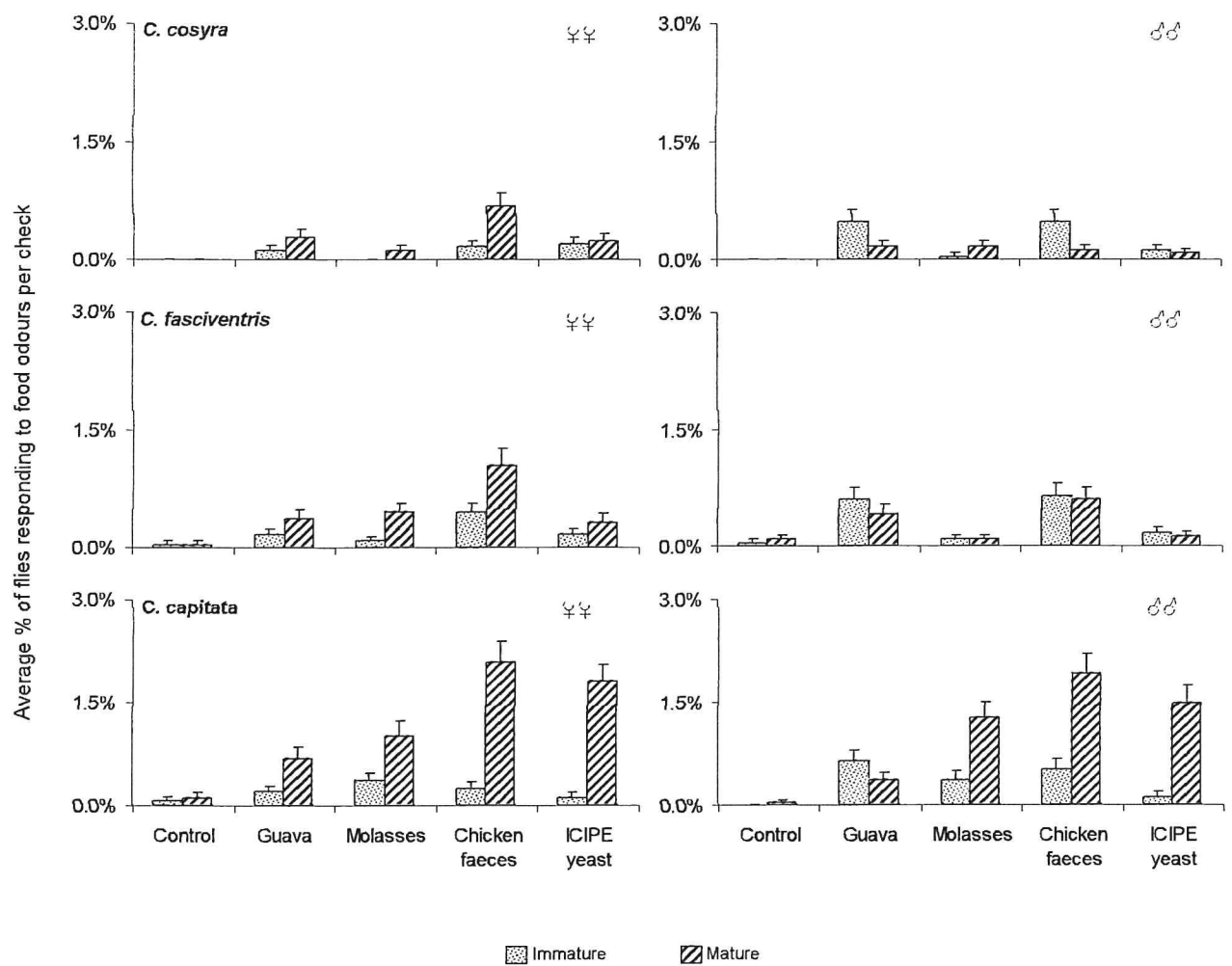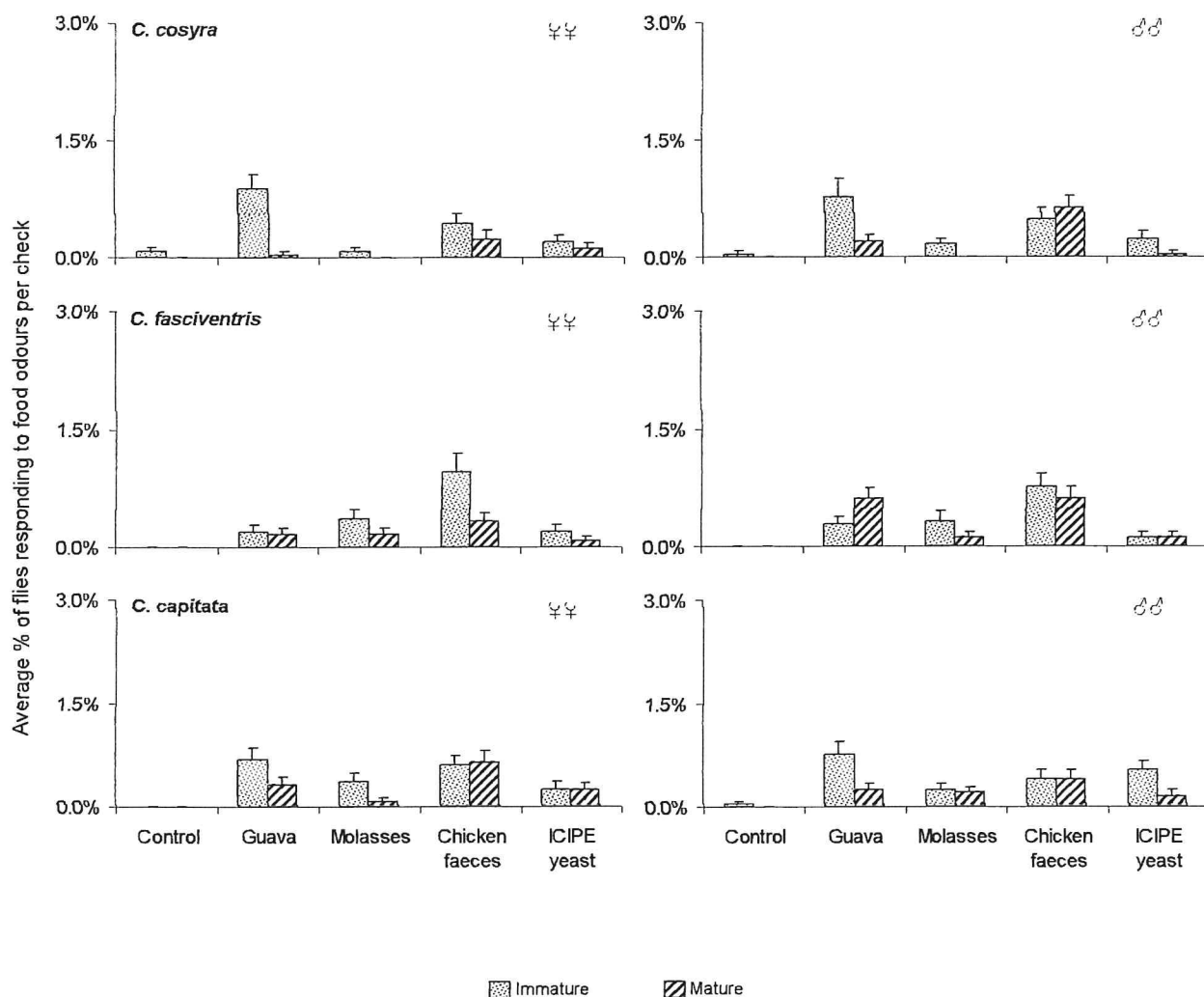
Fig. 5. Attractiveness of natural and artificial, sugar and protein, food sources to immature (1 – 2 days old) and virgin mature (14 – 17 days old) males and females of *C. cosyra*, *C. fasciventris* and *C. capitata* males which were fed on a complete diet of sugar and protein since emergence. Values represent means ± SE.

