FEEDING BEHAVIOUR OF THREE AFRICAN FRUIT FLIES: *CERATITIS COSYRA*, *C. FASCIVENTRIS* AND *C. CAPITATA* (DIPTERA: TEPHRITIDAE)

BY

ARUNA MANRAKHAN

A THESIS SUBMITTED FOR THE DEGREE OF DOCTOR OF PHILOSOPHY OF THE UNIVERSITY OF MAURITIUS

Host institution: International Centre of Insect Physiology and Ecology, Nairobi, Kenya

30 July 2004

TABLE OF CONTENTS

Acknowledgements

I would like to thank first of all my two supervisors, Dr Slawomir A. Lux and Prof Indurlall Fagoonee for their support in starting the Phd work, for their continuous guidance throughout the study period and for their constructive criticism of my work. Dr Lux, through you, I have learnt how to think in terms of goal oriented research. I would like to thank you for this. It has largely changed my perspectives of scientific thinking. Prof Fagoonee, your encouragement and advice on my work and university matters have been valuable throughout.

I would like to thank Mr Pascal Agolla Oreng who has provided me with technical assistance throughout this PhD work. I have learnt also a lot from you, Pascal and admired your patience in observing fly behaviour. I also appreciated a lot of innovative ideas from you in helping me design some materials for the study.

My sincere gratitude goes to Beatrice Gikaria who has been the vital link between my work in Mbita and in obtaining flies and materials from ICIPE Nairobi.

I wish to acknowledge Mr Peterson Nderitu, Mrs Faith Nyamu and Mr John Klilu in ICIPE Nairobi for providing me with flies for my experiments.

My thanks go to Dr Sunday Ekesi and Dr Nigel Price for their efforts in looking through some of my manuscripts. Your comments were highly appreciated.

I would also like to acknowledge Dr Robert Copeland for lending me some of the pictures that I presented in this thesis.

At the Mbita Point Field Station (MPFS), I had the constant support of my dear friend and neighbour, Dr Ulrike Fillinger. Uli, you have been with me throughout this thesis. You know it all, from helping me wash my petri dishes to reading my manuscripts. I owe a great deal to you and would like to thank you for all your help, care and encouragement. I will miss you here in Mbita.

My thanks also go to Theresa Wayuga, my househelper, who took good care of me during my whole stay in Mbita. Theresa, you did a great job in also maintaining the greenhouses, field cages and my lab during the whole study period!

Friends from Mbita: Hannah Nadel, Heather Ferguson, Odile Pfaehler, Jenny Lindh, Evan Mathenge, Rob Place, Hassan Ali Akelo, Johanna Schaedel, Ernst Jan Scholte, Egil Fischer, Richard Mukabana, Leunita Sumba, Hortance Manda and Tom Guda; I would like to thank you all for the good times we had together. Thank you for making me enjoy this place! Heather, learning about degrees of freedom and survival analysis was a total plus for my statistical background! Odile, the Swiss chocolate you fed me with certainly improved my quality of life here!

I would like to thank the ex station manager, Dr Bart Knols, for all his support when I first came here. Thanks for the big computer screen which definitely saved my eyes! I would also like to thank the Station Administrator, Dr Charles Mwendia for his support and encouragement of fruit fly work in Mbita.

Of the many people in MPFS whom I also wish to acknowledge are Godfrey Otieno Sune for his help during my experiments, Fred Orwa for rescueing me from IT problems, Mr Henry Ligedere, Mr John Kibera, Mr Georges Kisa and Mr Nyongesa for their support here.

My dear friend from ICIPE Nairobi, Andrea Rossbach, I would like to thank you so much for all your help during my stay in Nairobi. After our incident in May 2003, I developed an even higher esteem for you. You are a very brave one!

I would also like to thank some fellow students/ex-students in the AFFI project with whom I shared a lot with, especially during workshops and conferences, and also in Mbita. My thanks go to Mr Pontiano Nemeye for being my good housemate and work colleague in Mbita, Mr Maxwell Billah, Dr Susan Dimbi and Dr Samira Mohamed. Susan, I thank you for sheltering me during my one month stay in Nairobi for the statistical course back in 2000!

My sincere thanks go to Mr Anthony Wanjoya for all the statistical advice given to me for my work.

I would also like to acknowledge Prof Onesmo Ole Moi Yoi, Director of Research and Partnership, ICIPE, for supporting the study during financial problems. My thanks also go to the staff of Capacity Building, ICIPE, for their constant support, Ms Lucy Theuri, Mrs Wagendo Lizzie Chongoti, Ms Lisa Omondi and Ms Margaret Ochanda.

I would wish to acknowledge the help of Mrs Nadia Foondun, University of Mauritius, in university matters. Your prompt actions were highly appreciated.

This work was jointly funded by International Fund for Agricultural Development (IFAD) through the African Fruit Fly Initiative (AFFI) and by the International Center of Insect Physiology and Ecology (ICIPE) through the African Regional Postgraduate Program in Insect Science (ARPPIS) supported by the Human Resource Development for Scientific and Technological Capability in Arthropod Science.

Now to the ones who were miles away from me but always very close at heart;

I would like to thank Keith and Paulette for your constant support on e-mail! Keith, thanks so much for your comments on all the chapters of this thesis. Your patience in reading through all this was highly appreciated.

My dearest Chris, since we met here in 2002, you have been with me through everything. Your e-mails, calls and chats were essential in keeping me stable and happy in this place here. I would like to thank you very much for that.

Finally, I would like to convey my heartfelt thanks to my dearest family, my mother, Sunita, father Vijay, and my sisters, Nandita and Yashvee and their husbands Stuart and John, my uncle Ramesh and my grandmother, "Dadi", Gowtry for their support, care and encouragement throughout my stay in Kenya. We stayed knitted throughout my study period despite distances.

Abstract

In sub-Saharan Africa, three fruit flies: *Ceratitis cosyra* (Walker), *C. fasciventris* (Bezzi) and *C. capitata* (Wiedemann) (Diptera: Tephritidae) are important pests of commercial fruits. *C. cosyra* and *C. fasciventris* affect production of mangoes in the region, their damage causing high losses in local and export markets for fresh mango produce. *C. capitata* is a notorious pest of commercial fruits worldwide and yet in sub-Saharan Africa, it is the least important mango pest. It was considered here since it is the most thoroughly studied fruit fly in the world and can be set as a standard for comparison with other flies in the study.

One of the control methods being developed to control fruit fly pests is through the use of food baits (food attractants mixed with a killing agent). Control of fruit flies using food baits requires a thorough understanding of feeding behaviour of adult flies. The principal aim of this study was therefore to provide a rational basis for implementation of food baits in the management of *C. cosyra*, *C. fasciventris* and *C. capitata* by elucidating the biology of their feeding behaviour. The patterns and sites of feeding activities of the flies were determined at different scales, from laboratory sized cages to screenhouses. This was followed by a study on the contribution of natural food sources to longevity, fecundity and reproductive behaviour of the three fruit fly species. In order to determine differences in nutritional requirements of the three fruit fly species, the intake of sugar and protein by males and females were quantified. Feeding and olfactory responses of flies to natural and artificial food sources were determined in outdoor field cages.

Feeding activities of all three fruit fly species were found to be confined mostly to host trees, occurring mainly on the upper leaf surfaces which often contained honeydew, birds' droppings and accumulated dust but sometimes also bore no obvious sign of food. Feeding was also recorded on fruits. Flies exhibited diel patterns of feeding activities which varied according to species and sex. Males of *C. fasciventris* and *C. capitata* restricted their feeding activities during times which were not allocated to their sexual activities (calling and mating). *C. capitata* being a day mating species fed mostly during late afternoon hours while *C. fasciventris*, being a dusk mating species preferred to feed in the morning hours.

For *C. cosyra*, though also a dusk mating species, frequency of feeding by males did not differ significantly throughout the day.

Without a sugar source, flies of all three species did not survive beyond 3 days. Fly longevity was sustained by carbohydrate obtained from natural food sources such as fruit juice and honeydew for more than four weeks after adult emergence. Fecundity of flies was higher when fed on a source of protein than when fed on a source of sugar only. Nutrition significantly influenced reproductive behaviour of flies of the three species. Flies of all species fed on a protein rich diet had a higher frequency of calling, mating and oviposition than flies on a protein poor diet.

Intake of sugar for all adult flies was highest soon after emergence. Peak of protein intake for males and females of *C. cosyra* occurred in their third week of adult life, whilst for *C. fasciventris* and *C. capitata*, peak in protein intake by males and females occurred in the first week. Peak of protein intake for females of *C. fasciventris* and *C. capitata* was followed by peaks in egg laying. Total sugar intake was found to be similar for males and females of the three fly species. With respect to protein however, males and females of *C. cosyra* were found to consume less in total than males and females of *C. fasciventris* and *C. capitata*. Females of *C. cosyra* consume less protein than males while females of *C. fasciventris* and *C. capitata* consume more protein than males.

Responses to food sources were species and sex specific. Adult physiological states were found to affect fly responses to food odours. Nutritional state of a fly was the most influential factor in guiding responses to food odours. Effects of nutritional state and age were additive. When protein fed, there were no significant differences in attraction of food odours to juvenile and mature flies. However, attraction to food odours increased significantly with increasing age for protein-deprived females of all species. Mating status was the least important factor in influencing fly responses to food sources. Finally, odours from natural food sources were found to be more attractive than odours from artificial food sources for all fly species.

These findings have practical implications on the (1) establishment of protocol for evaluation of food baits, and (2) pattern of implementation of food baits in fruit fly infested

areas. Since flies of different species were found to have specific temporal patterns of responses to food sources, it is more appropriate to evaluate food baits at the time of maximum response to food sources with respect to both time of the day and age of the flies. Futhermore, feeding history of the flies was found to influence attractiveness to food odours, and therefore in testing of proteinaceous baits, flies should be deprived of protein for maximum response to the baits. Since natural food sources were found to influence fly survival, reproduction and feeding history, and artificial food baits were found to compete well with natural food sources, distribution of food baits in orchards during control programmes should be adjusted spatially according to composition and abundance of natural foods for the flies in the infested regions.

1. General Introduction

1.1 Distribution of fruit flies and general life cycle

Fruit flies belong to the family Tephritidae which includes about 4000 species. It is amongst the largest families of the order Diptera and one of the most economically important. The larvae of most species develop in the seed-bearing organs of plants, and about 35% of species attack soft fruits, including many commercial fruits; hence the name fruit flies (White & Elson-Harris, 1994). The larvae of about half of the species in the family develop in flowers, or their larvae are miners of leaf, stem or root tissue. Very few species are non-phytophagous. About twenty species of Tephritidae have been used as biological control agents of adventive weeds of the family Asteraceae (White & Elson-Harris, 1994).

