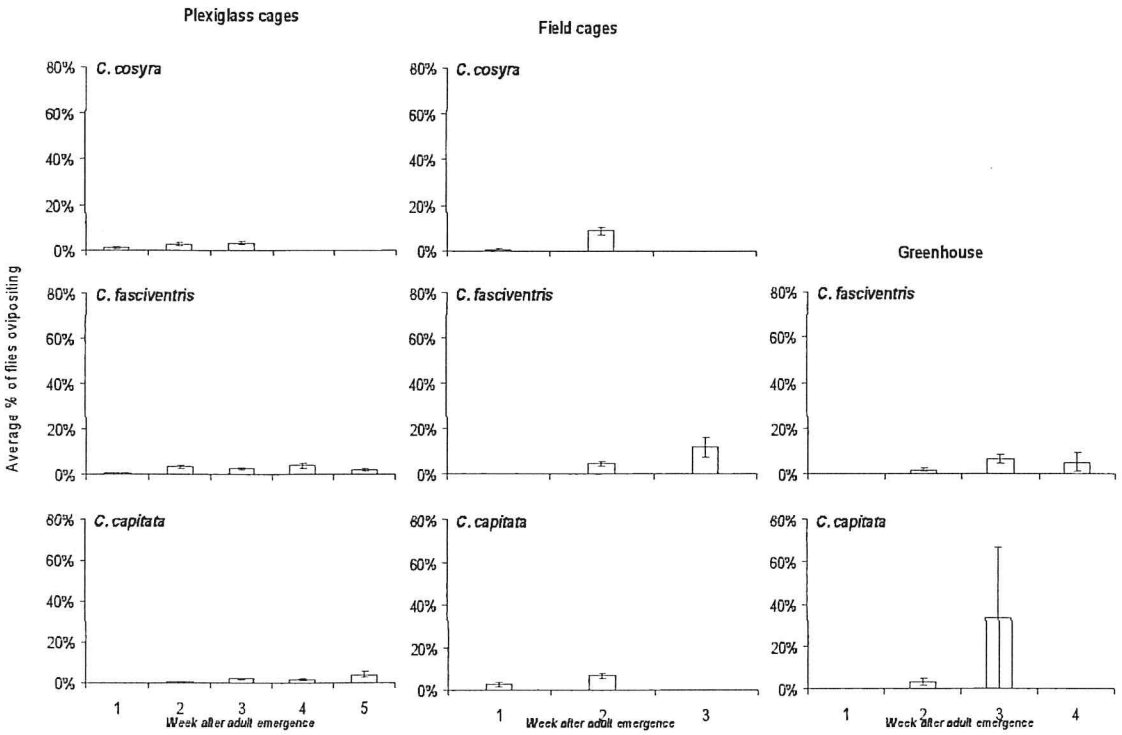

Fig. 16. Weekly oviposition activities in plexiglass cages, field cages and in the greenhouse for females of *C. cosyra*, *C. fasciventris* and *C. capitata*. Values represent means ± SE.

2.4 Discussion

Results from this study are among the first reports of systematic observations of feeding and reproductive activities (calling, mating and ovipositing) of *C. cosyra* and *C. fasciventris* in time and space under semi field conditions.

Presence of flies of *C. cosyra*, *C. fasciventris* and *C. capitata* and their feeding and reproductive activities was largely restricted to host trees. Only on few occasions were flies present on non-host trees. This is in contrast to some other fruit fly species, for instance the melon fly, *Bactrocera cucurbitae* (Coquillett), the papaya fruit fly, *T. curvicauda* and the tomato fruit fly, *Neoceratitis cyanescens* (Bezzi) whose activities are not closely confined to host plants (Fletcher, 1987; McQuate et al., 2003; Brevault & Quilici, 2000). In the tomato fruit fly, for instance, immature and mature flies of both sexes are normally more abundant on non-host plants where they feed, mate and rest and mature females make daily excursions to hosts only for oviposition (Brevault & Quilici, 2000).

2.4.1 Distribution of feeding activities

In field cages and in the greenhouse containing host and non-host trees, flies of all species fed mainly on the upper leaf surfaces. Upper leaf surfaces contained honeydew, bird's droppings and accumulated dust particles but also sometimes bore no obvious presence of food. Fruit fly feeding on leaf surfaces has been reported for other species such as *Rhagoletis fausta* (Osten Sacken), *R. mendax*, *R. pomonella* (Walsh) as well as *C. capitata* (Smith & Prokopy, 1981; Prokopy, 1976; Hendrichs & Hendrichs, 1990; Hendrichs et al., 1991; Hendrichs et al., 1993).

Honeydew has long been considered to form an important source of food for many adult tephritids (Christenson & Foote, 1960; Bateman, 1972). Honeydew represents a mixture of various sugars which constitute more than 98% of the dry weight, amino acids and secondary plant compounds (Volkl et al., 1999). Evolution of diptera has been credited in part with the availability of hondeydew produced by the homopterans who preceded them in evolutionary time (Downes & Dahlem, 1987). As to whether honeydew forms a complete diet for fruit flies, remains still a question since a few amino acids that are required by fruit flies such as tryptophan, cysteine and cystine are either absent or present in very little amounts in honeydew (Boush et al., 1969). Nonetheless, a diet of honeydew

has been found to sustain longevity and moderate egg production of some fruit fly species such as *Bactrocera oleae* (Gmelin), *R. pomonella* and *R. indifferens* Curran (Tsiropoulos, 1977; Hendrichs et al., 1993; Yee, 2003).

Bird's droppings as part of the diet of fruit flies in nature, have been reported in various studies including studies on *C. capitata* (Malavasi et al., 1983; Smith & Prokopy, 1981; Hendrichs & Hendrichs, 1990; Hendrichs et al., 1991; Hendrichs et al., 1993; Warburg & Yuval, 1997). In studies looking at contribution of natural food sources towards fly longevity and fecundity, fly longevity could not be sustained when flies were fed on a diet constituting of only bird's droppings, however when bird's droppings were combined with a source of sugar, natural or artificial, both longevity and fecundity were sustained (Hendrichs et al., 1991; Hendrichs et al., 1993; Yee, 2003). Therefore bird's droppings constitute mainly a proteinaceous food source for the flies in nature, protein being important for egg production.

Flies in this study were also found feeding on accumulated dust particles and sometimes on leaf surfaces bearing even no obvious sign of food. Studies conducted by Hendrichs et al. (1993) in field cages showed that survival and fecundity of apple maggot flies could be sustained only on foliage of host trees which were even devoid of honeydew. The authors proposed that these flies obtained nutrients leaching from leaf surfaces. Plant leachates are indeed an important source of carbohydrates for many dipterans (Stoffolano, 1995).

In this study, fruits were also found to be one of the feeding sites for all fruit fly species, especially in field cages. An increase in fruit feeding was even recorded for *C. fasciventris* females and males in the second week after emergence which coincided with increase in ovipositional activities. Fruit feeding is important for many tephritids. In nature, flies have been commonly found to feed on open fruits due to damage by birds or monkeys and on juices oozing out of these fruits following oviposition by female fruit flies (Smith & Prokopy, 1981; Malavasi et al., 1983; Hendrichs & Hendrichs, 1990; Hendrichs et al., 1991; Warburg & Yuval, 1997).

2.4.2 Diel Patterns of feeding and reproductive activities

C. cosyra, *C. fasciventris* and *C. capitata* exhibited distinct diel patterns of activity in the three set-ups in which they were studied. Interestingly the diel patterns of activities differed between species and between females and males of the same species. Moreover, patterns of some activities also differed between set-ups.

In all set-ups, flies were found to feed throughout the day. *C. cosyra* females and males were found to have the same feeding frequency throughout the day, except in greenhouses where females had a higher feeding frequency in the morning.

C. fasciventris males had a tendency to feed more during the morning hours in all set ups. *C. fasciventris* females, on the other hand fed constantly throughout the day in plexiglass cages and field cages but had two peaks of feeding in the greenhouse, one in the morning and one in the afternoon, following closely temperature, relative humidity and light intensity fluctuations in that particular set up.

C. capitata males had the opposite trend compared to *C. fasciventris* males, with a higher feeding tendency in the late afternoon hours whilst no significant variation in feeding frequencies was observed for *C. capitata* females.

This opposite trend between feeding patterns of males of *C. fasciventris* and *C. capitata* might be as a result of their timing in calling activities. *C. fasciventris* males started calling during the late afternoon hours and peaked at dusk thus leading to a shift in feeding activities to the morning hours. *C. capitata* males started calling soon after dawn and stopped calling in the late afternoon hours and therefore feeding activities of this group were shifted more to the latter period. This timing in calling activities by *C. capitata* males is in agreement to trends observed for *C. capitata* observed under natural conditions by Hendrichs & Hendrichs (1990), Hendrichs et al. (1991) and by Warburg & Yuval (1997) in different parts of the world, Egypt, Greece and Israel. The only notable difference in the calling patterns was that male calling in Egypt was bimodal, with a peak in morning and a peak in the afternoon hours, compared to a more unimodal peak observed in this study and in studies conducted in Greece and Israel, a peak occurring between 08 00 and 14 00 hours. In Egypt, temperatures at midday were higher compared to temperatures recorded in this study and in studies in Greece and Israel which might explain this difference in modality of calling. However, what was common in all studies including this study was that the peak in feeding activities by *C. capitata* males occurred in late afternoon hours, after disengagement of the males in calling activities.

C. cosyra males were also found to start calling at dusk, however, no change in their feeding frequency across the day was noted. *C. cosyra* males, though, started calling later than *C. fasciventris* (by 1 or 2 hours) and therefore this might not have affected their feeding activities so much. Moreover, *C. cosyra* had lower frequency of calling compared

to *C. fasciventris* and even more so compared to *C. capitata* which might also explain why its feeding activities were not shifted to another time.

Diel calling and mating patterns of *C. cosyra* and *C. fasciventris*, are similar to the majority of dacine fruit flies (Fletcher, 1987) which also call and mate at dusk under low light intensity. The long hours of mating of *C. cosyra* and *C .fasciventris* observed might either be to ensure a successful transfer of sperm or is imposed by the male to prevent other males from mating referred to as a post-insemination mate guarding in Taylor et al. (2000).

Lekking behaviour of *C. capitata* and *C. fasciventris* was observed in field cages and greenhouses. Lek formation by *C. capitata* has been described in various studies of behaviour of *C. capitata* in nature (Hendrichs & Hendrichs, 1990; Hendrichs et al., 1991; Warburg & Yuval, 1997). In this study, lek formation of *C. fasciventris* and *C. capitata* occurred in mango and guava trees, mostly on the lower leaf surfaces, which might have provided protection from predators, good abiotic conditions due to its foliage density and most attractive host odours. Lek mating systems have been described for the majority of the tropical and subtropical tephritid fruit flies. Males have to find and join leks (male aggregrations in mating arenas) within which they have to participate in aggressive encounters with other males in order to defend sites from which they would signal and court females (Sivinski & Burk, 1989). Receptive females are attracted by male pheromones emitted by males calling in leks, for the sole purpose of soliciting courtships from various males, thus comparing their performance and eventually accepting one for mating (Hendrichs et al., 2002).

In this study, mating of *C. capitata* was also recorded on fruits. Hendrichs et al. (1991) also found a second mating strategy for the medflies which is more of a resource-based mating system whereby males shift to host fruit in order to intercept ovipositing females. However, this strategy was described by the author to be less effective since females on fruits are usually less receptive. Sivinski & Burk (1989) proposed that on-fruit courtships are probably performed by subordinate males unable to establish a lek territory. Resource based mating increases risks of predation since high predation of fruit flies usually occur on fruit. Moreover, for polyphagous species, since female presence is less predictable in time and space due to greater variability of host fruit resources, a lek based mating system is more appropriate.

Patterns of oviposition activities in plexiglass cages were consistent for the three fruit fly species, with a peak occurring during late afternoon hours. While in the greenhouse, *C.*

fasciventris females were also found to peak in ovipostion during morning hours. So far, for all tephritids, reports indicate that oviposition occurs during the photophase with varying patterns for different species (Smith, 1989). Results on *C. capitata* oviposition activities in this study are in agreement to observations of the same activity observed in open field conditions in Egypt (Hendrichs & Hendrichs, 1990). In studies conducted on *C. capitata* in other parts of the world, Greece and Israel, patterns of oviposition activities were different, stretching throughout the afternoon hours in Greece and peaking at midday in Israel. Speculation on egg laying to be rather a dusk activity in order to lessen exposure of eggs to high temperatures at their most vulnerable stages of development was discussed in Brevault & Quilici (2000) who also found females of *N. cyanescens* to oviposit preferentially in the late afternoon hours in the field.