6.3.4 Effects of mating status and protein deprivation on attractiveness of flies to food odours

Three feeding history groups of mature males and females, both virgin and mated, of each species were tested for their responses to food odours. These were flies either completely deprived of protein since adult emergence (sugar only throughout adult life) or partially deprived of protein, notably in their second week of adult life (sugar throughout adult life) or fed on a complete diet of sugar and protein since emergence.

There were no significant differences in attractiveness to food odours between virgin and mated mature males and females of the three species when flies were fed on a complete diet of sugar and protein.

When deprived of protein partially, there was however a significant difference between virgin and mated mature females of *C. cosyra* whereby *C. cosyra* virgin females had a lower response to odours from guava and protein sources, compared to mated females (χ^2=4.05, df=1, P=0.04). While for *C. capitata*, when females were deprived completely of protein since adult emergence, virgin mature females had a significantly higher response to food odours, in particular to odours from protein sources (both chicken faeces and ICIPE yeast), compared to mated females (χ^2=4.10, df=1, P=0.04). There were no significant differences between mating states of *C. fasciventris* females and males of the three species in their responses to food odours either when completely deprived of protein or when partially deprived of protein.

6.4 Discussion

The questions addressed in this study were as follows: (1) Does deprivation of a nutrient influence readiness of a fruit fly to engage in food searching behaviour? (2) Does deprivation of a nutrient affect food preference for adult flies? (3) What are the influences of nutritional state, age and mating status on food searching behaviour of flies?

6.4.1 Food searching behaviour, a result of food deprivation

This study provided clear evidence that when immature flies (males and females) of all species were fed on sugar, their propensity of search for food odours decreased. A sugar source therefore seems to fulfill most of the nutritional requirements for immature flies and the latter are not motivated to look for other food sources when they are satiated with sugar compared to when they are sugar deprived. However, newly emerged flies seem to not only seek sugar but also protein. In this study, immatures of all fly species either deprived of all food (except water) or sugar fed (sugar only and sugar plus protein), were found to be attracted to odours from both sugar and protein sources. Similar findings were obtained by Cohen & Voet (2002) who found that immature *C. capitata* females exposed to low carbohydrate diet (2% sucrose) showed no preference between carbohydrate and proteinaceous sources. Protein is not mandatory for fly survival but flies require protein for normal reproductive development (Tsitsipis, 1989). The search for sugar remain of a higher priority as found here in this study for immature flies of *C. fasciventris* and *C. capitata* which when fed on protein only were more attracted to odours from guava juice, a natural sugar source compared to odours from protein sources. Robacker (1991) found that the newly emerged Mexican fruit flies, *A. ludens* (Loew), (up to 4 days old) fed preferentially on sugar over protein.

Protein deprivation was found here to increase the attractiveness of mature males and females of all species, to food odours, in particular to odours from a natural protein source, chicken faeces. Virgin and mated mature females of all species which were completely deprived of protein since emergence, were found to prefer odours from protein sources over odours from sugar sources. The preference of protein over sugar for protein deprived

mature females is consistent with previous studies suggesting that protein is an essential element in the diet of mature flies (Robacker, 1991; Robacker & Moreno, 1995; Cohen & Voet, 2002; Barry et al., 2003). Webster & Stoffolano (1978) found that a protein free diet for adult apple maggot fly, *Rhagoletis pomonella* (Walsh), led to reduced ovarian and accessory gland development in females compared to when they were fed with protein. Females of several tephritids were found to require a diet containing protein in order to realize peak egg laying (Webster et al., 1979; Hendrichs et al., 1991; Hendrichs et al., 1993; Nigg et al., 1995; McQuate et al., 2003). In results presented in Chapter 3 in this thesis, both *C. fasciventris* and *C. capitata* were found to realize the highest fecundity rates when fed on a protein rich diet compared to a protein poor diet. In addition, diet was found to significantly influence egg load (number of mature oocytes in the ovaries) in *A. ludens* and *A. obliqua* Macquart with a higher egg load for females fed sugar and protein than for females fed sugar only (Aluja et al., 2001). Interestingly in this study, for virgin and mated *C. cosyra* mature females which were fed on protein only partially, there was no difference in attractiveness between odours from sugar and protein sources while for partially deprived *C. fasciventris* and *C. capitata* protein odours were still highly preferred by females over sugar odours. In findings presented in Chapter 4, it was clearly shown that *C. cosyra* females and males had a lower overall protein intake compared to females and males of *C. fasciventris* and *C. capitata*. This was discussed as to their probable inherent lower fecundity potential due to their life history characteristic being more of an oligophagous nature compared to the highly polyphagous *C. fasciventris* and *C. capitata*.