The distribution of the Tephritidae is virtually worldwide. The major frugivorous pest genera are as follows:

- *Anastrepha* spp. are found in South and Central America and the West Indies.
- *Bactrocera* spp. are native to tropical Asia, Australia and the South Pacific regions with a few species found in Africa and warm temperate areas of Europe and Asia.
- *Ceratitis* spp. are native to tropical Africa.
- *Dacus* spp are mostly found in Africa infesting fruits of Cucurbitacea.
- *Rhagoletis* spp. are found in South and Central America, mostly on Solanaceae, and in the temperate areas of Europe and North America.

The family divides naturally into two major groups on the basis of physiological and ecological characteristics: (i) the univoltine species (having one generation per year), which usually have a winter diapause and inhabit the more temperate regions of the earth (e.g *Rhagoletis* spp) and (ii) the multivoltine species (having more than one generation per year) which have no obvious diapause and inhabit warmer regions (e.g. *Ceratitis*, *Dacus* and *Anastrepha* spp) (Bateman, 1972).

Host range of fruit flies varies considerably, often among closely related species. Many species are strictly monophagous, for example, *Bactrocera oleae* (Gmelin), which breeds only in olives, but some pest species are remarkably polyphagous, for example, *Ceratitis capitata* (Wiedemann), which has been reported from more than 300 hosts (Liquido et al.,

1990). Probably the majority of Tephritidae, however, are oligophagous, breeding in a few related or ecologically and chemically similar hosts (White et al., 2001).

In the typical developmental cycle of a frugivorous adult fly, the mature female inserts its eggs, using its long extendible ovipositor, beneath the skin of suitable host fruits. Eggs are laid either singly or in clutches. Depending on species, the number of eggs laid by a female fruit fly during her lifetime varies between 50 to over 1000 eggs, which usually hatch after 2 to 20 days (White & Elson-Harris, 1994). Immediately after hatching the larvae begin to feed and burrow into the pulp of the host. In larger fruits, larvae usually move towards the centre, which may offer them some protection from hymenopterous parasitoids and predators such as birds, bats and monkeys. Larvae of the frugivorous species in the family Tephritidae have a pair of mouthhooks, used for maceration of food substrates as well as cutting exit holes in fruit skins, and a simple median oral opening for intake of fluid (White & Elson-Harris, 1994; Drew & Yuval, 2001). There are three larval instars inside the fruit. The duration of the larval stage varies between species. It ranges from 1 to 5 weeks and in some species such as the temperate apple maggot fly, *Rhagoletis pomonella* (Walsh), larvae can over-winter in fruits.

The larvae of many of the fruit feeders can jump along the ground to find suitable pupation sites in the soil and this is a common feature of the Dacinae (e.g. *Bactrocera* and *Ceratitis* spp) (Christenson & Foote, 1960). Fruit flies stay as pupae between 1- 4 weeks and some species can over-winter as pupae. Emerging adults crawl upward through the soil.

Soon after adult emergence, an individual starts foraging for food. Adult tephritids require a variety of nutrients in order to survive, fuel their various activities and most species require a protein source to realise their reproductive potential (Tsitsipis, 1989). Natural food for adult flies include fruit juices, homopteran honeydew, nectar, plant sap exuding from trunk, stem, leaf or fruit injuries and glandular plant secretions (Christenson & Foote, 1960; Bateman, 1972; Hendrichs & Hendrichs, 1990; Hendrichs et al., 1991; Malavasi et al., 1983). As the fly matures, it starts engaging into other resource oriented behaviour such as mate-oriented behaviour and oviposition-site oriented behaviour. Adult longevity varies between 1 month to up to a year depending on species, fly size, their diet and climatic conditions (Christenson & Foote, 1960; White & Elson-Harris, 1994; Aluja, 1994).

The associations of bacteria with different stages in the life cycle of many fruit flies in relation to nutrition have been studied for many years (Drew & Lloyd, 1987; Fletcher, 1987; Drew & Lloyd, 1989; Howard, 1989). Drew & Lloyd (1989) found that the

predominant microflora within the tropical/subtropical Dacinae comprise of bacteria belonging to the family *Enterobacteriaceae* (termed Fruit Fly Type Bacteria or FFT bacteria). These FFT bacteria colonise the alimentary tract of adult flies and are distributed onto host fruit surfaces by mouthing and regurgitation of crop contents. During oviposition, some of these bacteria are introduced into the host fruit where they grow in association with developing larvae causing damage to host fruit tissue. FFT bacteria have also been shown to be an important natural source of protein for adult flies and the latter are strongly attracted to the odours produced by the bacteria (Drew & Lloyd, 1989).

1.2 Mango-infesting fruit flies in Africa

Horticulture is the fastest-growing agricultural sector in Africa, providing income and employment. There is an important and growing trade of fresh fruit produce from Africa and mango, *Mangifera Indica* L. is among one of the major fruits traded. In 2002, about 2 million metric tons of mangoes were produced in Africa, out of which 1.4% was exported to European and Middle East markets, worth over US$18 million (FAO, 2002). Mango production is however greatly hampered by fruit flies. An estimated 40% of the mango production in Africa is lost due to fruit flies. Fruit infestation rates vary among countries and seasons, ranging from 5% to 100% (Lux et al., 2003).

Mango, native to South East Asia, has been introduced to many other tropical regions including Africa. In each region where mango is grown, it is attacked by fruit flies from different genera: *Bactrocera* in Asia, *Anastrepha* in America and in Africa by the genus *Ceratitis* and *Bactrocera* (White & Elson-Harris, 1994; Lux et al., 2004; EPPO, 2004).

The genus *Ceratitis* is endemic to the Afro-tropical region and contains about 65 species, many of which are highly polyphagous. One species, *C. capitata*, the Mediterranean fruit fly (medfly), has spread to almost all tropical and warm temperate areas of the world (White & Elson-Harris, 1994). In California and Florida, U.S.A, repeated medfly introductions have threatened the exports of a multi-billion dollar fruit industry and these have required recurrent emergency eradication actions costing millions of U.S dollars using insecticide applications. Since 1994, costly prevention programmes using the Sterile Insect Technique (SIT) have been in place in that area to prevent major outbreaks of medfly (Hendrichs et al., 2002).

Results of surveys carried out in various countries in sub-Saharan Africa have revealed five species in the genus *Ceratitis* to be important pests of mangoes: *Ceratitis cosyra* (Walker), *Ceratitis fasciventris* (Bezzi), *Ceratitis rosa* Karsch, *Ceratitis anonae* Graham and to a much lesser extent *C. capitata*. A new invasive species in the *Bactrocera dorsalis* (Hendel) group, which is capable of devastating the mango industry, has recently been detected in Kenya (Lux et al., 2004). *B. dorsalis* (Hendel) group is a complex native to a region which extends between tropical Asia and northern Australia. It contains many highly polyphagous species and is of major quarantine concern to Europe and America.

1.2.1 C. cosyra

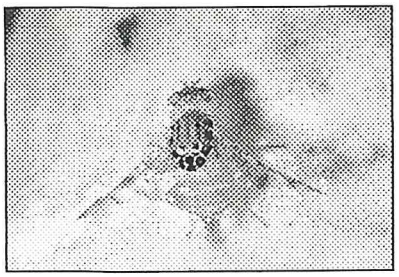

Fig. 1. Adult female of C. cosyra *(R. Copeland, ICIPE)*

C. cosyra, the mango fruit fly or marula fly has been known as *Pardalaspis cosyra*, *P. parinarii* and *Trypeta cosyra* (fig. 1). It is distributed across eastern, central and western Africa and also in parts of southern Africa (De Meyer, 1998). *C. cosyra* has often been reported to be the dominant fruit fly species attacking mango fruits in sub-Saharan Africa (Malio, 1979; Javaid, 1986; Mukiama & Muraya, 1994; Labuschagne et al., 1996). Lux et al. (2003) reported 20-30% loss in mango crops due to this pest alone. White & Elson-Harris (1994) list other commercial hosts for this species: common guava (*Psidium guajava* L.), sour orange (*Citrus aurantium* L.), avocado (*Persea Americana* M.), wild custard-apple (*Annona senegalensis* Pers.) and finally maroola plum (*Sclerocarya birrea* A. Rich.) which is also reported as its wild host and is native to Africa and related to mango. De Meyer (1998) and Steck (2000) list also other hosts for this species from various other plant families. Despite the importance of *C. cosyra* as an important mango pest in Africa, very few studies have been conducted so far on its biology. Malio (1979) made some observations on the life cycle of this fly. The first egg-mass was reported to be laid 5 days after emergence of the female. Pupal stages were found to last 9-12 days at 28.8-32.7° C. Adults of both sexes lived up to 41 days. The adult fly can be recognised by its characteristic pattern of yellow wing bands and three black areas in the apical half of the scutellum. Males of the mango fly do not respond to commercially available parapheromones or male lures such as Trimedlure, CueLure or Methyl Eugenol (White & Elson-Harris, 1994). Instead they

respond to terpinyl acetate and females respond to protein bait such as NuLure (Lux et al., 2003).

1.2.2 C. fasciventris and C. rosa

Fig. 2. Adult male of C. fasciventris *(R. Copeland)*

The next important pest species of mango are the Natal fly, *C. rosa* and its close relative *C. fasciventris* (White & Elson-Harris, 1994; De Meyer, 2001). *C. fasciventris* was formerly considered to be a mere variation to the Natal fruit fly but it is now considered to be a separate species (De Meyer, 2001). Both *C. rosa* and *C. fasciventris* are known to attack a wide variety of indigenous and commercial fruits (White & Elson-Harris, 1994; De Meyer, 2001). They are both also recorded from common guava, coffee (*Coffea* spp), avocado and peach (*Prunus persica* L.) among others. The Natal fruit fly is tolerant to a broad range of temperatures and seems capable of establishing into cooler areas than *C. capitata* (Lux et al., 2003). It is highly competitive and where both *C. rosa* and *C. capitata* occur, for example in Mauritius, *C. rosa* has been reported to even displace *C. capitata*, hence the reason for quarantine concerns in Europe and America (Hancock, 1989). *C. rosa* seems to be restricted to southern and eastern Africa while *C. fasciventris* has a more scattered distribution and it is mainly a Central Africa species venturing into Ethiopia and Kenya (De Meyer, 2001). Morphologically the males can be separated by distinct characters. The male *C. fasciventris* has setae covering the apical third of the tibia whilst the male *C. rosa* has setae covering more than half of the tibia (De Meyer, 2001). Females of the two species cannot be separated unambiguously. Again, despite the high quarantine concerns for these two fruit fly pests and their economic importance in Africa, their place of origin, very little has been published on their biology. Moreover, since *C. fasciventris* is recently described as a new species, its biology is totally unknown.