2.4.3 Weekly patterns of feeding and reproductive activities

Patterns of feeding activities were found to vary across the weeks, with a reduced frequency of feeding for all species on leaf surfaces 1-2 weeks after adult emergence. Similar observations were made on leaf feeding across time for *C. capitata* studied under natural conditions in Egypt (Hendrichs & Hendrichs, 1990). Possibilities for this decrease in leaf feeding might be (1) acquisition of adequate nutrients during the first 1-2 weeks for reproductive maturation and therefore flies required relatively smaller amounts of food for sustaining longevity and fecundity or (2) priorities to other resource-oriented behaviour were given such as calling, mating and oviposition which reduced overall frequency of feeding of flies including feeding on these susbrates.

In plexiglass cages, sugar feeding was found to decrease after the first week for females of *C. fasciventris* and *C. capitata* as well as for males of *C. cosyra* and *C. capitata*. For males of *C. fasciventris* and *C. capitata*, yeast feeding was found to decrease after the first 1 – 2 weeks. Webster et al. (1979) made similar observations for apple maggot flies whereby both sugar and protein intake were found to decrease during the first three weeks and remained to a lower level thereafter. The authors postulated that the greater initial sugar and protein consumption coincided with the period of maturation of the reproductive system and once females become sexually mature, protein meals are required only in small amounts to maintain asynchronous maturation of oocytes.

Calling activities for *C. capitata* were found to peak in the third and fourth week after adult emergence while no significant change in frequency of calling activities was observed for

3.2.3 Survival

Daily records of fly mortality (male and female) were taken from each cage. Mean longevity refers to the average duration of life of flies in each treatment. Maximum longevity refers to the one individual with the longest lifespan in each treatment.

3.2.4 Fecundity

Fecundity was assessed using an oviposition dome consisting of a plastic cup containing a small piece (approximate dimension of 1cmx1cmx0.5cm) of host fruit (mango (*Mangifera indica* L.) for *C. cosyra*, guava (*P. guajava*) for *C. fasciventris* and citrus (*Citrus* spp.) for *C. capitata*. Each host fruit was covered with humid black cloth and parafilm membrane (Parafilm 'M', American National Can, Greenwich, CT. 06836). This egg laying device was chosen because from preliminary tests carried out on *C. fasciventris* we found this surface to be more attractive to ovipositing females compared to a natural guava fruit skin (devoid of pulp). The parafilm was placed to simulate the waxy layer of the fruit skin. Moreover, the black colour eased counting and collection of eggs. Eggs were collected and counted daily from the oviposition domes and were then transferred to a wet black cloth placed on a petri dish for hatching. The oviposition domes were washed and later re-used whenever necessary but host fruits within the devices were renewed daily. Egg fertility was recorded 4 days after collection of eggs and was measured as the percentage of eggs that hatched into larvae. The 8 week period of assessment of both daily egg production and fertility were divided into three time periods according to the patterns of egg laying by adult females on all diets:

1. 0 to 14 days after emergence
2. 15 to 35 days after emergence
3. 36 to 56 days after emergence

Gross and net fecundity rates for each treatment were also determined. Gross fecundity is the lifetime egg production per average female that lives to the last day of possible life in the cohort. Net fecundity is the average lifetime production of eggs per female weighted by the survival probability (Carey et al., 1998).

3.2.5 Reproductive behaviour

At the peak time of reproductive activities, the number of males calling, of females ovipositing and of flies mating were counted daily in each cage. In *C. capitata*, calling and mating activities were recorded every 30 minutes between 09 00 and 11 00 h and in *C. fasciventris* and *C. cosyra*, calling and mating activities were recorded between 16 00 and 18 00 h. The ovipositional activities for the three species were recorded every 30 minutes between 16 00 and 18 00 h.

Fly reproductive activities were defined as follows:

1. "calling" involved wing fanning or conspicuous presence of a clear droplet in a pouch everted from the anal gland in males
2. "ovipositing" involved ovipositor insertion into oviposition dome
3. "mating" actual copulation.

Fly longevity, fecundity, egg fertility and reproductive activities were evaluated for 8 weeks.

3.2.6 Statistical analyses

Survival data were analysed using the Kaplan-Meier model (Proc Lifetest) (SAS, 2001). Life expectancy, fecundity, daily egg production, egg fertility and reproductive behaviour data were analysed by the General Linear Model (Proc GLM) (SAS, 2001). Life expectancy, fecundity and daily egg production data were log (x+1) transformed while the percentage egg fertility and percentage of flies calling, mating and ovipositing data were arcsine square root transformed, to stabilize variances. To compare female vs male life expectancy (table 1) for each treatment, t-tests were performed (SAS, 2001).

3.3 Results

3.3.1 Survival

For males and females of the three fruit fly species, there was a significant difference in survivorship when flies were fed on different diets (*C. cosyra*: females: Log Rank, χ^2=66.62, df=4, P<0.01; males: Log Rank, χ^2=29.87, df=4, P<0.01; *C. fasciventris*: females: Log Rank, χ^2=317.97, df=4, P<0.01; males: Log Rank, χ^2=169.55, df=4, P<0.01; *C. capitata*: females: Log Rank, χ^2=245.61, df=4, P<0.01; males: Log Rank, χ^2=96.69, df=4,

$P<0.01$). With the exception of chicken faeces treatment, survivorship of males and females of the three species on all other treatments, extended for more than 4 weeks after adult emergence (Fig. 1). Survivorship of females and males of all fly species was longest for flies fed on sucrose and yeast hydrolysate. For *C. cosyra* females and males, there was no significant difference in mean longevity between flies fed on diets of honeydew, guava and a combination of guava, honeydew and chicken faeces (table 1). Females and males of *C. fasciventris* and *C. capitata*, exhibited a lower mean longevity when fed on a diet of guava compared to a diet of either honeydew, or a combined diet of guava, honeydew and chicken faeces. Maximum longevity, in both females and males of *C. cosyra*, was attained by flies fed on sucrose and yeast hydrolysate and a combination of guava, chicken faeces and honeydew. Whilst for both *C. fasciventris* and *C. capitata* females and males, maximum longevity was attained by flies fed on sucrose and yeast hydrolysate, a combination of guava, chicken faeces and honeydew and honeydew only. With respect to mean longevity, no significant difference was found between the sexes of the three species.

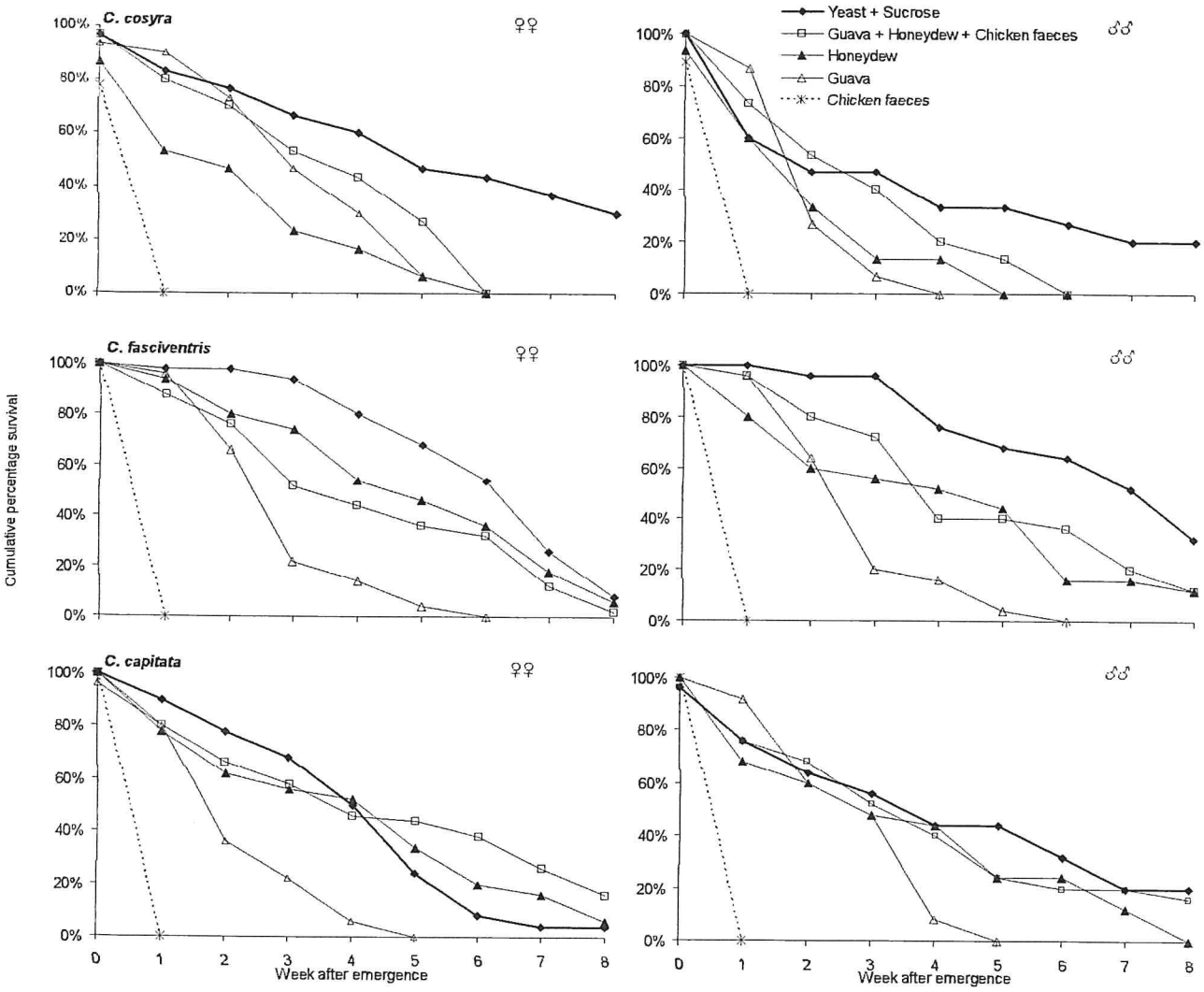

Fig. 1. Percentage survival of *C. cosyra*, *C. fasciventris* and *C. capitata* adult females and males fed on five different diets

Table 1: Effect of adult diet on mean life expectancy and maximal longevity of adult female and male (A) *C. cosyra*, (B) *C. fasciventris* and (C) *C. capitata*

C. cosyra

Treatment	Mean longevity (days)		Maximum longevity (days)	
	Females*	Males*	Females	Males
Chicken faeces	3.00 b	2.89 b	6	5
Guava	20.50 a	12.60 a	36	28
Honeydew	13.90 a	11.13 a	36	29
Guava, Honeydew and Chicken faeces	22.97 a	17.53 a	41	41
Yeast and sucrose	33.33 a	22.87 a	56**	56**

C. fasciventris

Treatment	Mean longevity (days)		Maximum longevity (days)	
	Females*	Males*	Females	Males
Chicken faeces	2.26 d	2.48 c	4	3
Guava	17.86 c	17.96 b	40	39
Honeydew	31.60 ab	26.96 ab	56**	56**
Guava, Honeydew and Chicken faeces	27.86 b	30.96 a	56**	56**
Yeast and sucrose	40.50 a	43.52 a	56**	56**

C. capitata

Treatment	Mean longevity (days)		Maximum longevity (days)	
	Females*	Males*	Females	Males
Chicken faeces	3.14 c	3.00 b	4	4
Guava	14.16 b	17.36 a	29	29
Honeydew	26.44 a	23.48 a	56**	52
Guava, Honeydew and Chicken faeces	29.02 a	25.36 a	56**	56**
Yeast and sucrose	26.66 a	28.32 a	56**	56**

* Means within a column followed by the same letter are not significantly different ($P<0.05$; Tukey HSD-test)

** Since study was carried out for only a period of eight weeks, maximal longevity in these cases might be even greater than 56 days

3.3.2 Fecundity

Since a diet consisting of chicken faeces only did not sustain longevity of flies until their maturation and therefore the start of their reproductive activities, this treatment was not considered in the assessment of food sources for their contribution to fecundity and fertility. There were no significant differences in preoviposition periods for *C. cosyra* and *C. capitata* flies fed on different diets. As for *C. fasciventris*, the shortest and longest preoviposition periods were observed for individuals exposed to sucrose and yeast hydrolysate and honeydew, respectively ($F=8.35$, $df=3,18$, $P<0.01$).