In general, in this study, protein deprived mature males of both mating states, except for virgin *C. capitata* males, did not show any food odour preference. Virgin mature protein deprived *C. capitata* males preferred odours from protein sources over odours from sugar sources. Protein in the diet of *C. capitata* males has been found in previous studies to enhance their sexual behaviour (Blay & Yuval, 1997; Papadopoulos et al., 1998; Taylor & Yuval, 1999; Kaspi et al., 2000; Kaspi & Yuval, 2000; Shelly & Kennelly, 2002; Shelly et al., 2002). *C. capitata* males were found to be more likely to call and to copulate when fed on a diet of protein compared to when fed on a protein free diet.

Findings in this study seem to indicate that odours from natural food sources, guava juice and chicken faeces, appeal more to sugar deprived and protein deprived flies respectively than do odours from artificial sugar and protein sources. Similarly, Robacker (1991) found that torula yeast, a commonly used proteinaceous bait for various Tephrititae, was a poor

attractant to protein-hungry Carribean fruit flies compared to bacterial filtrate, from a bacterium closely matching the bacterium isolated in Carribean fruit flies and possibly one of its natural food sources. Odours from natural food sources were previously found (Chapter 5) to be more attractive to flies of *C. cosyra*, *C. fasciventris* and *C. capitata*. Studies by Prokopy et al. (1993), Prokopy et al. (1992) and Pinero et al. (2003) also confirm a high attractiveness of natural food sources to *C. capitata* and *Anastrespha* spp, sometimes being even more attractive to them than commercial proteinaceous food baits used in control and monitoring fruit fly programmes.

6.4.2 Age, mating status and food deprivation

This study indicated that the nutritional state of a fly was more important than age and mating status in influencing its food searching behaviour.

A higher preference for odours from both sugar and protein sources occurred among exclusively sugar-fed females of all species as they aged. Whilst when females were fed on a complete diet of sugar and protein, there was no significant difference in attraction to odours from different food sources between immature and mature females. Similarly, Prokopy et al. (1996) found protein deprivation to be more important than fly age on degree of attraction of *C. capitata* flies to sources of protein. Robacker & Garcia (1993) also found increasing attraction to a protein feeding attractant (bacterial odour) by Mexican fruit flies exclusively fed on sugar.

Effects of mating status on attraction to semiochemicals have mostly been looked at for pheromones and host fruit odours (Cornelius et al., 2000; Jang, 2002). One of the rare reports on effects of mating status on attraction to food sources was by Heath et al. (1995) in a field evaluation in Guatemala, where they found that McPhail traps baited with proteinaceous baits captured a greater percentage of mated female medflies than unmated. From results presented in Chapter 5 on responses of mature flies of *C. cosyra*, *C. fasciventris* and *C. capitata*, mating status was not found to be influential in the attractiveness to odours from food sources. In that experiment, the flies were fed on a diet of sugar and protein throughout adult life and were deprived of food completely only for 14 hours before the tests.

In this study, also, when female and male flies of all species were fed on a complete diet of protein (with sugar), virgin flies responded with a similar frequency to food odours as mated flies. However, when deprived of protein, differences in responses to food odours

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were noted particularly for *C. cosyra* and *C. capitata* females. Mature virgin protein-deprived *C. cosyra* females had a lower response to odours from protein sources compared to their mated counterparts. While for *C. capitata* flies deprived of protein, virgin mature females had a higher response to odours from protein sources compared to mated females. It is highly likely that the higher need for protein by protein deprived mated *C. cosyra* females as compared to their virgin counterparts might occur as a result of higher egg laying by mated females compared to virgin females (Jang, 2002). *C. cosyra* females, as per the results presented in Chapter 4, have been found not to lay any eggs (up until 20 days of age) in the absence of males in cage. The reverse observation for *C. capitata* females might be due to experimental protocol. Since tests were conducted between 15 00 and 17 00 hours which usually marks the end of the mating time for *C. capitata*, protein deprived *C. capitata* males and females might have still been in copula and therefore not attracted to food odours. Copulation duration in other studies have been found to be higher for protein-deprived flies compared to protein-fed flies (Taylor & Yuval, 1999).

6.4.3 Practical implications of findings

It can be concluded from findings in this study that (1) nutritional state of a fly specific to the age category of the fly influences its readiness to engage in food searching behaviour, (2) nutritional state of immature flies did not influence food odour preference (sugar versus protein) while nutritional state of mature flies had a high influence in food odour preference, (3) natural sugar and protein sources were preferred over artificial sugar and protein sources for food deprived flies and (4) nutritional state of a fly was more important than age and mating status in influencing food searching behaviour.

These findings can have important practical implications on strategies for control of fruit fly pests using food baits. Strategies for fruit fly control could be adjusted in relation to the conditions in the natural setting, in other words in relation to the abundance of natural food for fruit flies in orchards (bird's droppings, honeydew secretions, fruit juices from fallen and rotting fruits or fruits on trees pecked by birds or bats or monkeys). In orchards where natural food sources for flies are abundant, the proportion of food deprived flies might be low and since food deprived flies might be more attracted to natural food sources compared to artificial food sources, the resulting proportion of flies responsive to baiting stations might be low. To improve response to baiting stations in an environment with abundant

food sources for flies, baiting stations should be combined with orchard sanitation or if sanitation is not practiced, then the number of baiting stations should be increased.

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7. Field evaluation of attractiveness of natural and artificial food sources to three African fruit flies: *Ceratitis cosyra, C. fasciventris* and *C. capitata* (Diptera: Tephritidae).

7.1 Introduction

Artificial food baits have been developed and used in combination with toxicants in order to suppress or eradicate populations of fruit fly pest species in several countries (Roessler, 1989). Food baits used in fruit fly control are usually made from hydrolysed proteins derived from industrial waste materials such as brewery yeast or corn syrup. However, food baits applied in fruit growing areas are often in competition with natural food sources for fruit fly pests which occur in these areas such as bird's droppings, fruit juices and honeydew. As found in the previous chapters, for the mango infesting flies of this region, odours from natural sources were more attractive than odours from artificial food sources that have been used for monitoring mainly and some control trials. Field cage studies by Prokopy et al. (1992) and Prokopy et al. (1993) have shown indeed that bird's droppings were more attractive than a widely used protein attractant PIB-7 (Staley's Protein bait 7) when tested in Hawaii with the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann). Studies in the field by Pinero et al. (2003) investigating on fruit fly attractants for resource-poor fruit growers, have shown that chicken droppings and human urine were attractive to *Anastrepha* spp., though less compared to commercially available protein baits such as hydrolyzed protein and torula yeast.