Quilici & Franck (1999) described the biology of the Natal fruit fly. Females of the fly lay eggs in clutches which hatch after 2-3 days. The larval stage lasts between 8-12 days and the pupal stage takes another 12-15 days. An adult fly lives for up to 2 months and the female has a total fecundity of 400 eggs. The preoviposition period of the female is between 10-12 days. The fly is known to be a dusk mating species (Quilici et al., 2002). *C.*

rosa and *C. fasciventris* respond both well to the male lure, Trimedlure, and females of both species respond to baits such as NuLure (White & Elson-Harris, 1994; Lux et al., 2003).

1.2.3 C. anonae

C. anonae, an important pest of mango in the western and central parts of Africa as well as in western parts of Kenya, has been recorded also from robusta coffee (*Coffea canephora*) Pierre ex Froehner and tropical almond (*Terminalia catappa* L.) (White & Elson-Harris, 1994; Lux et al., 2003). There are old records from other fruits: avocado, guava, soursop (*Annona muricata* L.) and strawberry guava (*P. littorale* Raddi) (White & Elson-Harris, 1994). The adult is recognised by its characteristic patterns of brown wing bands and three black areas in the apical half of the scutellum. The male *C. anonae* has broad feathering on the mid tibia. There is no publication as yet as to the biology of this fly.

1.2.4 C. capitata

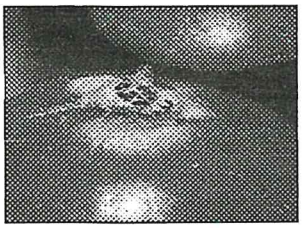

Fig. 3. Adult male of C. capitata *(R. Copeland, ICIPE)*

The Mediterranean fruit fly, *C. capitata*, is amongst the most studied tephritid in the world due to its extensive geographical distribution. It is considered as the most serious pest in the entire family (White & Elson-Harris, 1994). The species is highly polyphagous. The medfly has also a high reproductive potential with an adult female laying over 700 eggs during her lifetime under laboratory conditions when fed with sugar and protein (Carey et al., 1998). Eggs hatch after 2-5 days. The larval stage of the fly as well as its pupal stage last between 6 to 11 days. An adult fly can live for up to 2-3 months (White & Elson-Harris, 1994). Adult males respond to Trimedlure and also to terpinyl acetate whilst females respond to protein baits such as NuLure (White & Elson-Harris, 1994; Lux et al., 2003). The behaviour of the adult fly has also been well studied, in particular its mating behaviour due to the implementation of the Sterile Insect Technique for the control (eradication, prevention and suppression) of the pest in several countries in the world: Mexico, U.S.A, Latin America and recently in Tunisia, Israel and Jordan, Australia, and South Africa (Hendrichs et al., 2002). Despite being a highly destructive pest in other parts of the world, the medfly has been rated as being of rather minor importance to mango production in Africa (Lux et al., 2003).

1.3. Fruit Fly management strategies

Presence of several mango-infesting flies in Africa poses serious constraints to the production of fresh fruits in the area. The recent introduction of a uniform, strict quarantine and Maximum Residue Level regulations in the European Union (EU) will affect fruit production practices and the costs in producing these fruits.

For a highly lucrative market such as America, all the mango-infesting flies mentioned above are considered as serious quarantine pests. The status of quarantine pest even applies to *C. capitata* which poses a threat to fruit growing areas such as Florida and California in the U.S.A which have costly operational programs for preventing the entry of this pest species.

The development of high quality fruit industry in a region with several endemic fruit fly species is a challenge. In sub-Saharan Africa, fruit farms rarely exist as uniform production blocks. They are often surrounded with wild and abandoned fruit trees that can be good reservoirs for a range of pests including fruit flies.

Lux et al. (2003) reported that the majority of small scale fruit growers in Africa made no attempts to control fruit flies. The farmers tended to harvest fruits before they matured fully to evade damage by fruit flies. As such the fresh fruits produce are of poor quality and do not conform to the norms set by markets such as Europe. Moreover, such unripe fruits may still contain inconspicuous eggs or very young larvae which can still cause rejection of the fresh produce by stringent markets. In the few big fruit production farms, fruit flies are often controlled by blanket pesticide sprays. These blanket pesticide sprays provide control against not only the fruit fly pests but several other pest species. Some insecticides used have a systemic action and are absorbed into the fruit and also kill the larvae or eggs that may be present. There are several disadvantages however with this type of control: (1) deleterious effects on non-target organisms including beneficial insects (predators, parasitoids) (2) environmental damage and (3) residue problems with the harvested product.

Two fruit fly management strategies have been discussed by Vijaysegaran (1996) and Lux et al. (2003). The first strategy involves population control/suppression techniques to reduce yield losses. In order to be able to export fruits to quarantine sensitive markets, fruit fly control/suppression is often accompanied by postharvest quarantine disinfestations

treatments. The second strategy involves eradication of the pests to certify fly free zones which is very costly and can be justified only when a high productive industry is threatened. Moreover, in a situation where several pest species of the fruit are present and are endemic to the area, the eradication strategy is not viable. Therefore for the sub-Saharan region, the best approach for fruit fly control is through population control/suppression which is usually more successful when various techniques for control are applied in combination. The different techniques to achieve fruit fly control are outlined as follows.

1.3.1. Natural enemies

The eggs, larvae and puparia of fruit flies are attacked by a number of parasitic Hymenoptera, particularly by species of Opiinae belonging to the family Braconidae (Christenson & Foote, 1960). A few species of African fruit fly parasitoids have been used and established outside Africa to control *C. capitata* and some *Bactrocera* spp (Lux et al., 2003). Vargas et al. (2001) found high rates of parasitism for *C. capitata* (over 40%) in coffee field in Hawaii. Similar parasitism rates were also found for *C. capitata* in coffee in Kenya at the peak of the season. However, for most of the year, parasitism was lower ranging from 1% to 10% (Lux et al., 2003). Lux et al. (2003) found, however, negligible parasitism rates of fruit flies infesting other cultivated fruits such as mango. Certainly, parasitoids are capable of high parasitism rates at certain times of the year but they are not able to suppress fruit fly populations significantly when used on their own (Vijaysegaran, 1996; Lux et al., 2003). Therefore indigenous parasitoids should be encouraged in fruit growing areas and methods of fruit fly control that are not harmful to them should be adopted.

Tephritids are subject to predation at various stages in their lifetime. As eggs or larvae, they can be eaten by birds and rodents which eat fallen fruits or ripe fruits on the trees. Ants and other ground dwelling insects such as Coleoptera, Neuroptera, Dermaptera and Hemiptera can do significant damage to larvae in fallen fruits and pupae in the soil (Bateman, 1972). Conservation of these species in orchards may therefore be of practical importance.

Pathogens such as fungi and bacteria are known to cause mortality in tephritids (Fletcher, 1987). A number of strains of the entomopathogenic fungi *Metarhizium anisopliae* (Metsch) Sorok were found to be effective against puparia of *C. cosyra*, *C. fasciventris*, and *C. capitata* (Ekesi et al., 2002). Results of the latter study suggested that soil inoculation

with *M. anisopliae* under mango trees might form an important component of integrated pest management strategies in areas where these three species of fruit fly coexist. Moreover, possibilities for control of adult flies by entomopathogens have also been demonstrated in the field in Kenya (Lux et al., 2003). Baiting stations housing fungal spores of *M. anisopliae*, used as auto-inoculative devices were used to target the adult flies and have been found to be effective in reducing fruit fly populations.

1.3.2. Crop Hygiene

Fallen fruits are often breeding grounds for fruit flies. Crop hygiene has been encouraged in many individual orchards and gardens. This includes the destruction of these fruits by burning or burying deep in the soil.

Crop hygiene is not easy to implement and enforce but it is important to be included in an overall pest management programme.

1.3.3. Fruit wrapping/bagging

Wrapping or bagging of individual fruits to prevent oviposition by fruit flies has been extensively used in the Asian Region in carambola and mango production. The bags provide a continuous physical barrier from the time of bagging to harvest, which prevents female from laying their eggs in the fruit (Vijaysegaran, 1996).

Fruit wrapping is effective in producing fruit of good quality and it is also simple to apply and is safe to the environment provided the bags are well disposed and recycled.

There are some limitations as to the adoption of this technique on a large scale. The trees need to be at manageable heights to be easy to handle which is not the case in most African fruit farms. This technique is also labour intensive and can be very costly.

1.3.4. Male lures

Parapheromones or male lures are chemical compounds that attract the males of some fruit fly species (Cunningham, 1989). The most important male lures include methyl eugenol which attracts several *Bactrocera* species, trimedlure which attracts *C. capitata*, *C. fasciventris*, *C. rosa*, cue-lure which attracts *Bactrocera cucurbitae* Coquillet, pest of cucurbit crops and many other species, vertlure which attracts *Dacus Vertebratus* Bezzi, also another pest of cucurbit crops, and terpinyl acetate which attracts a range of *Ceratitis* species including *C. cosyra* (White & Elson-Harris, 1994). The strong attraction of males to

their respective parapheromones has been put to good use in male annihilation programmes as well as in detection and monitoring programmes.

Male annihilation programmes work on the mass trapping of wild males until they are completely eliminated. The parapheromones are usually mixed with insecticide and placed in traps or adsorbed in plywood or fibreboard for distribution in fruit growing areas. Methyl eugenol and cue-lure are highly attractive lures which have been used in eradication or suppression programmes of exotic fruit fly species in several places including the African region, more precisely in Mauritius (Vijaysegaran, 1996; Cunningham, 1989; Seewooruthun et al., 1998). Trimedlure which attracts a few mango pests in Africa has been used in several detection and monitoring programmes (Shelly & Pahio, 2002). Its attractiveness, however, is weak compared to methyl eugenol and cue-lure for their respective target species which makes it a less suitable candidate for use in male annihilation programmes. Response of terpinyl acetate to *C. cosyra* has also been discussed to be not strong enough for direct fruit fly control but only sufficient for reliable monitoring (Lux et al., 2003). Therefore, use of male lures in the control of the endemic mango-infesting *Ceratitis* spp in Africa, remains not an option for the time being. As for the new invasive mango pest, *Bactrocera* spp, methyl eugenol can be used in control programmes. However, one major disadvantage with male lures is that they are costly (Vargas et al., 2000) and have to be imported. Male lures used in control are required in large quantities since they are usually densely distributed over large areas. Use of male lures alone by an individual grower, in an area where fruit flies are endemic and also against a highly polyphagous invasive species appears to be of little value since large numbers of gravid females, unaffected by the lure, will always be present, both as resident populations and immigrating populations from surrounding areas (Vijaysegaran, 1996).