There were no significant differences in both gross and net fecundity for *C. cosyra* flies fed on different diets, despite that numerically fecundity was highest for those flies fed on sucrose and yeast hydrolysate. *C. fasciventris* and *C. capitata* flies exposed to guava exhibited the lowest gross (0.14, 4.32 eggs per female, respectively) and net (0.12, 2.63 eggs per female, respectively) fecundity (*C. fasciventris*: Gross: $F= 37.04$, $df=3,19$, $P<0.01$, Net: $F= 40.19$, $df=3,19$, $P<0.01$; *C. capitata*: Gross: $F= 60.40$, $df=3,19$, $P<0.01$, Net: $F= 60.98$, $df=3,19$, $P<0.01$). Gross and net fecundity were highest for *C. fasciventris* (342.81, 299.70 eggs per female, respectively) and *C. capitata* (384.53, 68.74 eggs per female, respectively) flies fed on sucrose and yeast hydrolysate, and progressively less on a combination of guava, honeydew and chicken faces and honeydew (table 2).

Results of daily egg production per female and egg fertility for the three species across the study period divided into three time periods (0-14, 15-35, 36-56 days after emergence) are summarized in table 3. No significant differences in daily egg production per female (E/F/D – eggs per female per day) and egg fertility were found across the study period for *C. cosyra* in the four treatments. On the other hand, both C. *fasciventris* and *C. capitata* peaked in daily egg production per female and egg fertility between 15 and 35 days after adult emergence in all four treatments (*C. fasciventris*: E/F/D: $F=31.82$, $df=2,59$, $P<0.01$; Fertility: $F=23.59$, $df=2,59$, $P<0.01$; *C. capitata*: E/F/D: $F=83.95$, $df=2,59$, $P<0.01$; Fertility: $F=88.42$, $df=2,59$, $P<0.01$).

There was no significant difference in egg fertility between treatments for *C. cosyra*. Egg fertility was highest in individuals of *C. fasciventris* and *C. capitata* exposed to sucrose and yeast hydrolysate and lowest when exposed to guava.

Significant differences in daily egg production per female and fertility were observed between the three species (E/F/D: F=10.03, df=2,155, P<0.01; Fertility: F=30.38, df=2,155, P<0.01). Daily egg production per female and egg fertility was highest for *C. capitata* when compared to the other two species.

Table 2. Effect of adult diet on mean preoviposition period, gross and net fecundity in (A) *C. cosyra*, (B) *C. fasciventris* and (C) *C. capitata*

C. cosyra

Treatment	Preoviposition period (days)*	Gross fecundity (eggs per female)*	Net fecundity (eggs per female)*
Guava	10.50 a	4.46 a	3.13 a
Honeydew	10.50 a	24.52 a	9.97 a
Guava, Honeydew and Chicken faeces	22.00 a	2.05 a	1.43 a
Yeast and sucrose	15.67 a	32.13 a	24.83 a

C. fasciventris

Treatment	Preoviposition period (days)	Gross fecundity (eggs per female)	Net fecundity (eggs per female)
Guava	10.50 ab	0.14 c	0.12 c
Honeydew	14.20 a	15.70 b	9.52 b
Guava, Honeydew and Chicken faeces	13.20 a	20.29 b	11.76 b
Yeast and sucrose	8.40 b	342.81 a	299.70 a

C. capitata

Treatment	Preoviposition period (days)	Gross fecundity (eggs per female)	Net fecundity (eggs per female)
Guava	9.60 a	4.32 c	2.63 c
Honeydew	7.60 a	87.73 b	33.84 b
Guava, Honeydew and Chicken faeces	7.80 a	121.90 b	25.82 b
Yeast and sucrose	6.40 a	384.53 a	68.74 a

* Means within a column followed by the same letter are not significantly different (P<0.05; Tukey HSD-test)

Table 3 . Mean daily egg production and egg fertility of laboratory reared (A) *C. cosyra*, (B) *C. fasciventris* and (C) *C. capitata* fed on water and natural food sources or enzymatic yeast hydrolysate and sucrose (1:4) for a period of 8 weeks from adult emergence.

C. cosyra

Food source	0 – 14 days after emergence*		15 – 35 days after emergence*		36- 56 days after emergence*	
	E/F/D	Egg fertility (%)	E/F/D	Egg fertility (%)	E/F/D	Egg fertility (%)
Guava	0.29 a	4.67 a	0.02 a	2.82 a	0.00 a	0.00 a
Honeydew	0.70 a	8.37 a	0.83 a	23.64 a	0.00 a	0.00 a
Guava, Honeydew and Chicken faeces	0.00 a	0.00 a	0.10 a	2.76 a	0.00 a	0.00 a
Yeast and sucrose	0.01 a	0.00 a	1.06 a	21.44 a	0.47 a	7.11 a

C. fasciventris

Food source	0 – 14 days after emergence*		15 – 35 days after emergence*		36- 56 days after emergence*	
	E/F/D	Egg fertility	E/F/D	Egg fertility	E/F/D	Egg fertility
Guava	0.01 b	0.00 c	0.00 b	1.11 b	0.00 b	0.00 c
Honeydew	0.01 b	0.00 c	0.57 b	39.24 a	0.18 b	11.57 ab
Guava, Honeydew and Chicken faeces	0.07 b	4.05 b	0.86 b	22.44 a	0.06 b	3.67 bc
Yeast and sucrose	5.46 a	17.76 a	11.08 a	45.62 a	1.46 a	17.55 a

C. capitata

Food source	0 – 14 days after emergence*		15 – 35 days after emergence*		36- 56 days after emergence*	
	E/F/D	Egg fertility	E/F/D	Egg fertility	E/F/D	Egg fertility
Guava	0.11 c	4.83 b	0.24 c	20.43 b	0.00 a	0.00 b
Honeydew	1.25 b	34.59 a	2.89 b	64.60 a	0.64 a	18.81 a
Guava, Honeydew and Chicken faeces	1.03 b	35.84 a	4.59 b	80.74 a	0.63 a	20.18 a
Yeast and sucrose	8.17 a	56.21 a	12.54 a	79.91 a	0.36 a	9.25 a

* Means within a column followed by the same letter are not significantly different ($P<0.05$; Tukey HSD-test). E/F/D = Eggs/female/day

3.3.3 Reproductive behaviour

The influence of different diets on the frequency of reproductive activities of the three fruit fly species is presented in figures 2-4. Similar to the assessment of food sources for their contribution to fecundity and fertility, the treatment consisting of chicken faeces only was not considered in the measurement of this parameter. Generally, *C. fasciventris* and *C. capitata* had higher frequency of calling and oviposition as compared to *C. cosyra* (Calling: $F=15.53$, $df=2,339$, $P<0.01$; Oviposition: $F=30.13$, $df=2,339$, $P<0.01$). There was no significant difference in frequency of mating among the three fruit fly species.

3.3.3.1 Calling

Among the three fruit fly species studied, there was a significant effect of diet on calling frequency by males (*C. cosyra*: $F=6.58$, $df=3, 65$, $P<0.01$; *C. fasciventris*: $F=32.04$, $df=3, 139$, $P<0.01$; *C. capitata*: $F=14.76$, $df=3, 133$, $P<0.05$). Males of all fly species on a diet of yeast and sucrose exhibited significantly higher level of calling activity than males on the diets consisting of natural food sources. Among the natural food sources, the frequency of calling was higher for males of all species on diets of either honeydew only or a combined diet of guava, honeydew and chicken faeces than for males on a diet of guava only. There was no significant interaction between effects of diet and age in all fly species indicating that the effects of diet did not vary significantly across weeks for all fly species.

The diet of *C. capitata* flies did not influence latency until calling (time period until the start of calling activities) (Fig. 2). On the other hand for *C. cosyra* and *C. fasciventris* diet was found to influence latency until calling in individuals. Males of *C. cosyra* provided yeast and sucrose started calling in the first week after emergence while males on natural food sources started calling rather in the second week after emergence. *C. fasciventris* males on all diets except guava started calling in the first week after emergence.

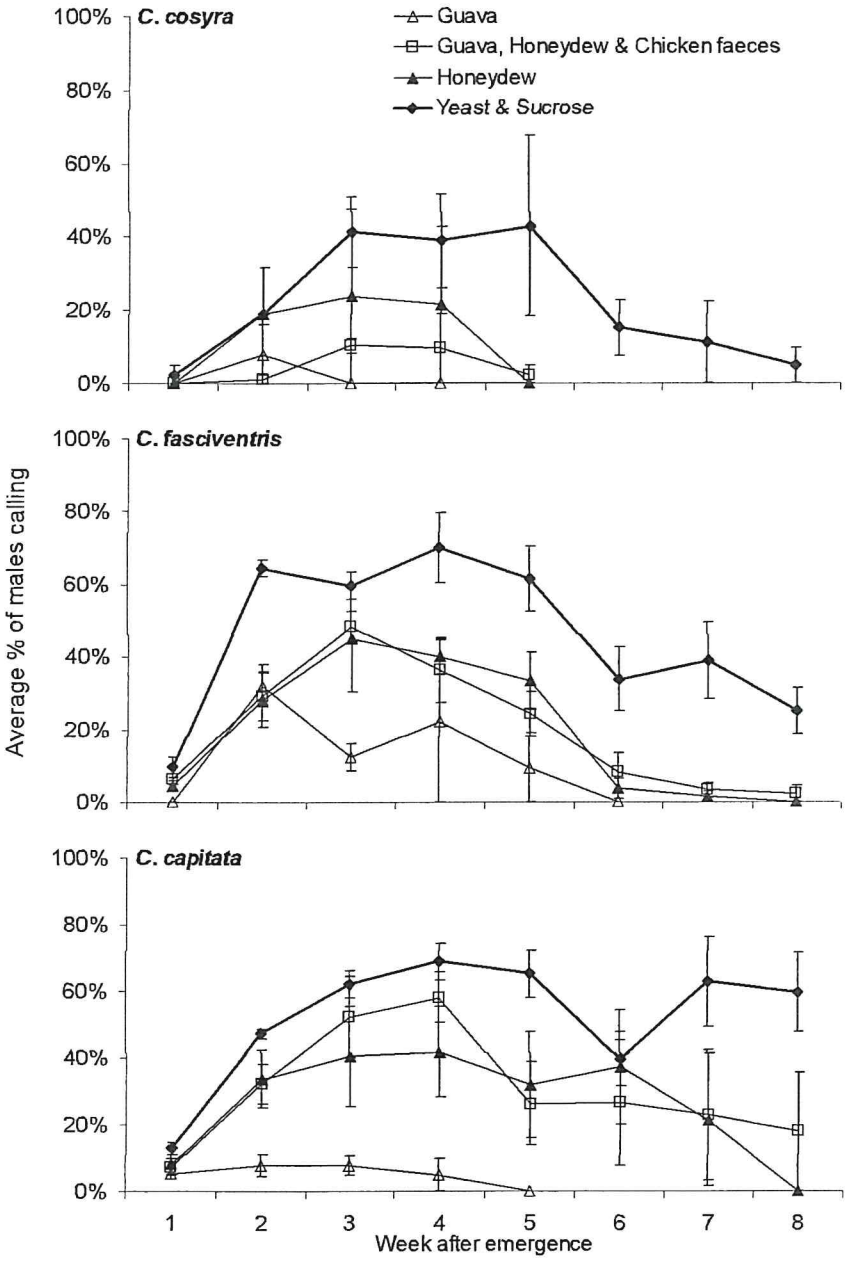

Fig. 2. Effect of diet on calling by males of *C. cosyra*, *C. fasciventris* and C. *capitata* (mean ± SE).