The aim of this field study was to determine relative attractiveness of wild *C. cosyra, C. fasciventris* and *C. capitata* flies, in their natural habitats, to natural and artificial, sugar and protein baits. The physiological condition of females responding to the different baits was also investigated. As such, results from this study were compared to results obtained in the previous field cage experiments using laboratory reared flies to evaluate responses to the same food sources.

7.2 Materials and Methods

7.2.1 Study area

Experiments were conducted in two unsprayed commercial plantations of coffee and mango in May 2003 and in June 2003 respectively. The size of each plantation was about 20 ha. The coffee plantation was located in Nairobi, Kenya, whereby fruit fly populations consisted mainly of *C. capitata* and *C. fasciventris*. The mango plantation was located in Muhaka, Coast Province, Kenya, whereby fruit fly populations consisted of *C. cosyra*. Mean daily temperature in Nairobi in the month of May ranged between 13.2 and 22.6°C. Mean daily temperature in Muhaka in the month of June ranged between 21.3 to 28.4°C.

7.2.2 Food baits

Food baits consisted of (1) chicken faeces from caged chickens, (2) ICIPE yeast, which is a modified local brewery by-product, (3) guava juice (*Psidium guajava* L.) made from mature fruits collected from trees, (4) sugar cane molasses obtained from the Muhoroni sugar factory, Kenya and (5) Nu-Lure® Insect Bait, liquid protein hydrolysate which is a standard bait often used in fruit fly monitoring and/or control programmes. All food baits were made into a 10% solution, diluted with water and were filled in McPhail traps, which are standard traps used in fruit fly monitoring programmes (White & Elson-Harris, 1994). In the McPhail traps, reservoirs were filled with 200 ml of food solution.
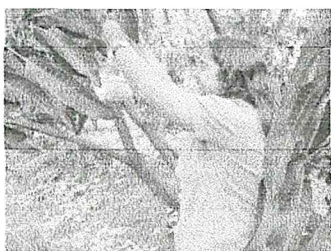
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7.2.3 Field evaluation

Fig. 1. Placement of McPhail trap on mango tree in plantation in Mombasa (June 2003). A Manrakhan, ICIPE.

The responses of wild *C. cosyra*, *C. fasciventris* and *C. capitata* flies to McPhail traps baited with different food baits were evaluated. Experiments were conducted in 5 blocks in each of the two plantations where trees contained a mixture of unripe and ripe fruits. A block consisted of five traps, each with one type of food solution. In each block, traps were placed at random and a distance of 20 m was kept in between traps. Traps were hung from branches of host trees, 1.5-2 m above ground (1 trap per tree) (Fig. 1). This experiment was replicated during 7 days, and traps were rotated daily within each block. Traps were emptied and flies were removed and collected in 70% alcohol. Traps were refilled daily with a fresh food solution of the same type that was in the trap before. Flies captured in each trap were identified, recorded and classified into species and sex. Females of *C. cosyra*, *C. fasciventris* and *C. capitata* were dissected to determine their physiological state. Dissections of females determined egg loads of females at the time of capture.

7.2.4 Data analyses

Fruit fly capture data for each field experiment were subjected to non parametric analysis of variance (SAS, 2001) since the data did not follow a normal distribution. Means were separated by first ranking the number of captures for a particular group of variables using the procedure Proc Rank in (SAS, 2001), then by Tukey's HSD test following Proc GLM based on the ranks of captures. For each species and bait, the number of females containing no mature eggs versus females containing mature eggs was compared using a nonparametric Wilcoxon rank sum test. For females containing mature eggs, the egg load between females captured in different baits were compared using non-parametric Kruskal-Wallis test.

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7.3 Results

7.3.1 Adult captures

For each species, captures of flies over the 7 days of trapping were pooled together for each bait type since there was no significant effect of day in capture of females and males of the three species.

Number of *C. cosyra* captured (9 in total) within a week from all food bait traps in the mango plantation was significantly less than the number of *C. fasciventris* (17 in total) and *C. capitata* (313 in total) captured in the same amount of time in the same traps in the coffee plantation (Fig.2). For *C. cosyra* and *C. capitata*, significantly more females were captured than males in traps containing food baits (*C. cosyra*: χ^2=4.60, df=1, P=0.03; *C. capitata*: χ^2=8.93, df=1, P<0.01).There was no significant difference between captures of males and females in traps containing food baits for *C. fasciventris*.