1.3.5. Sterile Insect Technique

Sterile Insect Technique (SIT) involves the release of sterilized males in massive numbers, which will mate with the wild females producing infertile eggs eventually decreasing to even eradicating the fruit fly population. Over the last years, there has been an increasing use of SIT in prevention, suppression and eradication of pest fruit fly species in several parts of the world (Hendrichs et al., 2002). Advantages with SIT are that it is highly species specific, it does not release exotic agents into new environments unlike other biologically-based control methods and also does not introduce new genetic material into existing

populations as the released organisms are not self-replicating. The use of SIT, however, involves a high degree of expertise, time and funds. Important components include: appropriate diets and mass rearing techniques to produce 500-1000 million individuals per week; to release males only for better efficiency of control (a technique which was made possible recently for medflies by the development of genetic sexing strains); suitable techniques to sterilise flies; handling, transport and release methods; methods to evaluate the progress of the control or eradication programme (Vijaysegaran, 1996). In addition to all these components, evaluation and improvement of sterile male performance have to be carried out for a better efficiency of the SIT and this entails advanced research into the mating behaviour of the sterile males. The problem with implementing SIT against one species in an area with multiple pest species is that elimination of a major pest can lead to resurgence of another fruit fly pest. And if fruit fly species native to a region are being the targets for eradication, then the conservation of our biodiversity is questioned.

1.3.6. Food baits

Female fruit flies need a supply of proteins for sexual maturation and baits based on such materials have been developed in the 1930's and used successfully from the 1950's onwards for control of these pests throughout the world (Roessler, 1989). For control of fruit flies, the protein baits are usually mixed with an insecticide and applied to delimited foliage areas (spots) on trees in the fruit growing and surrounding areas. The concept of a bait spray is based on the principal that the bait in the spray mixture should attract flies to the spray, where they feed and die.

Food baits attract both females and males, usually the former are attracted to a larger extent than the latter. They have a broad spectrum of attractiveness and as such are also used in detection and monitoring programmes where they can target many species at a time including invasive female flies and invasive males that do not respond to commercially available male lures. Standard food baits are produced by several companies in Europe and U.S.A. The baits produced are hydrolysed proteins derived from corn syrup or yeast. The baits themselves are not expensive but their formulation as liquid causes an increase in shipment costs (Lux et al., 2003). As such some countries such as Malaysia have developed their own commercial baits for fruit flies, derived from brewery waste (Vijaysegaran, 1996). In Africa, a bait developed by the African Fruit Fly Initiative (AFFI), International Centre of Insect Physiology and Ecology (ICIPE), made from locally available brewery

waste have been found to be attractive to mango-infesting flies (Lux et al., 2003) . Recently, dry food baits have been developed in America, following evaluation of volatiles emanating from protein hydrolysates. The dry food bait consists of three components: putrescine, trimethylamine and ammonium acetate. These baits are highly effective and are efficient for several weeks in the field but are relatively expensive.

Use of food baits in the control of fruit flies drastically reduces the use of insecticides as opposed to using insecticides alone. There is also reduced or no application of insecticide to the fruit thereby resulting in little or no insecticide residues in the fresh produce. Though the impact on beneficial insects is much less when fruit flies are controlled using food baits compared to insecticidal cover sprays, a few adverse effects on beneficial insects still do occur (Michaud, 2003).

Since the 1950's malathion-bait sprays have been used extensively around the world for the control of fruit flies (Roessler, 1989). Malathion, a broad spectrum organophosphate insecticide, has been the insecticide of choice due to its low mammalian toxicity, low price and low levels of fruit fly resistance (Roessler, 1989). Though highly effective in control of fruit flies, the use of malathion has been controversial because of human health concerns and harmful effects on beneficial insects (Peck & McQuate, 2000). As such replacements of malathion in the food bait mixture have been under consideration which can provide acceptable fruit fly control, are less harmful to beneficial insects and demonstrate lower mammalian and environmental toxicity (Burns et al., 2001; Vargas et al., 2001). One of these replacements include Spinosad which is an insecticide derived from metabolites of soil-dwelling bacteria. Spinosad kills primarily by ingestion, unlike malathion which is both a contact and stomach poison and thus has an increased risk of killing any insect, including non-target ones, landing on a leaf containing the poisoned bait (Vargas et al., 2002).

Pathogens as mentioned *earlier* can be an alternative to pesticides used in baits. Fungal pathogens incorporated in auto-inoculative devices containing food baits have been tried as a control strategy in Kenya and have been found to provide considerable reduction on fruit fly populations in orchards (Lux et al., 2003). Its effects on non-target organisms are yet to be evaluated but there are indications that these fungal isolates are quite benign to parasitoids.

There is currently a move towards the adoption of spatially localised baiting such as traps and bait stations for the control of fruit flies (Heath et al., 2002). In Israel, recently, control of medflies was carried out successfully by mass trapping using dry food bait exposed in plastic traps containing a toxicant (Cohen & Yuval, 2000). A bait station functions on a similar basis as a trap. It contains an attractant and a killing agent (Heath et al., 2002). In South Africa, a semi-dry baiting station was recently developed called the M3 baiting station. The latter consists of bait formulated as a paste mixed with a pesticide and placed in a protective housing. In this way, the use of pesticide is even more restricted and contact with the fruit and the environment is also limited (Lux et al., 2003).

Bait stations would therefore be an efficient and environmental friendly way of controlling fruit flies in an area with multiple pest species. This control technique is suitable in an area of fragmented horticulture since it targets also migrating gravid females from surrounding reservoir hosts. Bait stations would certainly be affordable to the farmers, even more so if the food baits are available locally. Use of food baits in fruit fly control should be integrated with recognized post harvest treatments when fruit is destined for export markets.

1.4. Problem definitions and goals

From the different fruit fly control techniques reviewed, use of food bait stations seems to be the most suitable method for fruit fly management in Africa. Lux et al., (2003) proposed an integrated approach to management of fruit flies in Africa which combines the use of food bait stations with orchard sanitation, conservation of natural enemies and post harvest treatments in order to permit quality fruit production for domestic and export markets.

Control of fruit flies using food baits requires a thorough understanding of feeding behaviour of adult flies. Despite the wide use of food baits all over the world for the control of fruit flies in suppression and eradication programmes, feeding behaviour is the least studied of all tephritid behaviour (Drew & Yuval, 2001). Yet studies on feeding behaviour can have potentially strong practical impact on strategies and tactics for managing frugivorous flies (Hendrichs & Prokopy, 1994). In relation to the application of food baits, knowing what drives the flies to food can help adjust temporal and spatial application of food baits. Various factors are likely to influence fly feeding behaviour: (1) time of day, as an influence of environmental parameters such as temperature, relative humidity, light or

possibly regulated by timing of other behaviours such as reproductive behaviour, (2) presence of host trees and (3) physiological factors such as age, mating status, hunger status (orchards with abundant natural food sources for the pests or food-scarce orchards). Moreover, natural food sources in fruit growing areas are likely to influence effectiveness of food baits and therefore it is important to know the extent of the competition between natural food sources of flies and food baits used in control. In relation to crop hygiene, it is also important to know the contribution of various natural food sources to survival and reproduction of flies in order to enhance practices in the orchard that will enable a reduction in these food sources.

The general objective of this study is therefore to provide a basis for rational implementation of food baits in the management of three important mango pests in Africa: *C. cosyra*, *C. fasciventris* and *C. capitata* by elucidating the biology of their feeding behaviour.

So far, no quantitative data exist concerning feeding behaviour of *C. cosyra* though it is, to date recorded as the most serious pest of mangoes in Africa (Lux et al., 2003). *C. fasciventris* is a newly described species (De Meyer, 2001). Nothing is yet known of the biology of this fly as well as its feeding behaviour. *C. capitata*, though the least important mango pest in Africa, is still the most notorious pest around the world. It is also the most studied tephritid. However, a lot of work has been focussed rather on its mating behaviour due to the increasing use of SIT for control of this pest in several countries. There is now increasing research on feeding behaviour of medflies in relation to their sexual behaviour in order to increase the sexual competitiveness of sterile flies that are released during SIT programmes (Blay & Yuval, 1997; Papadopoulos et al., 1998; Kaspi et al., 2000; Kaspi & Yuval, 2000; Shelly & Kennelly, 2002; Shelly et al., 2002) . Therefore findings in this study can also contribute to knowledge in this field. Feeding behaviour of *C. capitata* has been observed in natural conditions in Egypt, in the Mediterranean area and in Israel, however preferences in feeding time and feeding sites, have been found to differ in the areas of study (Hendrichs & Hendrichs, 1990; Hendrichs et al., 1991; Warburg & Yuval, 1997). Results from this study can therefore be compared to these findings. Attractiveness of medflies of different physiological states to natural food sources (bird faeces and fruit juices) and proteinaceous lure (Staley's PIB-7) have only been determined in field cages in Hawaii (Prokopy et al., 1993; Prokopy et al., 1992) whereby bird droppings were found to be more attractive than odours emanating from proteinaceous bait. Considering variability

of non-reproductive behavioural activities of fruit flies in different environmental conditions, no information yet exists on the feeding behaviour of medflies in sub-Saharan Africa.

The three species studied, though all belonging to the *Ceratitis* genus, show differences in their host range. It is therefore interesting to compare the adult feeding behaviour of these flies as well as their feeding requirements and other general behaviour.

The research on adult flies of *C. cosyra*, *C. fasciventris* and *C. capitata* presented in this thesis consisted of three major objectives:

- Determine the diel and lifetime patterns of feeding activities of flies
- Determine the contribution of natural food sources to fly survival and reproduction
- Elucidate the feeding and olfactory responses of fruit flies towards artificial and local food sources.

1.5 References cited

1. Aluja, M. (1994) Bionomics and management of *Anastrepha*. Annual Review of Entomology, 39, 155-178.
2. Bateman, M.A. (1972) The ecology of fruit flies. Annual Review of Entomology, 17, 493-518.
3. Blay, S. & Yuval, B. (1997) Nutritional correlates of reproductive success of male Mediterranean fruit flies (Diptera: Tephritidae). Animal Behaviour, 54, 59-66.
4. Burns, R.E., Harris, D.L., Moreno, D.S., & Eger, J.E. (2001) Efficacy of spinosad bait sprays to control Mediterranean and Carribean fruit flies (Diptera: Tephritidae) in commercial citrus in Florida. Florida Entomologist, 84, 672-678.
5. Carey, J.R., Liedo, P., Muller, H.G., Wang, J.L., & Vaupel, J.W. (1998) Dual modes of aging in Mediterranean fruit fly females. Science, 281, 996-998.
6. Christenson, L.C. & Foote, R.H. (1960) Biology of fruit flies. Annual Review of Entomology, 5, 171-192.
7. Cohen, H. & Yuval, B. (2000) Perimeter trapping strategy to reduce Mediterranean fruit fly (Diptera: Tephritidae) damage on different host species in Israel. J Econ Entomol, 93, 721-5.