3.3.3.2 Mating

Similar to male calling behaviour, there was a significant effect of diet on the frequency of mating for all fly species (*C. cosyra*: F=4.45, df=3, 65, P<0.01; *C. fasciventris*: F= 16.60, df=3, 139, P<0.01; *C. capitata*: F=15.90, df=3, 133, P<0.01). The effects of diet on mating frequency varied significantly across weeks for individuals of *C. fasciventris* and *C. capitata* while no significant variation occurred in the case of *C. cosyra* (*C. fasciventris*: F= 2.80, df=19, 139, P<0.01; *C. capitata*: F=2.43, df=17, 133, P<0.01). Flies of *C. cosyra* and *C. fasciventris* on a diet of yeast and sucrose copulated more than flies on the other diets. *C. capitata* flies on a combined diet of guava, honeydew and chicken faeces had the highest frequency of mating. For both *C. fasciventris* and *C. capitata*, as from the 4th week after adult emergence, we found no significant difference in frequency of mating between flies fed on different diets (Fig. 3). Diet did not influence latency until copulation in *C. capitata*. For *C. cosyra* flies, mating was recorded in the first week after emergence only for those on a diet of honeydew. The opposite was observed for *C. fasciventris*, where mating was recorded for flies on a diet of honeydew only in the second week while flies on all other diets started mating in the first week.

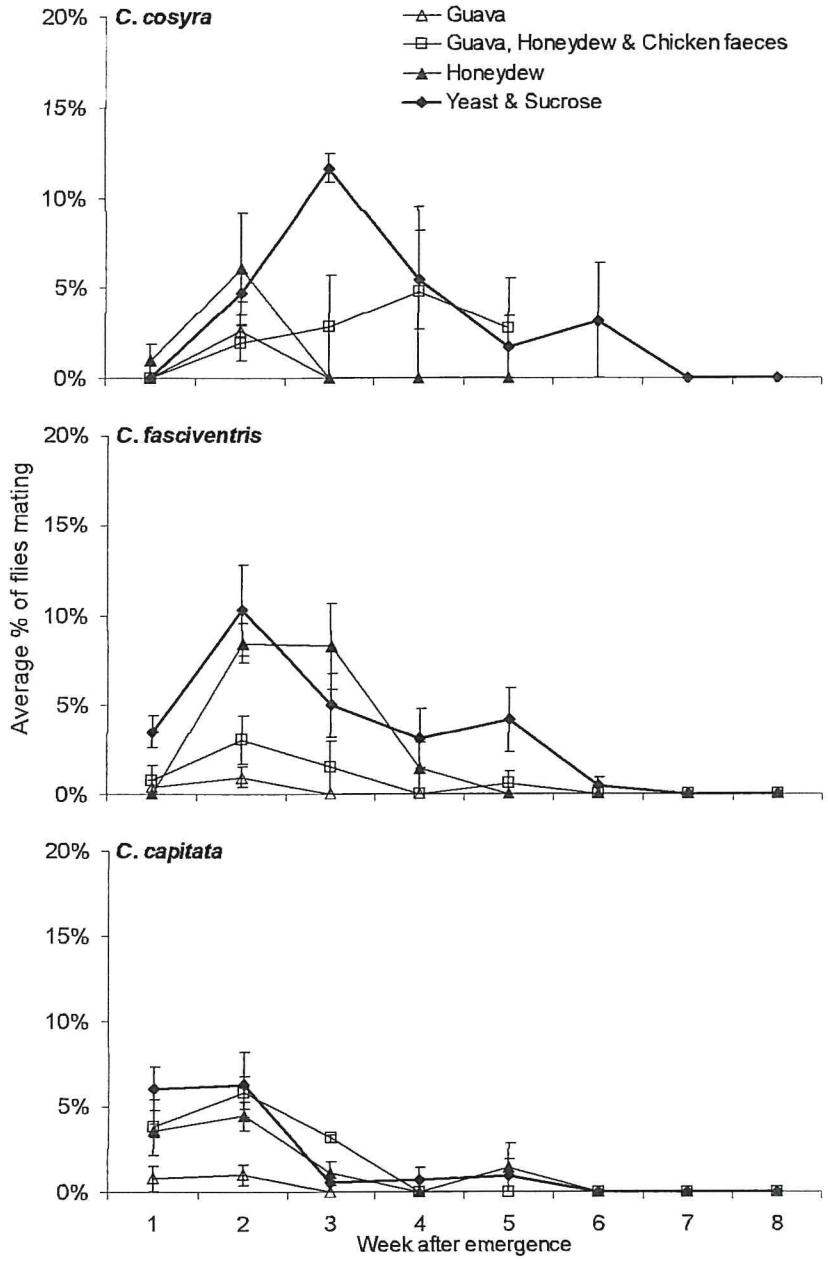

Fig. 3. Effect of diet on mating of *C. cosyra*, *C. fasciventris* and *C. capitata* (mean ± SE).

3.3.3.3 Oviposition

C. cosyra females on a diet of honeydew had a significantly higher frequency of oviposition than females on a diet of either yeast and sucrose or guava only or a combined diet of guava, honeydew and chicken faeces ($F=4.62$, $df=3, 65$, $P<0.01$). Females of both *C. fasciventris* and *C. capitata* on a diet of yeast and sucrose had a significantly higher frequency of oviposition compared to individuals fed on a diet of either honeydew only or a combined diet of guava, honeydew and chicken faeces or guava only (*C. fasciventris*: $F=58.07$, $df=3, 139$, $P<0.01$; *C. capitata*: $F=34.17$, $df=3, 133$, $P<0.01$). There was a significant interaction between diet and age of flies for *C. fasciventris* and *C. capitata* while this interaction was not significant for *C. cosyra* (*C. cosyra*: $F=2.00$, $df=12, 65$, $P=0.05$; *C. fasciventris*: $F=5.16$, $df=19, 139$, $P<0.01$; *C. capitata*: $F=2.32$, $df=17, 133$, $P<0.01$).

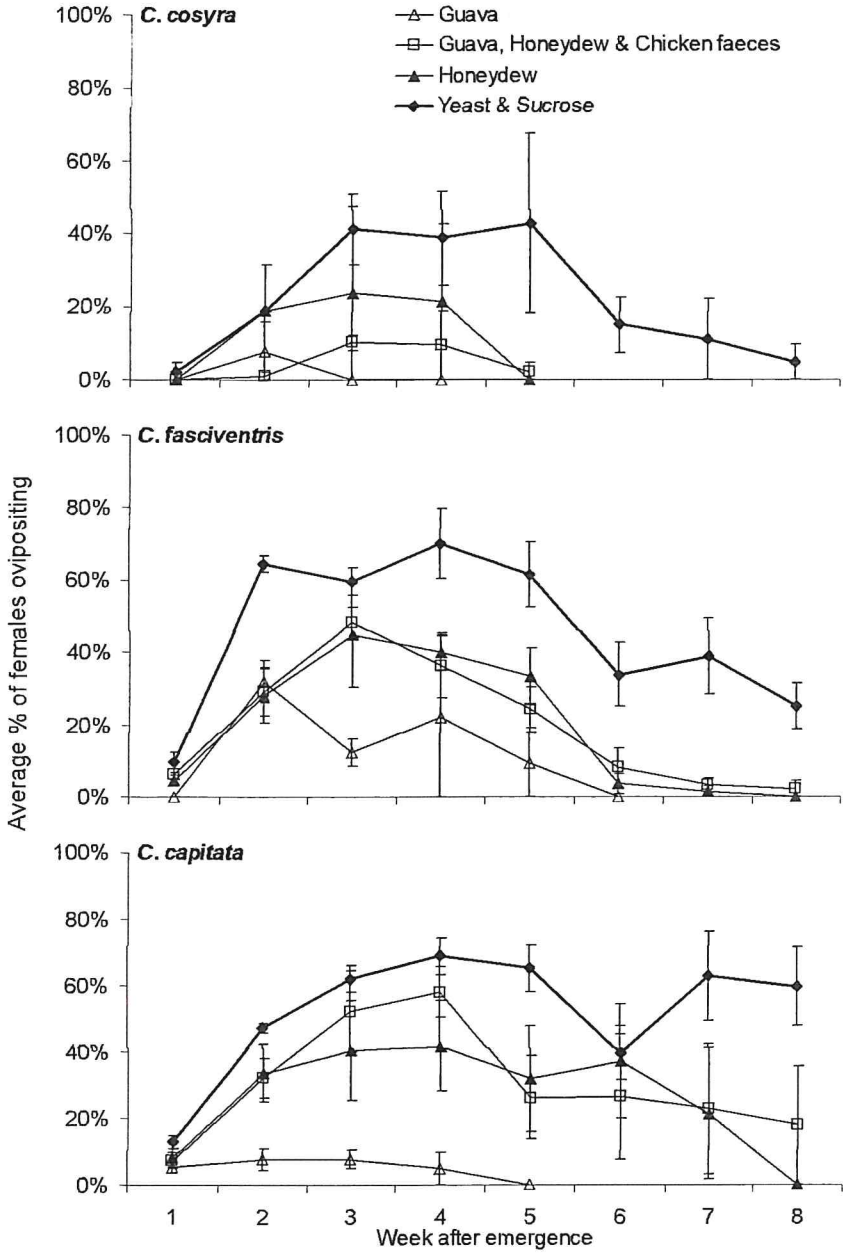

Fig. 4. Effect of diet on oviposition by *C. cosyra*, *C. fasciventris* and *C. capitata* (mean ± SE).

3.4 Discussion

3.4.1 Survival

The results of this study indicate as in previous studies on *Bactrocera oleae* (Gmelin), *C. capitata* and *Anastrepha suspensa* (Loew) (Tsiropoulos, 1981; Hendrichs & Hendrichs, 1990; Hendrichs et al., 1991; Cangussu & Zucoloto, 1992; Nigg et al., 1995; McQuate et al., 2003) that long term survival of fruit flies was poor without a sugar source. The results demonstrate that the three fruit fly species studied here require sufficient amount of carbohydrates in the form of either guava juice or honeydew (Stoffolano, 1995) to satisfy some basic energy requirements for survival and maintenance.

Herein, the results also demonstrated that a diet consisting of guava only (mostly a sugar source) did not adequately sustain longevity of males and females of *C. fasciventris* and *C. capitata* as compared to a diet of sugar and a substantial protein source such as honeydew only or a combined diet of guava, honeydew and chicken faeces or the standard adult diet of yeast and sucrose. Lower survival of flies on a diet consisting of only sugar as compared to flies on a diet of sugar and protein have been reported by some authors (McQuate et al. 2003;, Jacome et al. 1995; Cangussu & Zucoloto 1995). In contrast, studies by Hendrichs et al. (1991), Carey et al., (1998) and Kaspi & Yuval (2000) found that flies fed on sugar alone incurred lower mortality than those fed a diet of sugar and protein. Muller et al. (1997) found an early surge of mortality among female medflies under protein deprivation as compared to fully fed (protein and sugar) flies. However, the authors also observed a decrease in hazard rate at older ages in protein-deprived diet compared with protein-fed females. This early surge in female mortality under protein deprivation was associated to a weakening effect caused by transfer of proteins and other essential nutrients required for egg production that would otherwise be used for maintenance and repair. The increased hazard rates at older ages for protein-fed female flies were associated to the costly process of egg production.

Carey et al. (1998) discussed two physiological modes in the life history of female medflies: a waiting mode in which mortality and reproduction are low and a reproductive mode in which mortality is very low at the onset of egg laying but accelerates as eggs are laid. It was proposed that medflies stay in a waiting mode when they are fed only on sugar. Since in our studies, females of all three species on a diet consisting of guava only still laid

some viable eggs and flies on the same diet still engaged in reproductive behaviour (male calling and mating), this constant waiting mode was absent for the flies fed on guava only. The absence of the "waiting" mode for *C. fasciventris* and *C. capitata* flies on a diet consisting of guava only might be due to the sufficient protein reserves in the adult stage carried over from the larval stage. *C. fasciventris* and *C. capitata* flies used in this experiment were reared on artificial diets which are protein rich. Kaspi et al. (2002) found that a protein rich larval diet enhances reproductive maturation of adult medflies.