Traps baited with natural protein bait, chicken faeces, captured more *C. cosyra* females than traps containing other food baits (χ^2=12.79, df=4, P=0.01) (Fig. 2). The only *C. cosyra* male captured during one week of trapping was found in a trap containing chicken faeces. There were no significant differences in captures of males and females of *C. fasciventris* between traps containing different types of food baits. Significantly more *C. capitata* females were captured in traps containing the local sugar bait, molasses, compared to traps containing other baits though catches were also high in traps baited with natural protein and sugar baits, chicken faeces and guava juice, and commercial protein bait, Nu-Lure (χ^2=10.76, df=4, P=0.03) (Fig. 2). There were no significant differences in captures of males of *C. capitata* between traps containing different types of food baits.
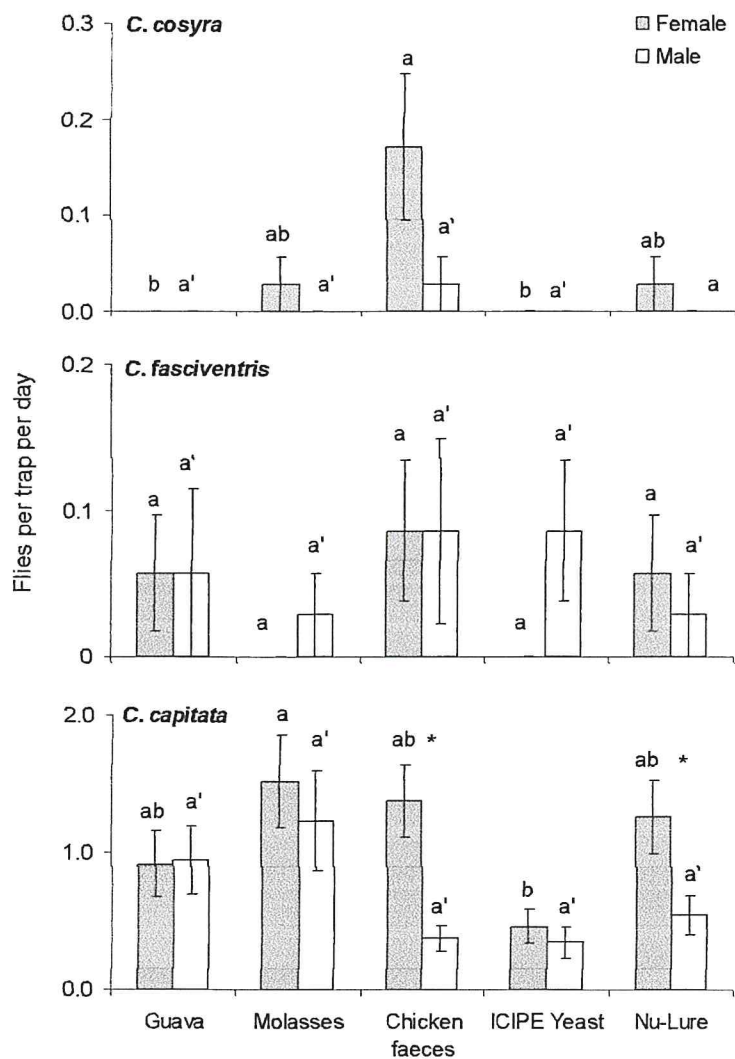
Fig. 2. Comparison between the average number (± SE) (n=35) of *C. cosyra*, *C. fasciventris* and *C. capitata* per trap per day captured in McPhail traps baited with natural and artificial sugar and protein sources. *C. cosyra* were captured in mango plantations in Muhaka, Coast Province, Kenya. *C. fasciventris* and *C. capitata* were captured in coffee plantations in Nairobi, Kenya. Statistical analysis was performed separately for females and males. Bars of the same colour headed by the same letter are not significantly different (Tukey mean separation test, P=0.05). An asterisk indicates a significant difference in the number of females versus males captured for the food source in particular (Wilcoxon 2-sample test).

7.3.2 Sexual maturity stage of captured females

The proportion of females, according to the presence or absence of mature eggs and therefore their physiological states, caught by traps containing different baits is shown in Table 1. There were no significant differences in physiological states of female flies captured by different baits for the three species. There were also no significant differences in number of mature eggs per female for those females of the three species containing mature eggs which were captured from traps with different baits.

Table 1. Number of *C. cosyra*, *C. fasciventris* and *C. capitata* females captured by McPhail traps baited with guava juice, molasses, chicken faeces, ICIPE yeast and Nu-Lure according to the sexual maturity stage; Average number of mature eggs per female (egg load), for females containing mature eggs with respect to the food bait.

Food bait	n	% females with no mature eggs	% females with mature eggs	Average egg load for females with mature eggs
C. cosyra				
Guava	0	-	-	-
Molasses	1	0.00	100.00	28.00
Chicken faeces	6	16.67	83.33	29.20 ± 1.26
ICIPE yeast	0	-	-	-
Nu-Lure	1	0.00	100.00	38.00
C. fasciventris				
Guava	2	0.00	100.00	21.50 ± 1.5
Molasses	0	-	-	-
Chicken faeces	2	0.00	100.00	29.50 ± 8.5
ICIPE yeast	1	0.00	100.00	20.00
Nu-Lure	3	33.33	66.67	36.00 ± 8
C. capitata				
Guava	43	51.16	48.84	17.62 ± 1.97
Molasses	63	39.68	60.32	17.00 ± 1.44
Chicken faeces	53	50.94	49.06	18.15 ± 1.87
ICIPE yeast	15	66.67	33.33	13.00 ± 3.16
Nu-Lure	54	37.03	62.97	18.26 ± 1.69

7.4 Discussion

Responses to the different types of baits varied between species. For *C. cosyra*, the natural protein bait in the form of chicken droppings was found to be 6 times more attractive than the only two other food baits which had captures, molasses and Nu-Lure There were no captures in the natural sugar bait, guava juice and the artificial protein bait, ICIPE yeast. The low captures of *C. cosyra* in the mango plantation were due to the outbreak of a new invasive mango fruit fly pest species recently in Kenya (Lux et al., 2003). During trapping in this field study, 97% of fly captures consisted of the invasive *Bactrocera* spp which is already an indication of competition with *C. cosyra*, the indigenous mango fly. For *C. fasciventris* and *C. capitata*, on the other hand, high numbers of flies were captured. For both species, natural sugar and protein baits were as attractive as artificial sugar and protein baits. In field cage studies on the laboratory reared flies of the same three species as those tested in the field, odours from natural food sources were found to be more attractive to all fly species as odours from artificial food sources. Pinero et al. (2003), found bird's droppings to be less attractive to *Anastrepha* flies compared to commercial protein bait, torula yeast and suggested that the pH of the bird's droppings could have played a role in lowering the attractancy, pH of bird's droppings being lower than that of the torula yeast tested. As per the results obtained in this study, it can be argued that food baits when used in control of the three mango-infesting flies, *C. cosyra*, *C. fasciventris* and *C. capitata* in fruit growing areas can compete with natural food sources of these flies present in these areas.

All food baits attracted larger numbers of *C. cosyra* and *C. capitata* females whilst for *C. fasciventris*, there were no significant difference between female and male captures in the different food baits tested. This finding for *C. cosyra* and *C. capitata* contrasted with the finding from a previous field cage study evaluating responses of the same fly species, laboratory reared, to odours emanating from the same food sources (Manrakhan, unpublished). In the latter study, there were no significant differences between females and males in their responses to food odours. Since in the field, the proportion of males and females were not determined, this leaves the possibility open to the fact that more females than males were present at the moment the study was conducted. The settings used were

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fruit plantations during the end of their fruiting seasons (both mango and coffee) and these might have influenced the abundance of females in the field since ripe fruits might have provided feeding as well as oviposition cues to them.