8. Cunningham, R.T. (1989). Parapheromones. In Fruit flies, their biology, natural enemies and control (eds A.S. Robinson & G. Hooper), Vol. 3A, pp. 221-229. Elsevier, Amsterdam.
9. De Meyer, M. (1998) Revision of the subgenus *Ceratitis* (Ceratalaspis) Hancock (Diptera: Tephritidae). Bulletin Entomological Research, 88, 257-290.
10. De Meyer, M. (2001) On the identity of the Natal fruit fly *Ceratitis rosa* Karsh (Diptera, Tephritidae). Entomologie, 71, 55-62.
11. Drew, R.A.I. & Lloyd, A.C. (1987) Relationship of fruit flies (Diptera: Tephritidae) and their bacteria to host plants. Annals of the Entomological Society of America, 80, 629-636.
12. Drew, R.A.I. & Lloyd, A.C. (1989). Bacteria associated with fruit flies and their host plants. In Fruit flies: Their biology, natural enemies and control (eds A.S. Robinson & G. Hooper), Vol. 3A, pp. 131-140. Elsevier Science Publishers, Amsterdam.
13. Drew, R.A.I. & Yuval, B. (2001). The evolution of fruit fly feeding behavior. In Fruit flies (Tephritidae): Phylogeny and evolution of behaviour (eds M. Aluja & A.L. Norrbom), pp. 731-749. CRC Press, Boca Raton.
14. Ekesi, S., Maniania, N.K., & Lux, S.A. (2002) Mortality in three African tephritid fruit fly puparia and adults caused by the entomopathogenic fungi, *Metarhizium anisopliae* and *Beauveria bassiana*. Biocontrol Science and Technology, 12, 7-17.
15. EPPO (2004) *Bactrocera zonata*. http://www.eppo.org/QUARANTINE/bactrocera_zonata/bactrocera.html
16. FAO (2002) FAOSTAT Agriculture Data. http://apps.fao.org/page/collections?subset=agriculture
17. Fletcher, B.S. (1987) The biology of dacine fruit flies. Annual Review of Entomology, 32, 115-144.
18. Hancock, D.L. (1989). Pest status; southern Africa. In Fruit flies; their biology, natural enemies and control (eds A.S. Robinson & G. Hooper), Vol. 3A, pp. 51-58. Elsevier, Amsterdam.
19. Heath, R.R., Schnell, R., Lavallee, S., Villatoro, D., Epsky, N.D., & Midgarden, D. (2002) Development of bait stations for suppressing fruit fly populations (abstract). In 6th International symposium on fruit flies of economic importance, pp. 11, Stellenbosch, South Africa.

20. Hendrichs, J. & Hendrichs, M.A. (1990) Mediterranean fruit fly (Diptera: Tephritidae) in nature: location and diel pattern of feeding and other activities on fruiting and nonfruiting hosts and nonhosts. Annals of the Entomological Society of America, 83, 632-641.
21. Hendrichs, J., Katsoyannos, B.I., Papaj, D.R., & Prokopy, R.J. (1991) Sexual differences in movement between natural feeding and mating sites and tradeoffs between food consumption, mating success and predator evasion in Mediterranean fruit flies (Diptera: Tephritidae). Oecologia, 86, 223-231.
22. Hendrichs, J. & Prokopy, R.J. (1994). Food foraging behaviour of frugivorous fruit flies. In Fruit flies and the sterile insect technique (eds C.O. Calkins, W. Klassen & P. Liedo), pp. 37-55. CRC press, Boca Raton.
23. Hendrichs, J., Robinson, A.S., Cayol, J.P., & Enkerlin, W. (2002) Medfly areawide sterile insect technique programmes for prevention, suppression or eradication: the importance of mating behaviour studies. Florida Entomologist, 85, 1-13.
24. Howard, D.J. (1989). The symbionts of *Rhagoletis*. In Fruit flies: Their biology, natural enemies and control (eds A.S. Robinson & G. Hooper), Vol. 3A, pp. 121-128. Elsevier Science Publishers, Amsterdam.
25. Javaid, I. (1986) Causes of damage to some wild mango fruit trees in Zambia. International Pest Control, 28, 98-99.
26. Kaspi, R., Taylor, P.W., & Yuval, B. (2000) Diet and size influence sexual advertisement and copulatory success of males in Mediterranean fruit fly leks. Ecological Entomology, 25, 279-284.
27. Kaspi, R. & Yuval, B. (2000) Post-teneral protein feeding improves sexual competitiveness but reduces longevity of mass-reared sterile male Mediterranean fruit flies (Diptera:Tephritidae). Annals of the Entomological Society of America, 93, 949-955.
28. Labuschagne, T., Brink, T., Steyn, W.P., & De Beer, M.S. (1996) Fruit flies attacking mangoes - their importance and post harvest control. Yearbook South African Mango Growers' Association, 16, 17-19.
29. Liquido, N., Cunningham, R.T., & Nakagawa, S. (1990) Host plants of the Mediterranean fruit fly (Diptera: Tephritidae) on the island of Hawaii (1949-1985) survey. J Econ Entomol, 83, 1863-1868.

30. Lux, S.A., Copeland, R., White, I.M., Manrakhan, A., & Billah, M. (2003) A new invasive fruit fly species from *Bactrocera dorsalis* group detected in East Africa. Insect science and its applications.
31. Lux, S.A., Ekesi, S., Dimbi, S., Mohamed, S., & Billah, M. (2003). Mango-infesting fruit flies in Africa: Perspectives and limitations of biological approaches to their management. In Biological control in IPM systems in Africa (eds P. Neuenschwander, C. Borgemeister & J. Langewald). CAB International.
32. Malavasi, A., Morgante, J.S., & Prokopy, R.J. (1983) Distribution and activities of Anastrepha fraterculus (Diptera: Tephritidae) flies on host and nonhost trees. Annals of the Entomological Society of America, 76, 286-292.
33. Malio, E. (1979) Observations on the mango fruit fly *Ceratitis cosyra* in the Coast Province, Kenya. Kenya Entomologist's Newsletter, 7.
34. Michaud, J.P. (2003) Toxicity of fruit fly baits to beneficial insects in citrus. Journal of Insect Science, 3.8, 1-9.
35. Mukiama, T.K. & Muraya, J.K. (1994) Ceratitid fruit flies infesting fruit crops in Kenya. Insect science and its applications, 15, 155-159.
36. Papadopoulos, N.K., Katsoyannos, B.I., Kouloussis, N.A., Economopoulos, A.P., & Carrey, J.R. (1998) Effect of adult age, food, time of day on sexual calling incidence of wild and mass-reared *Ceratitis capitata* males. Entomologia Experimentalis et Applicata, 89, 175-182.
37. Peck, S.L. & McQuate, G.T. (2000) Field tests of environmentally friendly malathion replacements to suppress wild Mediterranean fruit fly (Diptera: Tephritidae) populations. J Econ Entomol, 93, 280-9.
38. Prokopy, R.J., Hsu, C.L., & Vargas, R.I. (1993) Effect of source and condition of animal excrement on attractiveness to adults of *Ceratitis capitata* (Diptera: Tephritidae). Environmental Entomology, 22, 453-458.
39. Prokopy, R.J., Papaj, D.R., Hendrichs, J., & Wong, T.T.Y. (1992) Behavioural responses of *Ceratitis capitata* flies to bait spray droplets and natural food. Entomologia Experimentalis et Applicata, 64, 247-257.
40. Quilici, S. & Franck, A. (1999). The Natal fruit fly. In Technical bulletins on the crop pests of the Indian Ocean region. CIRAD, Reunion.

41. Quilici, S., Franck, A., Peppuy, A., Dos Reis Correia, E., Mouniama, C., & Blard, F. (2002) Comparative studies of courtship behaviour of *Ceratitis* spp. (Diptera: Tephritidae) in Reunion Island. Florida Entomologist, 85, 138-142.
42. Roessler, Y. (1989). Insecticidal bait and cover sprays. In World crop pests, fruit flies: Their biology, natural enemies and control (eds A.S. Robinson & G. Hooper), Vol. 3B, pp. 329-335. Elsevier.
43. Seewooruthun, S.I., Permalloo, S., Gungah, S., Soonnoo, A.R., & Alleck, M. (1998) Eradication of an exotic fruit fly from Mauritius. In Fifth international symposium on fruit flies of economic importance (abstract), pp. 49. University Sains Malaysia, Penang, Malaysia.
44. Shelly, T.E. & Kennelly, S.S. (2002) Influence of male diet on male mating success and longevity and female remating in the Mediterranean fruit fly (Diptera: Tephritidae) under laboratory conditions. Florida Entomologist, 85, 572-579.
45. Shelly, T.E., Kennelly, S.S., & McInnis, D.O. (2002) Effect of adult diet on signalling activity, mate attraction, and mating success in male Mediterranean fruit flies (Diptera: Tephritidae). Florida Entomologist, 85, 150-155.
46. Shelly, T.E. & Pahio, E. (2002) Relative attractiveness of enriched ginger root oil and trimedlure to male Mediterranean fruit flies (Diptera: Tephritidae). Florida Entomologist, 85, 545-551.
47. Steck, G. (2000). *Ceratitis cosyra* (Walker) (Diptera: Tephritidae), Rep. No. 403. Florida Department of Agriculture and Consumer Services, Division of Plant Industry.
48. Tsitsipis, J.A. (1989). Nutrition. In World crop pests, fruit flies: Their biology, natural enemies and control (eds A.S. Robinson & G. Hooper), Vol. 3A, pp. 103-119. Elsevier.
49. Vargas, R.I., Miller, N.W., & Prokopy, R.J. (2002) Attraction and feeding responses of Mediterranean fruit fly and a natural enemy to protein baits laced with two novel toxins, phloxine B and spinosad[a]. Entomologia Experimentalis et Applicata, 102, 273-282.
50. Vargas, R.I., Peck, S.L., McQuate, G.T., Jackson, C.G., Stark, J.D., & Armstrong, J.W. (2001) Potential for areawide integrated management of Mediterranean fruit fly (Diptera: Tephritidae) with a braconid parasitoid and a novel bait spray. J Econ Entomol, 94, 817-25.
51. Vargas, R.I., Stark, J.D., Kido, M., Ketter, H.M., & Whitehand, L.C. (2000) Methyl eugenol and cue-lure traps for suppression of male Oriental fruit flies and melon flies

(Diptera: Tephritidae) in Hawaii: effects of lure mixtures and weathering. Journal of Economic Entomology, 93, 81-87.

52. Vijaysegaran, S. (1996) Fruit flies of economic importance to the fruit industry and methods of their control in Malaysia. In Second symposium on tropical fruit flies (eds T.H. Chua & S.G. Khoo), pp. 61-79. The working group on Malaysian fruit flies, Kuala Lumpur, Malaysia.
53. Warburg, M.S. & Yuval, B. (1997) Circadian patterns of feeding and reproductive activities of Mediterranean fruit flies (Diptera: Tephritidae) on various hosts in Israel. Annals of the Entomological Society of America, 90, 487-495.
54. White, I.M. & Elson-Harris, M.M. (1994) Fruit flies of economic significance: Their identification and bionomics CAB International, U.K.
55. White, I.M., Headrick, D.H., Norrbom, A.L., & Carroll, L.E. (2001). Glossary. In Fruit flies (Tephritidae): Phylogeny and evolution of behaviour (eds M. Aluja & A.L. Norrbom), pp. 881-924. CRC Press, Boca Raton.