3.4.2 Fecundity

Fecundity for *C. fasciventris* and *C. capitata* peaked between the 3rd and 5th week after adult emergence which is comparable to the results obtained by Webster et al. (1979)for the apple maggot fly, *Rhagoletis pomonella* (Walsh) in which egg laying of the latter peaked between days 21 and 36 after adult emergence and also to results obtained by Jacome et al. (1999) for *Anastrepha serpentina* (Wiedemann) where net fecundity peaked between 20 and 60 days. In agreement with findings from Webster et al. (1979), Hendrichs et al. (1991), Hendrichs et al. (1993), Cangussu & Zucoloto (1995) and McQuate et al. (2003), egg production of females was found to be highest for flies on a rich protein diet such as the standard yeast and sucrose diet. Here, in this study, a diet containing bird droppings (chicken faeces) and honeydew was also found to contribute significantly to fly fecundity. Hendrichs et al. (1993) found that bird droppings could sustain egg production in the apple maggot fly but uric acid, one of the components of bird droppings besides microorganisms and other nutrients, could not be utilized by the fly for egg development. A diet of honeydew was also found to sustain fly fecundity in the apple maggot fly (Hendrichs et al., 1993) and the olive fly, *Bactrocera Oleae* (Gmelin) (Tsiropoulos, 1977). Honeydew was found to contain a high number of essential amino acids and a few amides (Boush et al., 1969) and in the field it might serve further as a substrate for microbial growth. With all the evidence, that protein availability highly influences egg production, this study also demonstrated that flies of all three species were still able to produce eggs, though in fewer numbers, on a protein deficient diet such as a diet consisting of guava only. It appears from these results therefore that these tephritid flies require protein only in small but constant quantities throughout their lifetime since oocytes might be matured asynchronously as found for the apple maggot flies (Webster & Stoffolano, 1978).

Egg fertility patterns for the three fly species followed closely the egg production patterns with the highest percentage occurring for flies on a protein rich diet such as that of yeast and sucrose. Egg fertility was found to be highly influenced by the number of matings (Webster et al., 1979).

3.4.3 Reproductive behaviour

There was a significant effect of nutrition on the reproductive behaviour of the three fly species in this study. Consistent with previous studies (Papadopoulos et al., 1998; Kaspi et al., 2000; Kaspi & Yuval, 2000), males of all species on a protein rich diet in this study (yeast and sucrose or a combined diet of guava, honeydew and chicken faeces or honeydew only) were more likely to call than flies on a protein poor diet such as a diet consisting of guava only.

In this study, individuals of *C. cosyra* and *C. fasciventris* on yeast and sucrose and other protein rich diets such as honeydew and bird's faeces were found to start calling earlier than flies on a protein poor diet such as guava. There was no difference in the start of calling activities for *C. capitata* males on different diets which is in agreement with findings by Papadopoulos et al. (1998) for mass reared males. Webster & Stoffolano (1978) likewise found only slight differences in the maturation of the reproductive system between protein-fed and protein-deprived males.

Mating behaviour of all fly species in this study was also found to be affected by diet. Flies on a protein rich diet were found to copulate more than flies on a protein poor diet which is in agreement with findings from studies on both laboratory reared medflies (Kaspi & Yuval, 2000; Taylor & Yuval, 1999) and wild medflies (Kaspi et al., 2000; Shelly et al., 2002). However, Shelly & Kennelly (2002) reported differences between mass reared males and wild males in their mating success as influenced by their diets. Wild protein-fed males were found to enjoy a significant mating advantage over wild protein-deprived males whilst there was no difference in mating frequency between protein-fed and protein-deprived mass reared males. Moreover, Shelly & Kennelly (2002) found that addition of protein to the diet did not boost the mating success of mass-reared males in competition with wild or mass-reared males for wild females.

The finding that *C. cosyra* had in general lower frequencies of calling, oviposition as well as egg laying than *C. fasciventris* and *C. capitata* might indicate different nutritional

requirements for *C. cosyra* compared to the other two species. A possibility might be a lower need for protein sources for *C. cosyra* compared to *C. fasciventris* and *C. capitata.*

3.4.4 Practical implications of findings

Results from this study have provided further insights into the nutritional ecology as well as life history traits of adults of the three tephritid fruit flies which could have practical implications into improved laboratory rearing of these flies or for control purposes in the case of *C. capitata* using the Sterile Insect Technique (SIT).

With regard to laboratory rearing of the flies, the results indicate the need for inclusion of sugar and protein in the diet of adult flies in order to maintain colony production. Moreover, the finding that peak egg laying occur between the 3rd and 5th week after adult emergence has important implication in the maintenance of the colonies because it provides valuable information as to when possible increase in production can be achieved during one generation.

The importance of protein in the reproductive behaviour of fruit flies has clearly been demonstrated in all the species studied which included *C. capitata*. There is an increasing debate in scientific literature with regard to inclusion of protein in the diet of sterile *C. capitata* males that are meant for release in SIT control programmes. The benefit of incorporating protein in the pre-release diet have been discussed by some authors (Papadopoulos et al., 1998; Kaspi & Yuval, 2000; Shelly et al., 2002) and shown to contribute to increase in copulatory success in released males thereby increasing the success of SIT. Kaspi & Yuval (2000) however found that protein-fed males were less likely to survive a period of starvation than protein deprived males which was in contrast to findings of Shelly & Kennelly (2002) who found no difference in survivorship between these two diet groups following a period of starvation when using a higher protein content diet compared to what was used in the study by the former author. Results from this study demonstrated on the contrary that *C. capitata* flies on high protein diets had even a higher survival rate compared to flies fed on mostly a sugar source. Further evaluation therefore needs to be conducted to determine the benefits as well as risks of including protein in the pre-release diet of *C. capitata* sterile flies.

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4. Variability in nutritional requirements of three African fruit flies: *Ceratitis cosyra*, *C. fasciventris* and *C. capitata* (Diptera: Tephritidae).

4.1 Introduction

The consumption of a nutritional substrate by an adult fruit fly is the result of an interplay between a) an external sensory excitation, coming from the peripheral tarsal and labellar chemoreceptors of the fly and b) an internal, post ingestive inhibition coming from its gut and body wall stretch mechanoreceptors, as well as from its thoracic ganglion locomotor centre (Tsiropoulos, 1992). Thus, the amount of food a fruit fly consumes reflects both the phagostimulatory characteristic of that food and the physiological state of the fly (age, mating status, nutritional history).

Adult flies of *Ceratitis cosyra* (Walker), *C. fasciventris* (Bezzi) and *C. capitata* (Wiedemann), like many frugivorous tephritids consume water, carbohydrates and proteins throughout their lives. Water and sugar sources are critical for survival of adult fruit flies while proteins are important for their reproductive development (sexual behaviour and egg production) as well as for improved survival (Tsiropoulos, 1977; Webster et al., 1979; Webster & Stoffolano, 1978; Hendrichs et al., 1991; Hendrichs et al., 1993; Cangussu & Zucoloto, 1992; Kaspi et al., 2000; Kaspi & Yuval, 2000; McQuate et al., 2003; Papadopoulos et al., 1998; Shelly & Kennelly, 2002; Shelly et al., 2002). In terms of the quantitative nutritional requirements of a fly, however, little is published on the amount of sugar and protein required and therefore consumed at different stages of an adult life. Webster et al. (1979) and Landolt & Davis-Hernandez (1993) studied the long-term intake of sugar and protein by Carribean adult flies, *Anastrepha suspensa* (Loew) and both found age-related and sex differences in food consumption.

This study investigates the amount of sugar and protein consumed by males and females of *C. cosyra*, *C. fasciventris* and *C. capitata*. It was hypothesized that the three species might differ in their food intake in particular in their protein intake. From studies on life history characteristics of these three species, it was noted that *C. cosyra* had in general lower frequencies of sexual activities, calling and egg laying, compared to *C. fasciventris* and *C. capitata* and thus a difference in their nutritional requirements was expected. Moreover the three species also differ in their life history characteristics. Tephritid flies can be

categorised into four different categories: monophagous, stenophagous, oligophagous and polyphagous in order of increasing width of host range. Monophagous feeds on only one plant species, stenophagous feeds on members of a single genus, oligophagous feeds on members of a single family and polyphagous feeds on several plant families (White et al., 2001). *C. cosyra*, compared to *C. fasciventris* and *C. capitata* has a narrower host range having two important hosts in the same family Anarcadiaceae: mango (*Mangifera indica*) and maroola plum (*Sclerocarya birrea*). *C. cosyra* therefore approaches an oligophagous life history characteristic. *C. fasciventris* and *C. capitata*, are highly polyphagous attacking a wide range of fruits from several families (De Meyer, 2001; Liquido et al., 1990). Oligophagous species are known to have a lower potential fecundity compared to polyphagous species and their rate of egg production is more directly influenced by the availability of hosts (Fletcher, 1987). As such, a lower protein requirement is expected for *C. cosyra* compared to *C. fasciventris* and *C. capitata*, in particular for females under laboratory conditions (in absence of normal host availability conditions).

The objectives of this study therefore were: (1) to compare the amount of sugar and protein consumed between flies of different species, (2) to compare sugar and protein intake between males and females of each species, (3) to determine age-related patterns of sugar and protein intake and (4) to determine the relationship between food intake and fly fecundity for each species.

4.2 Materials and Methods

4.2.1 Insect material

Flies used in this study originated as puparia from *C. cosyra*, *C. fasciventris* and *C. capitata* colonies maintained at the International Centre of Insect Physiology and Ecology (ICIPE) in Nairobi, Kenya. For each species, upon emergence, 20 males and 20 females were placed separately in plexiglass cages (15 cm x 15 cm x 15 cm). Flies were kept under laboratory conditions at ambient temperatures ranging from 23°C to 30°C, relative humidity between 45-57% and on a photophase from 0600 to 1900 hours.

4.2.2 Food

Flies in all cages had constant access to water, sucrose (sugar source) and Torula yeast (protein source). Food sources were changed daily. Sucrose and Torula yeast were presented separately, each as a dried paste of 7 parts of food to one part of water in glass petri dishes (5.5 cm diameter) and dried for 48 hours in the oven at 80 degrees before introduction in cages. About 0.2 g of each food source was weighed before the addition of water and drying. The same amount of sucrose and Torula yeast were placed in three control cages without flies to determine evaporation. The dry weight of the food paste presented to the flies was measured on a Sartorius R2000 balance.

4.2.3 Consumption of sugar and protein by adult flies

After 24 hours of fly feeding, food sources were transferred in an oven at 80^0C for 48 hours. The dry weight of the food consumed by the flies was recorded after 48 hours on a Sartorius R2000 balance. This procedure was carried out each day beginning with newly emerged flies and continuing until flies were 20 days old. There were four replicate cages for males and females of each species. Mortality was recorded daily.

Ingestion was then calculated by the method of (Cangussu & Zucoloto, 1992), using the following formula:

$$DI=\frac{TDM-RDM}{N}$$

Where

DI = daily ingestion by each fly

TDM= total dry matter corresponding to the control cage

RDM= remaining dry matter corresponding to the treatment cage

N= number of flies.

In cages containing females, an artificial oviposition device was placed which consisted of a plastic cup containing a small piece of cut host fruit at the green mature stage (mango (*Mangifera indica*) for *C. cosyra*, guava (*Psidium guajava*) for *C. fasciventris* and citrus (*Citrus* spp) for *C. capitata*), and covered with humid black cloth and parafilm (Parafilm 'M', American National Can, Greenwich, CT. 06836). The number of eggs laid in the device for each cage containing female flies was counted daily.

4.2.4 Statistical analyses

Data was analysed by non-parametric analysis of variance (Kruskal-Wallis test) (SAS, 2001) since food intake data did not follow a normal distribution. Relationship between fecundity and food intake were analysed by Spearman Rank correlation.

4.3 Results

4.3.1 Species and sex differences in food intake

For the three species studied, males and females consumed more sugar than protein throughout the study period (for males and females of all species: $\chi^2 = 5.33$, df=1, P=0.02). There were neither significant differences in sugar consumption between species nor sex differences in sugar consumption for each species (Table 1). However, with respect to protein, *C. cosyra* males and females consumed significantly less than males and females of *C. fasciventris* and *C. capitata*. There were also significant differences between males and females of each species in their protein intake. *C. cosyra* females consumed in total less protein than *C. cosyra* males though this was not highly significant ($\chi^2 = 4.08$, df=1, P=0.04). On the other hand, females of *C. fasciventris* and *C. capitata* consumed in total significantly more protein than males (*C. fasciventris*: $\chi^2 = 5.33$, df=1, P=0.02; *C. capitata*: $\chi^2 = 5.33$, df=1, P=0.02).