Herein, for all fly species, there were no significant differences in numbers of immature and mature females trapped in the different food baits. Similarly studies by both Vargas et al. (1997) and Cornelius et al. (2000) have shown that protein and fruit odours attracted both immature and mature flies in the field. Moreover, results from previous field cage studies have shown that odours particularly from protein sources were equally attractive to both immature and mature *C. fasciventris* and *C. capitata* flies. On the other hand, in the same latter studies, for *C. cosyra*, mature flies responded more to food odours, either from fruit or sugar or protein, compared to immature flies. Response of flies to food baits is not only a function of age but also depends largely on the feeding history of the flies as seen in the previous chapter. Irrespective of fly age, flies on sub-optimal diets with respect to either sugar or protein are more likely to search for sources of sugar and protein respectively. In the field, feeding history of flies depends largely on abundance of natural sugar and protein sources which might be either in the form of honeydew, bird's droppings or fruit juices.

In conclusion, the findings herein in particular on the wild pest fruit flies, suggest that control of *C. cosyra*, *C. fasciventris* and *C. capitata* using food baits should be adjusted both spatially (density of application) and temporally (frequency of application) according to estimates, composition and abundance of natural foods for the flies in the infested regions mainly because of (1) likely competition of artificial food baits with odours from natural foods and (2) influence of natural foods on feeding history of flies in orchards.

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8. General discussion

8.1 Model of fruit fly feeding

Based on the findings herein, an attempt can be made to construct the flow of events leading to a feeding event of a fruit fly. The physiological bases regulating this flow are discussed. A summary is presented in Fig.1 based on a feeding model of the blowfly, *Phormia Regina* (Stoffolano, 1995).

8.1.1 Food foraging behaviour- Habitat, patch and food items

For most animals, eating begins with search, a restless kind of locomotory behaviour that tends to increase the probability of an individual's encountering food (Dethier, 1976).

The three fruit fly species studied here confined their search for food mostly within host trees. Hassell & Southwood (1978) proposed a model of an insect food foraging behaviour which occurred at three different hierarchical levels: the habitat, the patch and the food item. The food items in the case of the fruit flies studied here constituted of sugar and protein sources occurring in nature as honeydew, fruit juices and bird's droppings. The patch is an aggregation of food items. In this case the patch is the host tree where upper leaf surfaces contain honeydew and bird's droppings and where fruits are also present on the tree during fruiting season. The habitat is a collection of the patches, possibly a fruit growing area that might contain both host and non-host trees. Following Hassel and Southwood's model, one must therefore ask whether the fly first needs to distinguish between different types of trees (e.g host and non host trees) and then searches for food or does it randomly search for aggregation of food items (be it on host or non host trees) and then decides to increase its residence time in that patch which is profitable for feeding and profitable for other resources (such as finding mates or oviposition substrates or shelter).

Bernays & Chapman (1994) described the general process of host-selection of insects as including first an odour-induced attraction followed by visual attraction which might be due to the shape, size and colour. In a study conducted by Light et al. (1988) on responses of medfly to a spectrum of plant volatiles, they found that flies were most responsive to the general leaf volatiles that is likely to emanate from most plants (hosts and non hosts).

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Response to these odours therefore leads to simply a general attraction to vegetation. Production of these volatiles is the result of oxidative degradation of life lipids (Bernays & Chapman, 1994).

In distinguishing between host and non host trees, therefore, more specific host odours are likely to be involved. The findings herein have shown that flies of *C. cosyra*, *C. fasciventris* and *C. capitata* of different age groups, both males and females, were highly attracted to odours from fruit juices. Indeed, several studies conducted on other fruit flies have also demonstrated that flies are highly attracted to host fruit odour (Robacker, 1990; Aluja & Prokopy, 1992; Nigg et al., 1994), not only emanating from the pulp (Vargas et al., 1997; Cornelius et al., 2000) but also; as in the case of male medflies, to odours from the peel (Katsoyannos et al., 1996). Moreover, in recent studies by Shelly & Villalobos (2002), it was found that male medflies were strongly attracted to certain parts on the bark of the main trunk or branches of guava trees that eventually give them a mating advantage. Aluja & Prokopy (1993) found significant host odour and visual stimulus interaction during intra tree host finding behaviour of apple maggot flies. Although this study was mainly looking at host finding in relation to oviposition behaviour of females, it can be speculated that increase in residency of flies in host trees due to successful fruit finding (fruit odour and visual stimulus from fruit) somehow determines the fly's feeding distribution. This will be more so if feeding also occurs on the fruit. Moreover, Dalby-Ball & Meats (2000) found that female *B. tryoni* flies responded to higher fruit abundance by visiting more leaves and hence tended to scan leaves more thoroughly which consequently increases their chance of encountering food items. Another item that also plays a role in patch selection by fruit flies is foliage both in terms of type and density (Aluja & Birke, 1993; Prokopy & Papaj, 1986). And further, for the fruit flies studied here, it was found that host trees were not only feeding sites but also sites for the search for mates and shelter. Utilization of the same patch for obtaining other resources, I believe, might also play a role in confining feeding activities of the flies to host trees, the same way as either apple maggot flies and medflies were found to either increase their frequency of visits to fruits in close proximity to food sources (Averill & Prokopy, 1993; Prokopy et al., 1996).

The search for food by fruit flies could also be a random process as opposed to finding the host and thereafter finding food. The fly, through sampling various patches eventually selects the patch that it finds more profitable in terms of quality and quantity of food (Bell, 1990). In that case, it is likely to happen both in host and non host trees, such as found for

medfly females foraging for fruits on host and certain non-host plants in a like fashion (Prokopy et al., 1986). Upper leaf surfaces of trees containing bird's faeces might attract a nearby fly to that tree. As found here, flies were highly attracted to odours from bird's droppings. The odour emanating from bird's droppings on the leaf surfaces might evoke local search or success-motivated search which can restrict the insect to this particular patch (Bell, 1990). Similarly with honeydew, though it was found here that flies of the three species were not particularly attracted to odours from honeydew but liked to feed on it, contact with it might elicit a positive phagostimulatory response from the fly which eventually will restrict its search to that profitable patch.