2. Diel and lifetime patterns of feeding and reproductive activities of *C. cosyra*, *C. fasciventris* and *C. capitata*

2. 1 Introduction

A tephritid fly exerts different types of behaviour, all necessary for survival and reproduction. Many types of fly behaviour compete with each other for expression. Switching between different behaviours occurs frequently, in response to changes in stimulus conditions and levels of motivation and due to interactions between behaviours such as inhibition and facilitation. In many tephritids, diel patterning of behaviour has been developed to such a degree that the day is to an extent partitioned by the major motivational systems so that conflict between them is minimised (Smith, 1989). Browne, (1993) suggests two possible mechanisms leading to a separation of specific resource-oriented behaviour during the day. The first is that the timing of different behaviours is determined by endogenous circadian rhythmicity. The second possibility is that the priority that an insect gives to any resource-oriented behaviour is lowered by the recent acquisition of the relevant resource or as a result of the performance of that behaviour, thus allowing the emergence of a behaviour that previously had lower priority.

Diel patterning of activities, feeding and reproductive, has been found to vary between different species of Tephritids. Feeding of all tephritids occur only during the day with different patterns for different species, for different sexes of the same species and for flies of the same species but under different environmental conditions (Smith, 1989; Hendrichs & Hendrichs, 1990; Hendrichs et al., 1991; Warburg & Yuval, 1997). Timing of reproductive activities of tephritids has also been found to vary between different species. In some species mating is limited to the late afternoon hours and dusk such as for *Bactrocera tryoni* (Frogg.) and *Ceratitis rosa* Karsch, whilst for other species such as *C. capitata* (Wiedemann), *Rhagoletis mendax* Curran, *Anastrepha fraterculus* (Wiedemann), *A. striata* Schiner, *Toxotrypana curvicauda* Gerstaecker, mating occurs during the day (Hendrichs & Hendrichs, 1990; Tychsen & Fletcher, 1971; Smith & Prokopy, 1981; Malavasi et al., 1983; Aluja et al., 1993; Aluja et al., 1997; Quilici et al., 2002).

Timing of reproductive activities in medflies has been found to influence timing of feeding activities of the males of this species. Warburg & Yuval (1997) found that male medflies rarely feed during times allocated to sexual activities and rather feed after disengagement from calling and mating activities.

In addition to diel patterning of behaviour, resource-oriented behaviour of a tephritid changes across the adult stage. As flies become sexually mature, mate-oriented behaviour and oviposition site-oriented behaviour are expressed. Moreover, changes in feeding patterns on different food sources, protein and sugar, have been found to occur in fruit flies such as the Carribean fruit fly, *Anastrepha suspensa* (Loew). For this species, feeding on both sugar and protein was found to increase as the flies mature (Landolt & Davis-Hernandez, 1993).

Control of fruit fly pests by behavioural methods such as with the use of food baits, requires an accurate knowledge of the behavioural ecology of the flies to be targeted. In other words, before adoption of these control methods in the field, it is important to have a basic understanding on the temporal and spatial distribution of activities of these pests. So far, no quantitative information yet exists on the distribution and activities of two important mango infesting flies, *C. cosyra* (Walker) and *C. fasciventris* (Bezzi), in sub-Saharan Africa. The general behavioural ecology of the Mediterranean fruit fly, *C. capitata* (medfly), another important mango infesting fruit fly in the region, has been studied in different areas of the world, Egypt, Greece and Israel. However no such study has been carried out yet in sub-Saharan Africa. The first objective of this study was therefore to determine the distribution, diel and lifetime patterns of feeding and reproductive activities of *C. cosyra*, *C. fasciventris* and *C. capitata*. This study will, at the same time, enable comparison of behavioural patterns of medfly in this area with the other areas where medfly behaviour has previously been studied.

In tephritid literature, behavioural ecology of different flies was found to be studied under different set-ups ranging from small laboratory cages to open field conditions (Prokopy, 1976; Smith & Prokopy, 1981; Malavasi et al., 1983; Hendrichs & Hendrichs, 1990; Hendrichs et al., 1991; Aluja et al., 1993; Landolt & Davis-Hernandez, 1993; Nigg et al., 1995; Warburg & Yuval, 1997). Whether within the same study site, that is under similar external environmental conditions, set-ups do have an influence on expression of behaviour within a tephritid species has not been questioned so far. As such, another objective of this study was to determine changes in patterns of adult behaviour due to set up. This study was

therefore conducted under three different semi-field conditions varying from a small area with constant environmental conditions to a large area with more variable environmental conditions (closer to open field conditions). This has important practical implications in studies on evaluation of behavioural responses of flies to attractants as well as for quality control of pre-released sterile medflies in the context of the Sterile Insect Technique used for control of medflies in other parts of the world.

2.2 Materials and Methods

2.2.1 Study area

The diel and lifetime patterns of activities of *C. cosyra*, *C. fasciventris* and *C. capitata* were studied under three different semi-field conditions on the ground of ICIPE research field station in Mbita, Western Kenya. The station is situated at an altitude of 1240 meters between latitudes 0° 25 S and 0° 30 S and longitudes 34° 10 E and 34° 15 E . The average annual rainfall in the area is 1, 152 mm and the average minimum and maximum temperatures are 17.9°C and 33.6°C. The study was carried out in three different set-ups:

1. Three Plexiglass cages (50 cm x 50 cm x 50 cm)
2. Two cylindrical nylon screen field cages (height 190 cm , diameter 287 cm)
3. One screen-walled greenhouse (length 10m, width 6.6m, height 4.4m) with metal frame.

2.2.2 Plexiglass cages

The cages were placed on a table (1m high) under the shade of a non-host tree. One cage was used for each species. Each cage contained five objects: sucrose, enzymatic yeast hydrolysate, water, a twig and a fruit from a host tree for each species: mango (*Mangifera indica* L.) for *C. cosyra*; guava (*Psidium guajava* L.) for *C. fasciventris* and citrus (*Citrus spp*) for *C. capitata*. The objects were placed at the four corners of each cage. Sucrose, enzymatic yeast hydrolysate and water were placed in 9 cm diameter plastic petri dishes while the fruits were placed on the jar containing the twigs placed in water. Fruits were provided at the mature green stage and were changed every four days. Yeast hydrolysate and sucrose were changed every two weeks. The twigs were changed every week. The objects were allocated at random in each cage and were rotated daily.

Observations were conducted every day for 30 days from November to December 2000. Observations were initiated at 06H30 (shortly after sunrise) and ended at 22H30. Every two hours, the location and activities of the flies were recorded in each cage. Fly locations within the cage were defined as follows: upper or lower sides of the leaf with respect to the twig; sucrose, yeast hydrolysate, water, fruit and cage wall. At 22H30, three red lights over each cage were switched on to rotate the food sources and at the same time observation of fly activities were carried out. The number of dead flies was counted at the beginning of each observation day to determine the total number of flies in the cage.

2.2.3 Screen field cages

The cages were placed under the shade of 3 trees, *Pterolobium stellatum*. Four types of potted fruit trees were placed inside each cage as follows: mango, citrus, banana (*Musa spp*) and guava. The average height of the trees was 120 cm. Water, yeast hydrolysate, sucrose and the respective host fruit were placed on each host tree: Citrus, mango and guava. Only water, yeast hydrolysate and sucrose were placed on the non-host banana trees. Yeast hydrolysate was renewed every week and fruits were renewed after four days.

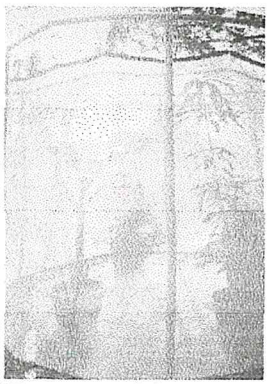

Fig. 2. Field cage set-up containing host and non-host trees. A. Manrakhan, ICIPE

Observations were conducted over two weeks from the 22.08.00 when the flies were 3 days old till the 3.09.00. Systematic observations were carried out in shifts by two observers every day. Observations were initiated at 07H00 and the last observations were made at 18H00. Every hour, the location and activities of the flies were recorded. Fly activities were categorized according to site (mango, banana, guava, citrus). Fly locations within trees were defined as follows: on fruit, lower side of leaf, upper side of leaf, on yeast hydrolysate, sucrose or water.

2.2.4 Screen-walled greenhouse

The greenhouse walls and roof were totally covered with a dark green PVC shade netting material of 1mm mesh size. The greenhouse contained 8 host trees, 4 guava trees, and, 4 citrus trees and one non-host tree, banana. Petri dishes containing yeast hydrolysate, sucrose, water and fruit were suspended on each host tree with respect to direction in a latin square design.

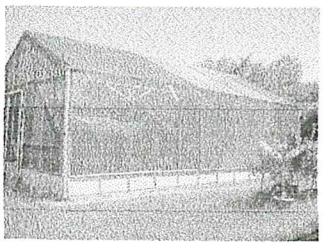

Fig.1. Greenhouse set-up containing host and non- host trees. A. Manrakhan,

Systematic observations were conducted on a 24-hour basis by two observers for 2 weeks for *C. cosyra* and *C. capitata* everyday. *C. fasciventris* had a better survival rate as compared to the two other species and observations for this species were made for three weeks.

The study took place from 31 January to 20 February 2001. Every two hours, the location and activities of flies on each tree inside the greenhouses were systematically recorded. Location of fly activities within and among trees in the greenhouse was defined similar as in the field cages. The order of the observation sites on the different trees was rotated systematically between observation periods. A ladder was used for observations on higher canopy.

2.2.5 Insect material

Flies used in all experiments were obtained as pupae originated from *C. cosyra*, *C. fasciventris* and *C. capitata* colonies maintained for at least 70 generations in the rearing facilities at the ICIPE headquarters in Nairobi, Kenya. Upon eclosion, 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 between 23 – 30 ^{0}C, relative humidity between 45-57 % and on a photo phase from 0600 to 1900 hours before release in each of the three set ups.

Three day old adult flies were released into each of these set-ups the day prior to start of observation: (i) 50 males and 50 females of each species were released into each of the plexiglass cages, (ii) 60 males and 60 females *C. cosyra*, *C. fasciventris* and *C. capitata* adult flies were released concurrently inside each of the field cages, and (iii) 200 *C. cosyra*, 400 *C. fasciventris*, and 400 *C. capitata* adult flies were released concurrently in the greenhouse.