Table 1. Comparison across males and females of three different species: *C. cosyra*, *C. fasciventris* and *C. capitata* of the total (mean) 20-day intake of sucrose and yeast hydrolysate (protein).

Food Type	**Sex**	**Species – Total (mean) 20-day food intake, mg/fly**		
		C. cosyra	***C. fasciventris***	***C. capitata***
Carbohydrates	**Male**	16.00 a[1] (a)[2]	19.04 a (a)	17.88 a (a)
(sucrose)	**Female**	16.92 a (a)	18.56 a (a)	16.78 a (a)
Protein	**Male**	0.80 c (a)	2.67 a (b)	1.55 b (b)
(yeast	**Female**	0.29 b (b)	3.64 a (a)	3.32 a (a)
hydrolysate)				

[1] Any means followed by the same letter across each row are not significantly different at the 0.05 level (Tukey's HSD test following Proc Rank for the sex food category).

[2] Any means followed by the same letter in parentheses between rows for each food type are not significantly different at the 0.05 level (Kruskal-Wallis test)

4.3.2 Age-related patterns of sugar and protein intake

4.3.2.1 Sugar

The patterns of sugar consumption across the 2 weeks after fly emergence for the three species studied are presented in Figure 1. Sugar intake by males and females of all species was highest soon after fly emergence, decreased by about 30% after this early peak to then remain relatively constant (about 1.4 mg/fly/day) throughout the rest of the study period, though for all flies tested this difference was not significant (*C. cosyra*: males: $\chi^2 = 16.99$, df = 20, P = 0.65; females: $\chi^2 = 28.27$, df = 20, P = 0.10; *C. fasciventris*: males: $\chi^2 = 30.30$, df = 20, P = 0.07; females: $\chi^2 = 23.04$, df = 20, P = 0.29; *C. capitata*: males: $\chi^2 = 24.20$, df = 20, P = 0.23; females: $\chi^2 = 25.17$, df = 20, P = 0.19).

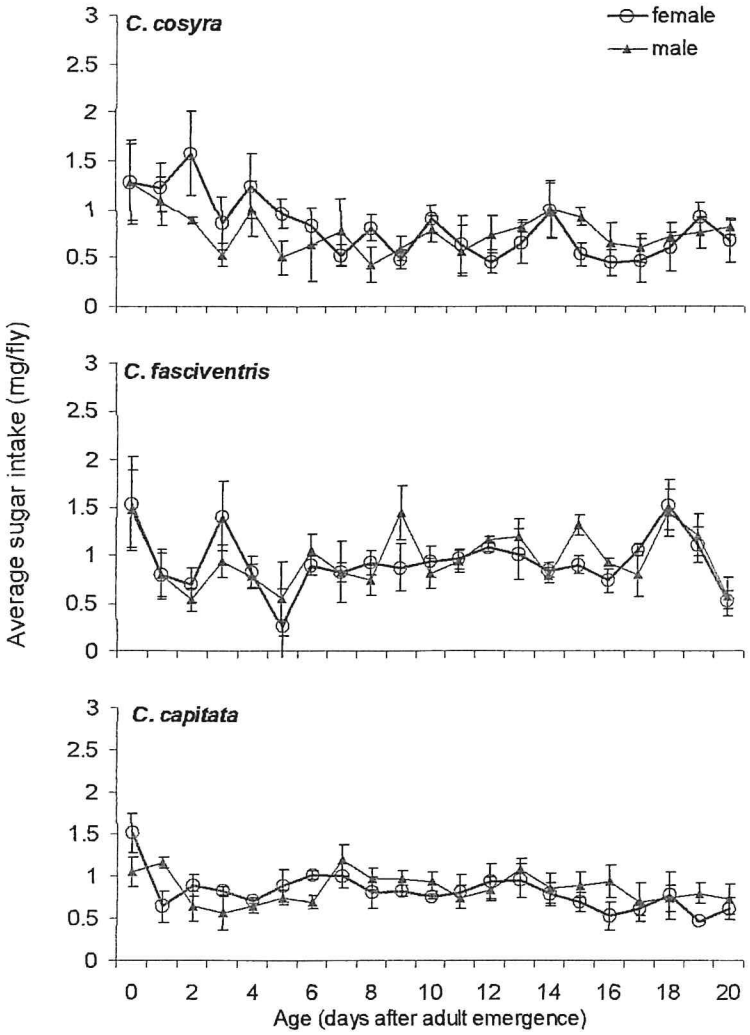

Fig. 1. Age-related patterns of sugar consumption by males and females of *C. cosyra*, *C. fasciventris* and *C. capitata*. Values represent means ± SE

4.3.2.2 Protein

Males and females of *C. cosyra* gradually increased their consumption of protein despite some fluctuations from the time of adult emergence to reach a peak of about 0.15 mg/fly/day for both males and females at 16 days, thereafter which the intake decreased significantly (males: $\chi^2 = 38.34$, df = 20, $P < 0.01$; females: $\chi^2 = 43.00$, df = 20, $P < 0.01$) (Fig. 2).

One female of *C. fasciventris* was found to consume on average 0.20 mg of protein per day, an intake which did not vary significantly across the different ages, though a slight decrease was observed at the end of the second week and protein intake increased again at the end of the third week ($\chi^2 = 19.00$, df = 20, $P = 0.52$) (Fig. 2). *C. fasciventris* males peaked in protein consumption in the first week (at 4 days – 0.26 mg/fly/day), after which intake gradually decreased by about 30% in the second week, then rose slightly by the end of the third week ($\chi^2 = 34.90$, df = 20, $P = 0.02$).

Protein consumption by both *C. capitata* males and females increased after fly emergence to reach a peak of intake of 0.18 mg/fly and 0.38 mg/fly for males and females respectively at 2 days, thereafter which intake decreased by about 50% in the course of the first week to gradually increase again in the second and third weeks (males: $\chi^2 = 33.94$, df = 20, $P = 0.03$; females: $\chi^2 = 33.09$, df = 20, $P = 0.03$) (Fig. 2).

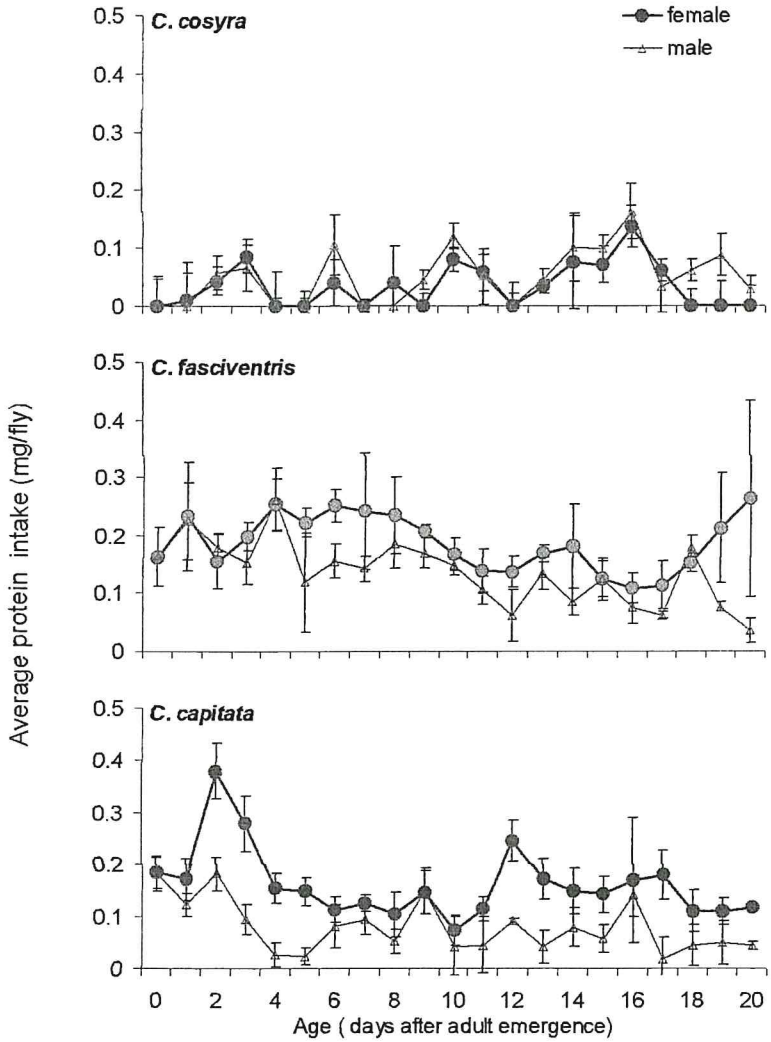

Fig. 2. Age-related patterns of protein consumption by males and females of *C. cosyra*, *C. fasciventris* and *C. capitata*. Values represent means ± SE

4.3.2.3 Fecundity and food consumption

No egg laying data were obtained for *C. cosyra*. For *C. fasciventris* and *C. capitata*, egg laying started at 2 days and 6 days respectively and for both species, there was a peak in fecundity at the end of the second week after adult emergence (*C. fasciventris*: $\chi^2 = 93.02$, df = 20, $P < 0.01$; *C. capitata*: $\chi^2 = 116.51$, df = 20, $P < 0.01$). *C. fasciventris* produced on average 3.90 ± 0.40 eggs per female per day and *C. capitata* produced on average 2.31 ± 0.26 eggs per female per day throughout the study period. Fecundity (eggs/female/day) was not significantly correlated with sugar intake for both flies (Spearman Rank correlation: *C. fasciventris*: N = 80, r = 0.10, P = 0.40; *C. capitata*: N = 82, r = -0.04, P = 0.69) (Fig. 3). However, with respect to protein intake of females, although no significant direct correlations with fecundity for *C. fasciventris* and *C. capitata* were obtained, there was nonetheless some coinciding patterns between protein intake and fecundity for both species (*C. fasciventris*: N = 78, r = -0.14, P = 0.23; *C. capitata*: N = 82, r = -0.20, P = 0.07). For both species, increase in protein intake by females was often followed by an increase in egg laying or even coincided with an increase in egg laying in the case of *C. fasciventris* (Fig. 4). For *C. fasciventris* females, the peak in egg laying at the end of the second week was followed by a renewed bout of protein consumption which was however not observed for *C. capitata* females.

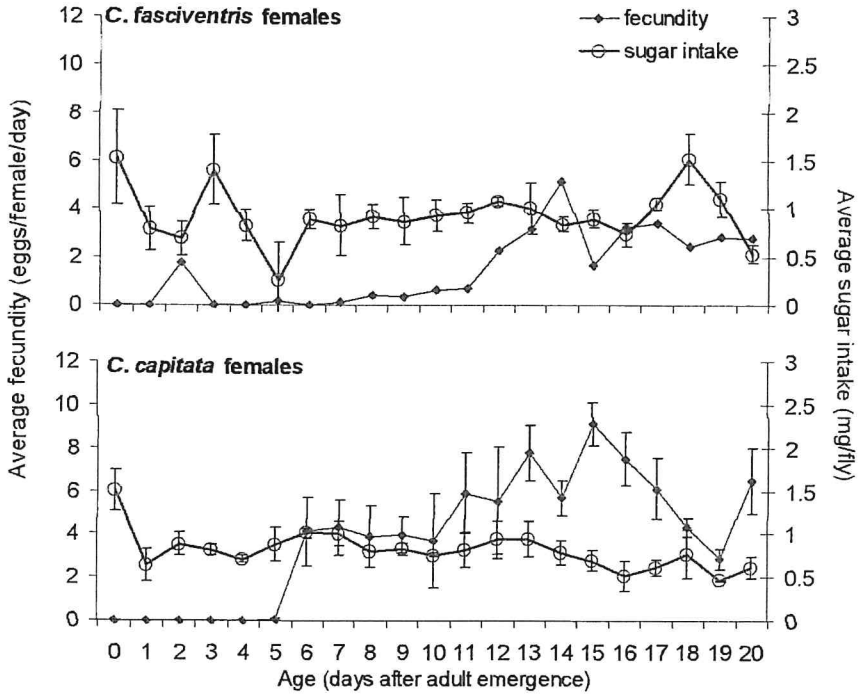

Fig. 3. Fecundity and sugar intake patterns for females of *C. cosyra*, *C. fasciventris* and *C. capitata*. Values represent means ± SE.