In so far as the distribution of feeding activities of a fly is concerned, it is likely to be the result of both, preference for host trees and random sampling. As found here, there is definitely a preference to stay and forage for resources including food in host trees compared to non host trees. But there was also a preference for feeding on certain host trees compared to others, this tends to indicate that patches varied in quality and quantity on different types of tree which thereby led to the sampling process before selection.

8.1.2 Food discrimination and preference

The initial discrimination of potential food items by a fly is largely said to be achieved through olfaction (Dethier, 1976). Results from this study demonstrated that odours from sugar and protein sources, such as fruit juices and chicken faeces were highly attractive to males and females of the three fruit fly species. Olfactory receptors, located on the antennae and on the palps of fruit flies are sensitive to the gaseous products from amino acid breakdown which is mainly ammonia but are also thought to include other products (Galun et al., 1985; Rice, 1989; Tsiropoulos, 1992).

Though odours from sugar and protein sources were highly attractive to flies, eventually flies of all ages preferred feeding on sugar-based sources compared to protein-based sources. A hierarchy of decision making occurs once the fly is in contact with the food source. If stimulation from the peripheral tarsal or labellar chemoreceptors is positive, proboscis extension takes place. If it is negative, the fly takes off searching for another food source. If now, after contact of the proboscis receptors with the substrate, a positive stimulation entails, the labellar lobes spread allowing contact of their chemoreceptors with the substrate. If then in turn, the input is positive, the pharyngeal pump starts sucking, while food quality is monitored by special chemoreceptor sensilla (Tsiropoulos, 1992). In this

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study, it therefore seems that the right stimulation provided from chemoreceptors came mostly from sugars (fruit juices, honeydew) and relatively less from protein based sources (bird's faeces). Galun et al. (1985) found that addition of sugar to protein sources increased the number of *C. capitata* flies aggregating on these sources. Dethier (1976) argues that whether or not a food will be ingested or which of several will be preferred depends on stimulating effectiveness of that food and the nutritional state of the fly. For all three fruit fly species in this study, measurements of food consumed have indeed shown that sugars are ingested in higher amounts compared to protein. A male/female fruit fly (any of the three species) was found to consume approximately 1 mg of sucrose per day. As for yeast (protein), it was found to vary between species with a lowest consumption for *C. cosyra* males and females (approximately 0.04 mg/fly/day and 0.01 mg/fly/day, respectively) and highest for *C. fasciventris* and *C. capitata* males and females (for both species, approximately 0.13 mg/fly/day for males and 0.18 mg/fly/day for females).

8.1.3. Factors influencing feeding behaviour

8.3.1.1 Food deprivation

Results from this study have shown that when flies were deprived of food, their propensity for food searching behaviour increased. Juvenile flies deprived of sugar responded more to food odours than juvenile flies fed with sugar. Protein deprivation of juvenile flies did not affect their response to food odours. On the other hand for mature flies, protein deprivation increased their propensity to search of food. There was also a tendency for protein deprived mature flies to exert their preference for odours from protein sources compared to odours from sugar sources.

Dethier (1976) describes the link between nutritional state and food foraging behaviour. According to him, a fly that has been deprived of all food flies in a random pattern until the odour of food is encountered and then the flight is oriented upwind. Upon contact with the food, when appropriate gustatory stimuli activate the external chemoreceptors, the sensory input to the central nervous system not only initiates feeding, but also inhibits locomotion. When feeding has ceased, internal stimuli inhibit any further incoming chemosensory input, which in turn no longer holds locomotory centres in check. Simpson & Raubenheimer (1993) discussed the role of haemolymph in regulating food location, food selection as well

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as ingestion. The authors proposed a possible mechanism linking levels of nutrient in the haemolymph and propensity for search for food.

8.1.3.2. Age

Flies of three species in this study were found to change their food intake with time. Sugar consumption was highest soon after emergence while protein peaked during the first or second week after adult emergence, depending on species. Protein by itself was found to be inadequate for survival but, in combination with sugar, was found to be important for reproductive behaviour of males (male calling) and also for egg production.

In all frugivorous species, females are anautogenous, that is, they need to feed on protein to realize their reproductive potential (Drew & Yuval, 2001). A definite "hunger" for protein was demonstrated for females of all three species in this study. When females were protein deprived, mature flies oriented more to odours from protein sources compared to juvenile flies. The female fly needs protein, not only to initiate hormonal secretion but also to supply raw materials for synthesis of yolk (Dethier, 1976). In fruit flies, nutrition apparently plays an important role in the activity of the endocrine gland, corpus allatum, in governing oocyte maturation as well as male accessory gland development (Williamson, 1989) In females, the corpus allatum is activated by protein ingestion, which in turn releases juvenile hormone to activate the fat body to synthesise special proteins, vitellogenin. These proteins are thereafter released into the blood and they are taken by oocytes for the formation of yolk (Dethier, 1976).

The physiological bases regulating changes in protein intake with the flies have been discussed by Dethier (1976) and by Browne (1995) and are likely to involve concentration of proteins in the haemolymph as well as abdominal distentions from the gut and from developing egg masses perceived by mechanoreceptors.

8.1.3.3. External environmental factors

Though this study did not address directly and individually the effects of temperature, humidity and light on feeding behaviour, a combined effect of the variation in the three factors with respect to time of day on feeding behaviour was investigated.

Flies of the three species did not feed at night and were found to spend the whole night either resting or in the case of *C. cosyra* and *C. fasciventris* in copula. After a whole night without feeding, food reserves in the fly are depleted and therefore one of the first priorities

of the flies following sunrise would be the search for food which involves movement including flight. Various factors promote flight and these involve light stimulation, wind-speed, humidity, temperature among others (Chapman, 1982).

Interestingly for *C. capitata* males, compensating for the food deficit in the morning hours was not a priority after a whole night of not feeding. They compensated the deficit however by feeding in a short window of time in the late afternoon hours before sunset when they stopped engaging in calling and mating. Similar tendencies of regimentation of feeding and calling activities were found for *C. fasciventris* males, which fed mostly in the morning and called in the late afternoon hours and engaged in mating the whole night.