2.2.6 Behaviour

Fly activities in all three set-ups were defined as follows: "feeding" as repetitive lowering of the proboscis to touch the surface on which the fly was situated; "ovipositing" involved ovipositor insertion into a fruit and ovipositor dragging; "calling" involved wing fanning or conspicuous presence in a male of a clear droplet in a pouch everted from the anal gland and "mating" (actual copulation).

2.2.7 Environmental conditions

Temperature and Relative Humidity in all set-ups were measured every two hours with a hygrothermometer (Cole-Parmer Instrument Company, Chicago, Illinois). Light intensity was measured every two hours with a photometer (Li-Cor, USA).

2.2.7 Data analysis

Data was analysed by nonparametric ANOVA (Kruskal-Wallis test) since it did not follow a normal distribution. When comparing two samples, Wilcoxon signed rank tests were run.

2.3 Results

2.3.1 Environmental conditions

In all three set ups temperature was lowest in the early morning hours at 08 00 hours and highest between noon and early afternoon hours (14 00 hours). Variations in mean daily temperatures were higher in field cages and in the greenhouse compared to the plexiglass cage. Relative humidity in all set ups was lowest between 12 00 to 14 hours and relatively higher during the early morning hours at 08 00 hours and at dusk. Fluctuations in light intensity in the field cages and plexiglass cages followed the same trend with the highest light intensity occurring at midday. In the greenhouse, however, light intensity had two peaks, one before midday and one after midday.

2.3.2 Diel pattern of fly distribution among trees in field cages and greenhouses containing host and non-host trees

Flies of all species were significantly more abundant on host trees in both field cages and greenhouses (Tables 1 and 2). Among the host trees, flies of all species were more abundant on guava trees. The male/female ratio was constant among the different trees in both set-ups.

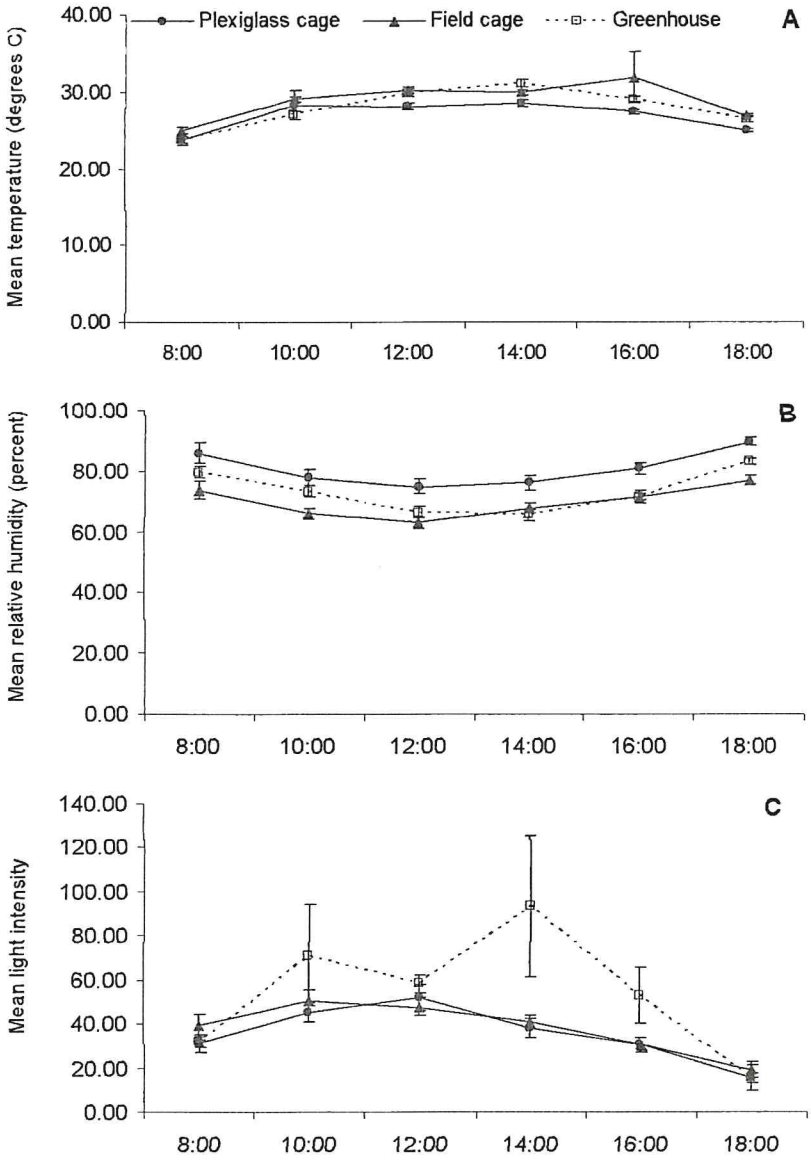

Fig. 4. Conditions prevalent during study in the field cages, greenhouse and plexiglass cages. (A) 2-hourly Temperature. (B) Relative humidity. (C) Light intensity. Values represent mean ± SE.

Table 1. Percentages location of flies on different trees in field cages

Species	Sex	N[a]	Host Trees			Non-host tree	Kruskal Wallis Test
			Citrus	Guava	Mango	Banana	P
C. cosyra	Female	1106	13	51	31	5	<0.01
	Male	668	9	51	37	3	<0.01
C.fasciventris	Female	1416	19	41	37	3	<0.01
	Male	1357	18	41	37	3	<0.01
C.capitata	Female	1195	28	30	36	6	<0.01
	Male	1491	21	39	38	2	<0.01

[a] Total number of sightings of flies on trees

Table 2. Percentages location of flies on different trees in greenhouses

Species	Sex	N[a]	Host trees			Non-host tree	Kruskal Wallis Test
			Citrus	Guava	Banana		P
C. cosyra	Female	977	22	75	3		<0.01
	Male	812	20	75	5		<0.01
C.fasciventris	Female	2933	19	75	6		<0.01
	Male	3221	16	78	6		<0.01
C.capitata	Female	558	33	63	3		<0.01
	Male	1237	32	67	2		<0.01

[a] Total number of sightings of flies on trees

2.3.3 Diel Pattern of types of fly activity

Feeding. Flies of all three species were seen feeding throughout the day in all three set-ups (Figs. 5 – 7). Little or no feeding occurred at night in both plexiglass cages and in the greenhouse. A few feeding events were recorded for all fly species at night in plexiglass cages, mostly under the influence of red lights used during observations at night and when switched on fully to rotate food sources within the cages. Therefore, data collected on feeding only at day time (from 6 00 to 18 00 hours) were selected for analysis of feeding behaviour in the three set ups.

In plexiglass cages, frequency of feeding by males and females of *C. cosyra* and females of both *C. fasciventris* and *C. capitata* did not vary significantly throughout the day (6 30 to 18 00 hours). Feeding frequency of *C. fasciventris* males was higher during morning hours and remained high till the early afternoon hours to decrease significantly then in late afternoon hours between 16 00 and 18 00 hours (χ^2= 26.95, df=6, P<0.01). In contrast, *C. capitata* males, had a significantly higher frequency of feeding during late afternoon hours around 16 00 hours (χ^2= 15.40, df=6, P=0.02).

In field cages, there were no significant differences in frequency of feeding at different times during the day for males and females of *C. cosyra* and *C. fasciventris* as well as for females of *C. capitata*. *C. capitata* males in field cages, like in plexiglass cages, peaked in feeding at dusk (χ^2= 20.09, df=3, P=0.04).

In the greenhouse, a significantly higher number of feeding events were recorded for *C. cosyra* females in the early morning hours (χ^2= 15.92, df=6, P=0.01), unlike the patterns of feeding for this group of flies observed in the plexiglass cage and field cages. There were no significant differences in feeding frequencies of *C. cosyra* males throughout the day similar to the patterns in the two other set ups. *C. fasciventris* females had two peaks in feeding, one in the morning hours at 08 00 and one in the late afternoon between 16 00 and 18 00 hours (χ^2= 38.49, df=6, P<0.01). This again, was in contrast to the patterns of feeding of *C. fasciventris* females recorded in the two other set ups. *C. fasciventris* males were found to feed more frequently in the greenhouse during morning hours, like the pattern observed in the plexiglass cage but unlike that observed in the field cages (χ^2= 29.68, df=6, P<0.01). Feeding frequencies of *C. capitata* females in the greenhouse did not differ

significantly throughout the day, whilst for *C. capitata* males, similar to its patterns of feeding in the plexiglass cage and field cages, a peak in their feeding frequency was observed in late afternoon between 16 00 and 18 00 hours (χ^2= 22.25, df=6, P<0.01).

Differences in feeding frequencies between males and females varied according to species and set up. In plexiglass cages, there were no significant differences in feeding frequencies between males and females of *C. cosyra* and *C. capitata*. While, in the same set up for *C. fasciventris*, females were found to feed more frequently than males (Wilcoxon signed rank test, P<0.01). In field cages, there were no significant differences in feeding frequencies between males and females of all fruit fly species. In the greenhouse, there was no significant difference between male and female feeding events for *C. cosyra*, however, frequency of feeding was significantly higher for *C. fasciventris* and *C. capitata* females as compared to males (Wilcoxon signed rank test, P<0.01 for both *C. fasciventris* and *C. capitata*).

Differences between species in their frequency of feeding varied according to sex and set up. Females of *C. fasciventris* in plexiglass cages, had on average, a significantly higher frequency of feeding compared to females of *C. cosyra* and *C. capitata* (χ^2= 9.75, df=2, P<0.01). In field cages, females of *C. cosyra* had a significantly higher frequency of feeding compared to females of the two other species (χ^2= 101.57, df=2, P<0.01). While in the greenhouse, no significant difference was found in frequency of feeding between females of the different species. In all three set ups, males of *C. cosyra* had the highest frequency of feeding compared to males of *C. fasciventris* and *C. capitata* (Plexiglass cages: χ^2= 13.66, df=2, P<0.01; Field cages: χ^2= 66.36, df=2, P<0.01; Greenhouse: χ^2= 11.86, df=2, P<0.01).

Comparing the three different set ups, significantly more feeding events were recorded in field cages compared to plexiglass cages and in the greenhouse (*C. cosyra*: χ^2= 282.32, df=2, P<0.01; *C. fasciventris*: χ^2=344.69, df=2, P<0.01; *C. capitata*: χ^2= 163.44, df=2, P<0.01).