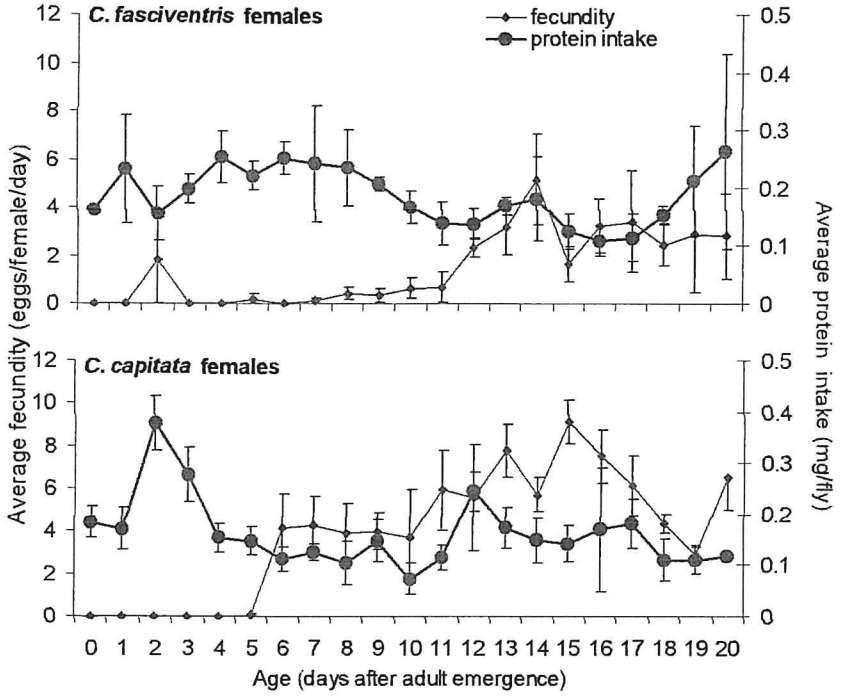

Fig. 4. Fecundity and protein intake patterns for females of *C. cosyra*, *C. fasciventris* and *C. capitata*. Values represent means ± SE.

4.4 Discussion

The daily intake of sugar and protein by males and females of the three fly species studied indicate a continuous requirement of both sugar and protein during the adult life of these flies. Sugar was consumed in larger quantities than protein. Sugar is not only highly phagostimulatory for flies, an important factor in controlling food intake (Dethier, 1976), but is also required as a daily source of energy to fuel daily activities of the flies. Protein intake is required mostly for reproductive maturation of flies (Webster et al., 1979; Webster & Stoffolano, 1978).

4.4.1 Sugar intake

Adult males and females of all species had similar sugar requirements during the study period. Differences in sugar intake across ages of flies were not significant for most species sex category except for *C. fasciventris* males and *C. capitata* females which peaked in sugar intake after fly emergence. Similarly, Robacker (1991) reported maximal visitation of caged male and female Mexican fruit flies, *Anastrepha ludens* Loew at sucrose feeding stations when flies were reproductively immature and number of flies older than 5 days at sucrose feeding stations was greatly reduced. Webster et al. (1979) also reported a decrease in sugar intake of male and female apple maggot flies, *Rhagoletis pomonella* (Walsh), following the first week after emergence. The peak in sugar intake following adult emergence observed for the flies in this study might be as a result of a depletion of nutrients carried over from the immature stages. These nutrient stores might have been expended in adult development such as wing expansion. The peak in sugar intake might also be due to the need of precursors such as protein and carbohydrates for synthesis of lipids used for egg production and pheromone production. Warburg & Yuval (1996) found that post-eclosion starvation of Mediterranean fruit flies led to a significant decline in lipid levels in both males and females, which was thereafter recovered following substantial feeding on either carbohydrate alone or on carbohydrate and protein.

4.4.2 Protein Intake

Protein intake, differed, as expected, between the three different species studied for both females and males. Females and males of *C. cosyra* had a lower protein intake overall compared to females and males of *C. fasciventris* and *C. capitata*.

Whether for females, total protein intake by each fly species can be associated with their eg production cannot be concluded from this study since in the case of *C. cosyra* no egg laying was recorded. This result might be due to the lack of appropriate host stimulus for *C. cosyra* which might have either delayed maturation of oocytes or caused the females to retain their mature oocytes (Aluja et al., 2001). Moreover, mating which usually stimulates oviposition behaviour (Jang, 2002) also did not occur for females of all species as they were kept separate from males in the same cages. Reference can be made however from Chapter 3 on fly survival and reproduction on different food sources whereby egg laying was recorded for the three species *C. cosyra*, *C. fasciventris* and *C. capitata* present in mixed sexes in cages and fed on a diet of yeast and sucrose. In that particular study, the average fecundity (eggs/female/day) at the peak of egg laying for *C. cosyra* was found to be 1.06 ± 0.97 while fecundity values for *C. fasciventris* and *C. capitata* were 11.08 ± 1.17 and 12.54 ± 1.27 respectively.

Sex differences in protein intake were observed in this study for flies of all three species studied. Results on *C. fasciventris* and *C. capitata* conform to results obtained by Webster et al. (1979) and Landolt & Davis-Hernandez (1993) whereby females were found to consume more protein than males. Unlike *C. fasciventris* and *C. capitata*, *C. cosyra* females were found to consume less protein than males though this difference was not highly significant. Female tephritids require a protein meal for reproductive development and vitellogenesis whilst for males little protein is required for the development of accessory gland and they do not require protein for spermatogenesis (Webster & Stoffolano, 1978). Protein affects pheromone production by males, though pheromones can be produced without a protein source (Epsky & Heath, 1993).

Age-related differences in protein intake was observed for males and females of the three species studied. *C. cosyra* males and females peaked in protein intake in the third week after adult emergence. There was no significant change in protein intake for *C. fasciventris*

with respect to age. *C. capitata* females peaked in protein intake in the first week after adult emergence. For both *C. fasciventris* and *C. capitata* females, high protein consumption was often followed by peaks in egg laying which supports the contention that females require protein for egg development and maturation. For males of *C. fasciventris* and *C. capitata*, peaks of protein intake in the first week of adult emergence can be related to the start of calling activities by these males, which have been recorded in this study to start at the end of the first week of adult life.

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5. Responses of Three African Fruit Flies: *Ceratitis cosyra*, *C. fasciventris* and *C. capitata* (Diptera: Tephritidae) to Natural and Artificial Food Sources.

5.1 Introduction

Flies locate their food by airborne food volatiles. Odours from artificial food sources have been used in trapping fruit flies for control and monitoring purposes. These food baits include protein hydrolysates, autolysed yeast, ammonium salts, molasses, syrups and fruit juices (Roessler 1989; Smith & Nannan 1988; Vijaysegaran 1996).

Efficient control of fruit fly pests using food baits depends on an understanding of their feeding behaviour. As yet, no quantitative data exists on feeding and food searching patterns of *Ceratitis cosyra* (Walker) and *C. fasciventris* (Bezzi). Behavioural responses (feeding and olfactory) of the Mediterranean fruit fly, *C. capitata* (Wiedemann), medfly, to different types of food sources (natural or artificial) under different set-ups ranging from natural field conditions to laboratory tests have been studied in different parts of the world. Galun et al. (1985) and Vargas et al. (2002) reported differences between female and male attraction and feeding responses to protein sources, with females being more attracted and feeding more than males. Prokopy et al. (1992) and Cohen & Voet (2002) found that age of flies significantly influence their attractiveness to protein sources with mature flies showing an increased attraction to protein. In field evaluation of proteinaceous food baits conducted in Guatemala, (Heath et al., 1995) found that a greater percentage of unmated females were attracted to protein baits compared to mated females. Therefore feeding and olfactory responses to food sources are expected to vary with respect to the physiological state of the flies. Finally, in tests conducted in Hawaii, evaluating concurrently attractiveness of an artificial food source (a proteinaceous lure –Staley's PIB-7) and natural food sources for the flies (animal excrement including bird's faeces), Prokopy et al. (1993) and Prokopy et al. (1992) found that natural food sources were more attractive to medflies than protein baits that are often used in control and monitoring of fruit flies. With regards to medflies therefore, quite some information have been gained on its feeding and food searching patterns. However, considering the variability of feeding behaviour of fruit flies expressed

under different environmental conditions, little or no information yet exists from elsewhere as to feeding behaviour of medflies with respect to natural and artificial food sources.

Feeding and olfactory responses to natural and artificial sugar and proteinaceous food sources of male and female *C. cosyra*, *C. fasciventris* and *C. capitata* are reported here. The principal objectives of this study were to determine (1) when flies were most likely to respond to various food sources and their odours, (2) whether or not feeding events were correlated to responses to food odours, and (3) relative attractiveness of natural versus artificial food sources.

5.2 Materials and Methods

5.2.1 Study Area

All experiments were carried out at the Mbita Point Field Station of the International Centre of Insect Physiology and Ecology (ICIPE), at Lake Victoria, Western Kenya (altitude of 1240 meters between latitudes 0° 25 S and 0° 30 S and longitudes 34° 10 E and 34° 15 E). Tests took place from July 2001 to March 2002 when daily average temperature ranged from 22°C to 28°C with a peak in the early afternoon hours (13 00 hours) and relative humidity ranged from 64% to 77% with the lowest humidity in the early afternoon hours (13 00 hours).

5.2.2 Insect Material

Flies were obtained as puparia from *C. cosyra*, *C. fasciventris* and *C. capitata* colonies maintained at the ICIPE headquarters in Nairobi, Kenya. Upon emergence, flies were maintained in cages supplied with water and a mixture of enzymatic yeast hydrolysate and sucrose. Flies were kept under laboratory conditions at ambient temperatures ranging from 23 ^{0}C to 30 ^{0}C, relative humidity between 45-57 % and on a photo phase from 0600 to 1900 hours. The following categories of flies were used in the tests to compare flies of different age groups: (a) "young immature", 1-2 days old, (b) virgin, "young mature", 4-7 days old, (c) virgin, "old mature", 14-17 days old. To compare flies of different mating states, flies which were 2 weeks and above were used in the tests. In looking at feeding responses, 20-23 day old mated and unmated flies were used. In looking at responses to food odours, 14-17 day old mated and unmated flies were used.

5.2.3 Food sources evaluated

Food sources consisted of (1) chicken faeces from caged chickens– 1 day after collection and storage at 4^0C, (2) ICIPE yeast which is a local brewery by-product, (3) honeydew collected for about 2 days in petri dishes suspended beneath leaves of an aphid infested *Citrus* spp tree, (4) guava juice (*Psidium guajava*) made from fruits which were collected at a mature stage from trees, and (5) sugar cane molasses from the Muhoroni sugar factory, Kenya.

The food samples were categorized into sugar and protein sources, with respect to their most abundant constituent. For example, honeydew contains amino acids and other substances as well as sugars, but sugars amount to >80% of the honeydew product (Downes & Dahlem 1987). Hence, guava juice, molasses and honeydew were grouped into sugar sources, while chicken faeces and ICIPE yeast were grouped into protein sources.

5.2.4 Experimental set-up

Experiments were conducted in two cylindrical (2 m tall x 3 m diameter) clear-nylon-screened field cages (Fig. 1). A clear plastic sheet covered the top of each field cage to exclude rain and direct sunlight. Four potted non-host banana (*Musa* sp) plants (0.75 cm tall) were placed at four sides of each field cage. 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 conducted, all food sources were offered simultaneously in petri dishes of 8.5 cm diameter. All foods except honeydew were presented as a paste made of 3 parts of food substance and 1 part of water and smeared on the petri dishes. In the first experiment, the feeding responses of flies to natural and artificial food sources were assessed. In the second experiment, the responses of flies attracted to odours emanating from the same food sources were assessed. During tests on feeding, flies had free access to the food sources whilst in the tests on food odours, flies were prevented to touch or taste the food by a net (1 mm mesh size) covering each petri dish. A control (an empty petri dish) was included in the second experiment. The food sources tested were arranged in 5x5 Latin Square experimental design during evaluation of feeding response (Fig. 2). A 6x 6 Latin Square experimental design was used during evaluation of the food odours (Fig. 3).