8.1.4 Feeding behaviour – Species specificity

Studying three species at a time, gave an interesting perspective in feeding behaviour of fruit flies in that not all species feed in the same patterns (diel and lifetime) and more interestingly, not all species need the same amount of nutrients as adults. Here, *C. cosyra* males and females were found to require less protein compared to *C. fasciventris* and *C. capitata*. They also tended to have a lower response to odours from protein compared to *C. fasciventris* and *C. capitata*.

In laboratory studies, here, fecundity of *C. cosyra* was found to be lower than that of *C. fasciventris* and *C. capitata*. This explains their lower need for protein compared to the two other species. The amount of reserves that are carried over to the adults from the immature stages might also be a likely possibility in creating this difference in nutritional needs as adult flies. Part of the nutrient reserve in a fruit fly pupa from larval feeding is usually transferred to the adult stage (Tsitsipis, 1989). Kaspi et al. (2002) found that larval diet did influence the amount of nutritional reserves in the adult stage. Canato & Zucoloto (1998) found that carbohydrate deprivation during the immature phase of *C. capitata* dramatically alters the discrimination threshold for sugars in adult flies. The impact of larval diet on consequent adult responses to food odours and feeding has not been extensively studied and would certainly be interesting to look at for various species and also certainly interesting in practical point of view in the context of application of both SIT and food baits in fruit fly control.
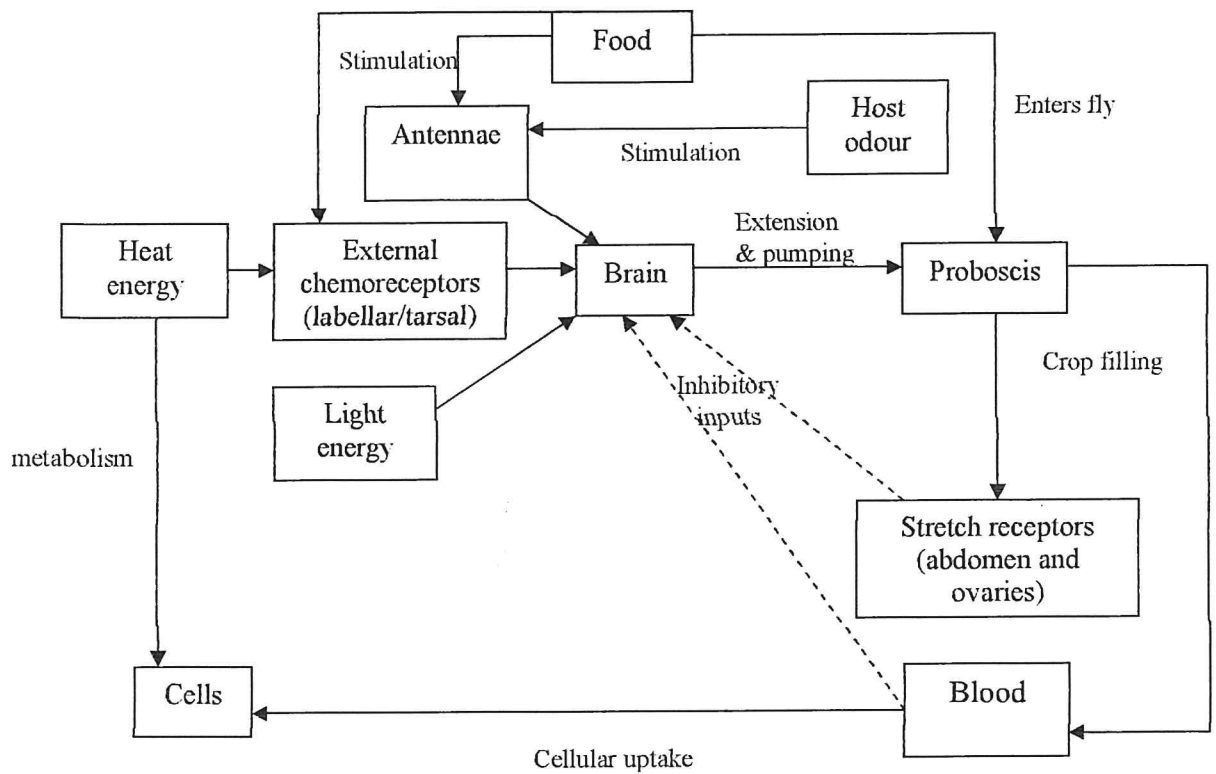
175

Fig. 1. Fruit fly feeding model based on feeding model of blowfly, *Phormia regina* after (Stoffolano, 1995)

8.2 Practical Implications

Various factors have been shown to influence the feeding behaviour of the three fruit fly species studied here. These included nutritional status of the fly, age of the fly and time of the day. Such information is vital in establishing protocol for evaluation of food baits to be used in control of these fruit flies. For instance, it can be expected that a fully fed fly will not respond strongly to food baits and therefore some degree of deprivation must be induced to the fly before carrying out any tests. Moreover, appropriate age of the flies and time of the day should be selected whereby maximum response of flies to food baits can be expected. Interestingly, the behavioural ecology studies on the three fly species conducted at three different scales in the same area have also highlighted that semi field set-up does influence the overall behaviour of flies and depending on the objective of the evaluation, the type of set up to be used must be selected accordingly. Finally, it is not to be expected that food baits will attract flies of all species with the same efficiency. Studies herein have

This work was mostly based on laboratory reared flies. In this way, a large range of questions could be answered in a limited time for the three fruit fly species. A valid question therefore would be: Do we expect the same feeding patterns and responses from wild flies? An attempt was made to determine responses of wild flies to food sources, in coffee plantations and mango plantations during the fruiting season. Some similar trends of fly responses to food sources could be observed between field collected data and semi field data, however no valid comparison can be made since the field data was limited.

To strengthen or compare data collected from semi field evaluation, it will be interesting to conduct some field studies investigating the responses of flies to food sources (natural and artificial) in fruit plantations across the year: before, during and after the fruiting seasons. This time series evaluation will give an indication of fly responses to food sources under different conditions, host phenology, natural food abundance and abiotic conditions. This can provide further information that can help improve strategies for managing fruit flies. An interesting feature also could be analyzing the physiological state (age and nutritional status) of wild flies attracted to different food sources, an area largely untouched in fruit fly research.

8.4 References

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