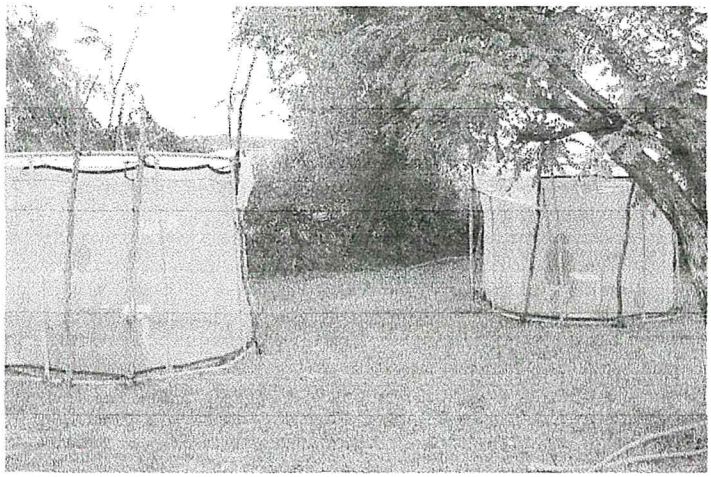

Fig. 1. Field cages used for evaluation of fly responses to natural and artificial food sources.

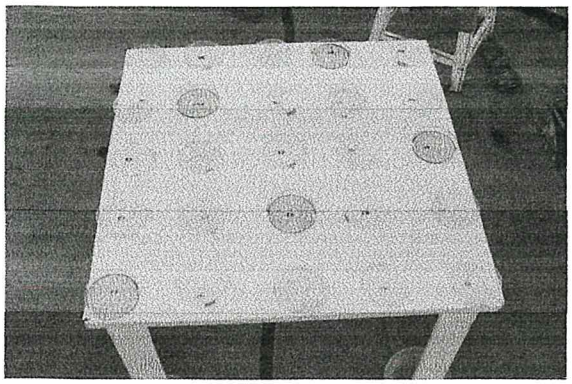

Fig. 2. Arena of food sources exposed on table for evaluation of fly feeding responses to natural and artificial food sources.

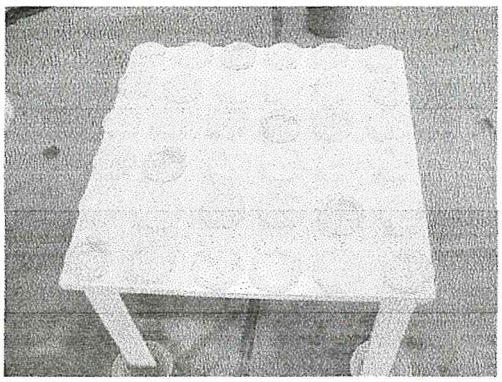

Fig. 3. Arena of food sources exposed on table for evaluation of fly responses to odours from natural and artificial food sources.

In each experiment, the responses of flies to the food sources were tested in three time periods (1) 07 00 to 09 00 hours (morning) (2) 11 00 to 13 00 hours (midday) and (3) 16 00 to 18 00 hours (afternoon). Four replicates were run for each experiment.

In the first experiment testing feeding responses of flies to food sources, the latter were freshly prepared in the morning and used throughout the three time periods on a test day. Whilst in the second experiment testing responses of flies to odours from food sources, freshly prepared food sources were presented at each time period.

Flies had no access to food 14 hours before release in the cages for each experiment. The number of flies released in a cage varied from 20 - 100, depending on availability of flies of various species and their age or mating status category.

In the first experiment, the same flies were used for the four replicates of each fly category tested. The number of flies present in the cage for each replicate was recorded at the end of each test day. While assessing the effects of mating status on feeding response, flies were tested at a time period that did not coincide with their peak mating time. Therefore 20-23 days old *C. cosyra* and *C. fasciventris* were tested in the morning hours 0700 to 0900 hours and 20-23 days old *C. capitata* were tested in the afternoon hours 1600 to 1800 hours.

In the second experiment, flies released for each replicate of each fly category tested were removed at the end of each test day. The removed flies were fed on a mixture of enzymatic yeast hydrolysate and sucrose for at least one day before being re-used again for tests.

In both experiments, the number of flies feeding or landing on the different food sources was recorded at 5 minutes intervals during each time period. The table was rotated by 90^0 every 30 minutes. Data for the replicates of each test substance for every 5-minute interval during one time period were pooled for analysis.

Temperature and Relative Humidity in the cages were determined every hour during the observation using a hygrothermometer (Cole-Parmer Instrument Company, Chicago, Illinois). Light intensity was also measured every hour with a photometer (Li-Cor, USA).

5.2.5 Data analysis

Data were analyzed by a parametric analysis of variance using the procedure GENMOD (SAS 2001) which fitted the data to a log-linear model. The data was assumed to follow a Poisson distribution. The two hour interval data were subjected as repeated measures since flies feeding or landing at one check point (5 minute interval) might or might not have been

the same flies feeding or landing at the next check point. The probability level for all tests was set at 0.01.

5.3 Results

Patterns of feeding and responses to food odours varied significantly between the three species tested (Feeding: χ^2 =56.35, df=2, P>0.01; Food odour χ^2 =65.54, df=2, P>0.01). There was however no significant difference in patterns of responses to food sources between males and females of all species in both experiments. Hence values are presented for both sexes combined for the three species.

5.3.1 Diel patterns of feeding and olfactory responses

5.3.1.1 Sugar sources

For *C. cosyra* and *C. fasciventris*, there was no significant difference in frequency of feeding on sugar sources between the different times of the day (Fig. 4). *C. capitata*, on the other hand, fed more frequently on sugar during the afternoon hours (guava: χ^2 =36.13, df=2, P<0.01; honeydew: χ^2 =9.50, df=2, P<0.01 and molasses: χ^2 =10.53, df=2, P<0.01). Flies of all three species responded significantly more to odours from guava in the afternoon hours (*C. cosyra*: χ^2 =14.25, df=2, P<0.01; *C. fasciventris*: χ^2 =10.28, df=2, P<0.01 and *C. capitata*: χ^2 =13.08, df=2, P<0.01) (Fig. 5). Odours from other sugar sources, honeydew and molasses, were highly attractive to *C. cosyra* in the afternoon hours (honeydew: χ^2 =10.48, df=2, P<0.01; molasses: χ^2 =13.50, df=2, P<0.01). For *C. fasciventris* and *C. capitata*, there was no significant difference in responses of flies to odours from honeydew at different times of the day (*C. fasciventris*: χ^2 =0.44, df=2, P>0.01; *C. capitata*: χ^2 =3.70, df=2, P>0.01). *C. capitata*, like *C. cosyra* responded also strongly to odours from molasses in the afternoon hours (χ^2 =16.09, df=2, P<0.01).

5.3.1.2 Protein sources

Flies of the three species, fed more frequently on chicken faeces in the morning hours (*C. cosyra*: χ^2 =9.50, df = 2, P<0.01; *C. fasciventris*: χ^2 =19.54, df=2, P<0.01 and *C. capitata*: χ^2 =9.17, df = 2, P=0.01). There was also a higher frequency of feeding on the other protein source, ICIPE yeast, in the morning hours, however this was not significantly different from frequencies of feeding on the same observed at the other times of the day for all three species (*C. cosyra*: χ^2 =2.62, df = 2, P>0.01; *C. fasciventris*: χ^2 =2.41, df=2, P>0.01 and *C. capitata*: χ^2 =0.00, df = 2, P>0.01).

In comparison to the feeding responses on chicken faeces, odours from fresh chicken faeces were highly attractive to flies of *C. cosyra* in the afternoon hours (χ^2 =14.75, df =2, P<0.01). As for *C. fasciventris* and *C. capitata*, responses to odours from chicken faeces were numerically higher in the afternoon hours, though not significantly different from the rest of the day. *C. capitata* flies responded significantly more to odours from the local protein source, ICIPE yeast in the afternoon hours (χ^2 =16.51, df =2, P<0.01). *C. cosyra* and *C. fasciventris* flies had a numerically higher frequency of response to odours from ICIPE yeast in the afternoon hours and midday respectively, though these were not significantly different from frequencies of response observed at other times of the day.

Interactions between age of flies and time of day in both experiments were not significant (Feeding: χ^2 =12.16, df=4, P>0.01; Food odour: χ^2 =1.31, df=4, P>0.01) indicating that the diel patterns of feeding and responses to food odours were consistent across the different life stages of the flies.

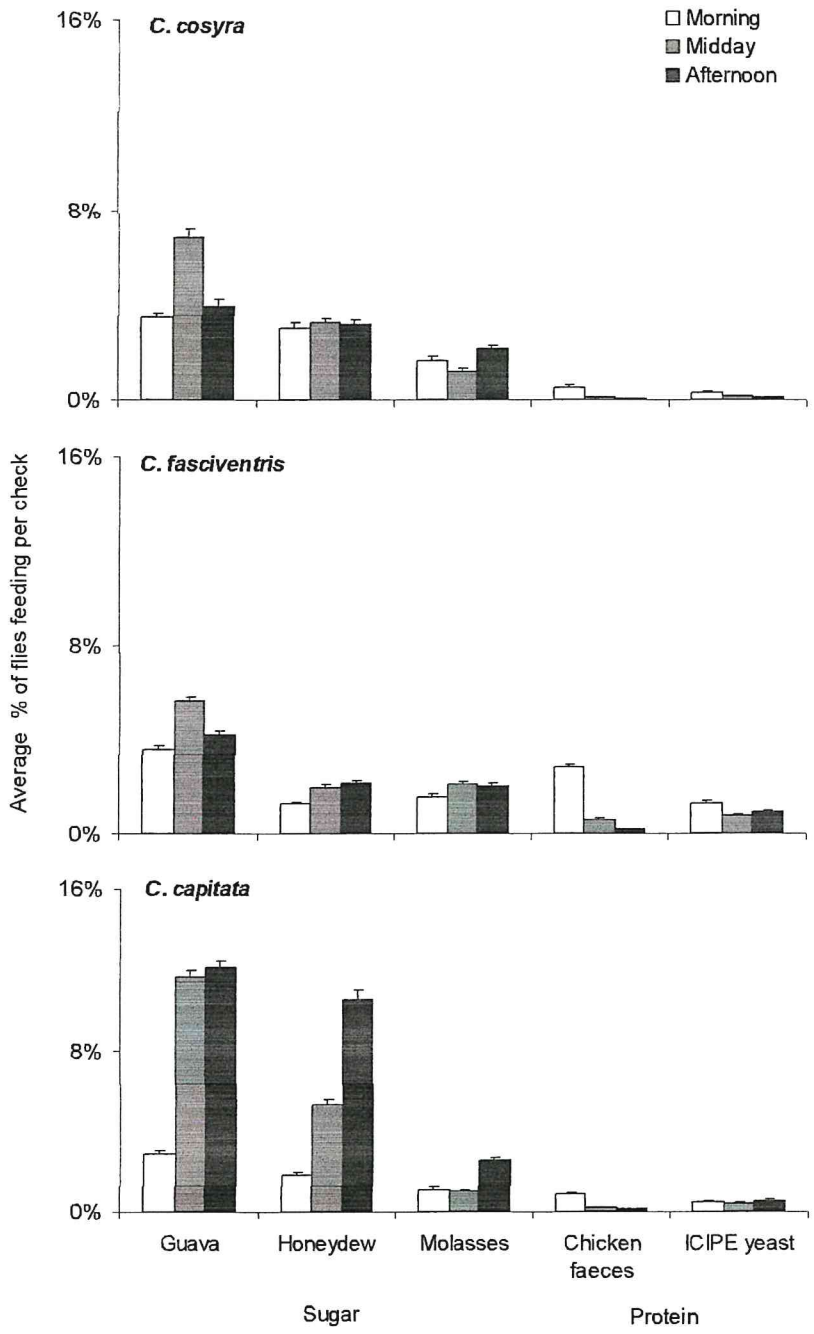

Fig. 4. Diel patterns of feeding on natural and artificial, sugar and protein, sources by (A) *C. cosyra*, (B) *C. fasciventris* and (C) *C. capitata*. Values represent means ± SE. Results are presented for all age categories combined.