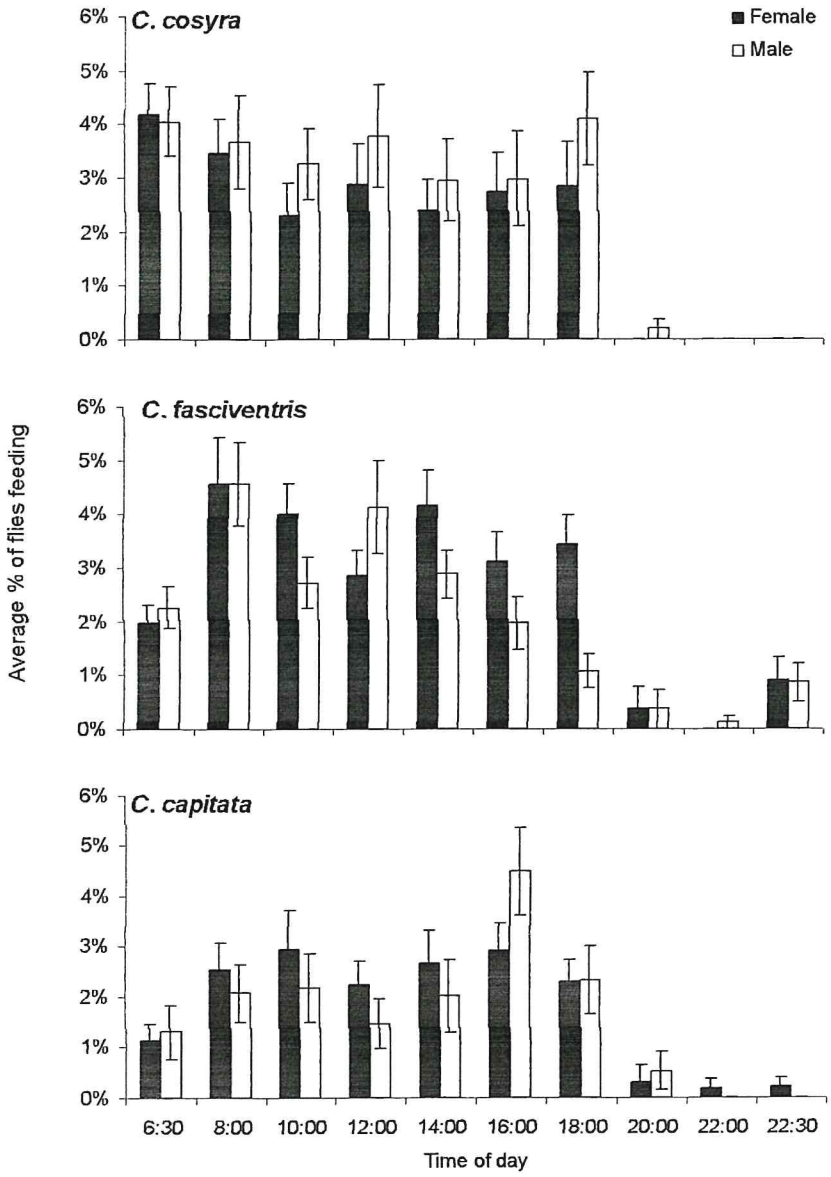

Fig. 5. Distribution of male and female *C. cosyra*, *C. fasciventris* and *C. capitata* feeding activities in plexiglass cages during the day. Values represent means ± SE.

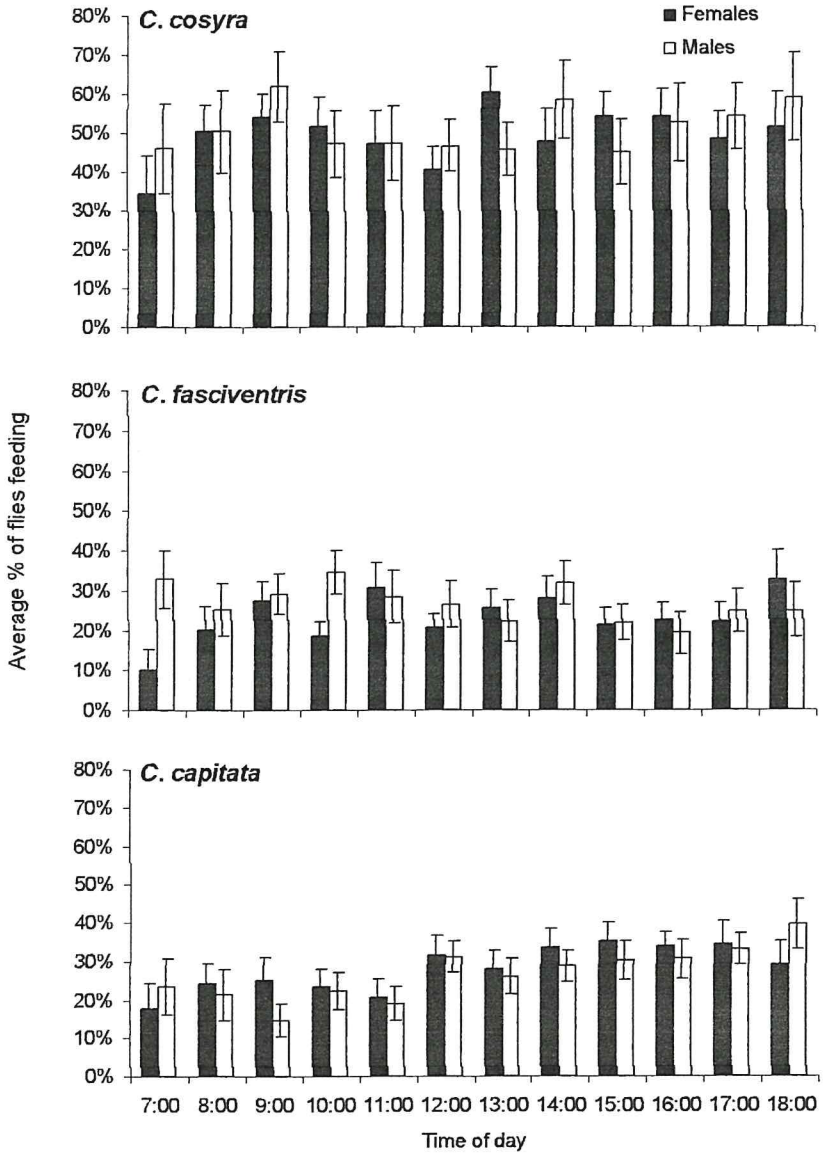

Fig. 6. Distribution of male and female *C. cosyra*, *C. fasciventris* and *C. capitata* feeding activities in field cages containing host trees and non-host trees during the day. Values represent means ± SE.

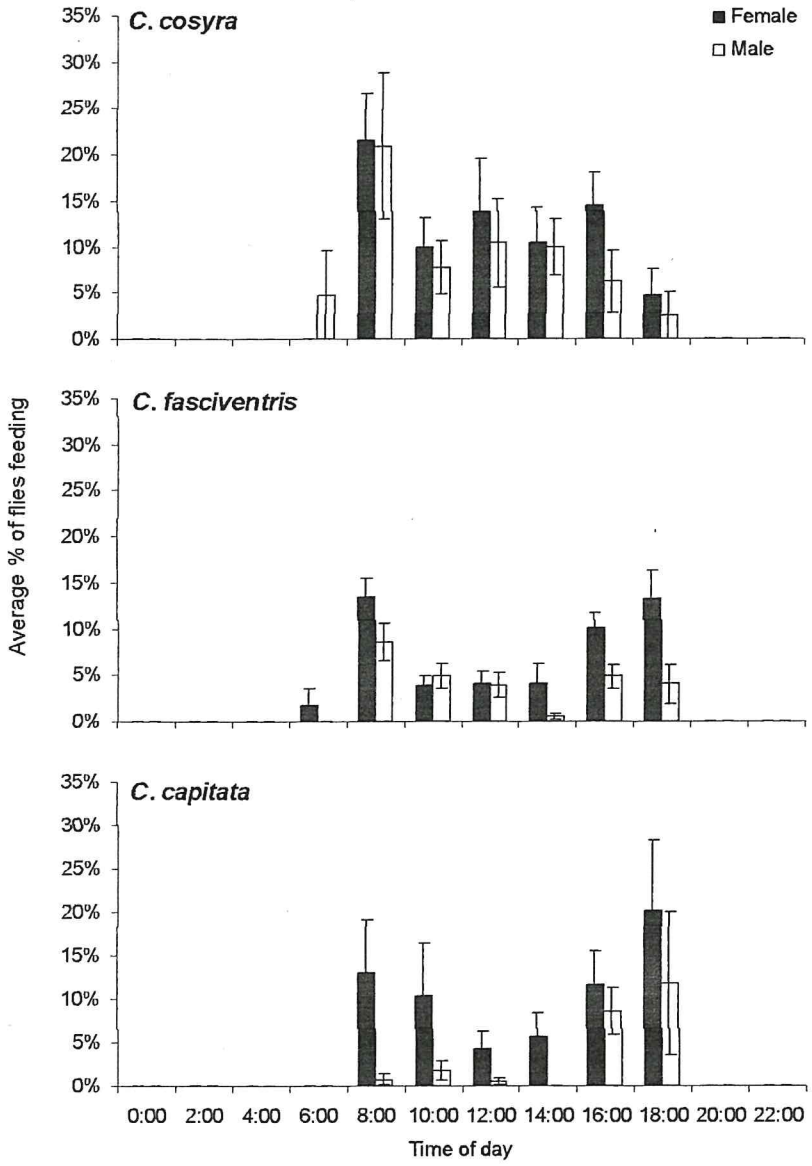

Fig. 7. Distribution of male and female *C. cosyra*, *C. fasciventris* and *C. capitata* feeding activities in greenhouse containing host and non-host trees during the day. Values represent means ± SE.

Calling. Calling behaviour was observed for all species in plexiglass cages (Fig. 8). However in field cages and in the greenhouse, calling activities were only recorded for *C. fasciventris* and *C. capitata* males.

Calling activities of *C. cosyra* males in plexiglass cages took place in the late afternoon hours close to dusk. In field cages, no calling activities were recorded despite that males were seen during that time mostly on the cage walls. In the greenhouse, however, *C. cosyra* flies were found to move to the ceiling of the greenhouse and to the light around dusk which made observation of their activities difficult during that period.

C. fasciventris males, in all set ups, started calling in the late afternoon, close to dusk. *C. fasciventris* were usually found to call in leks of 3 to 5 males.

Calling activities of *C. capitata* males, unlike the two other species, took place during day time, starting soon after dawn and ending in the late afternoon around 16 00. In all set ups, *C. capitata* males peaked in their calling activities between 08 00 in the morning to 14 00 in the afternoon (Plexiglass cage: χ^2= 67.60, df=6, P<0.01; Field cages: χ^2= 52.86, df=11, P<0.01; Greenhouse: χ^2= 60.78, df=6, P<0.01). Like *C. fasciventris*, *C. capitata* were usually found to call in leks of 3 to 5 males.

For *C. fasciventris* and *C. capitata*, a higher percentage of males were found calling in the greenhouse compared to plexiglass and field cages (*C. fasciventris*: χ^2= 20.25, df=2, P<0.01; *C. capitata*: = 43.24, df=2, P<0.01).

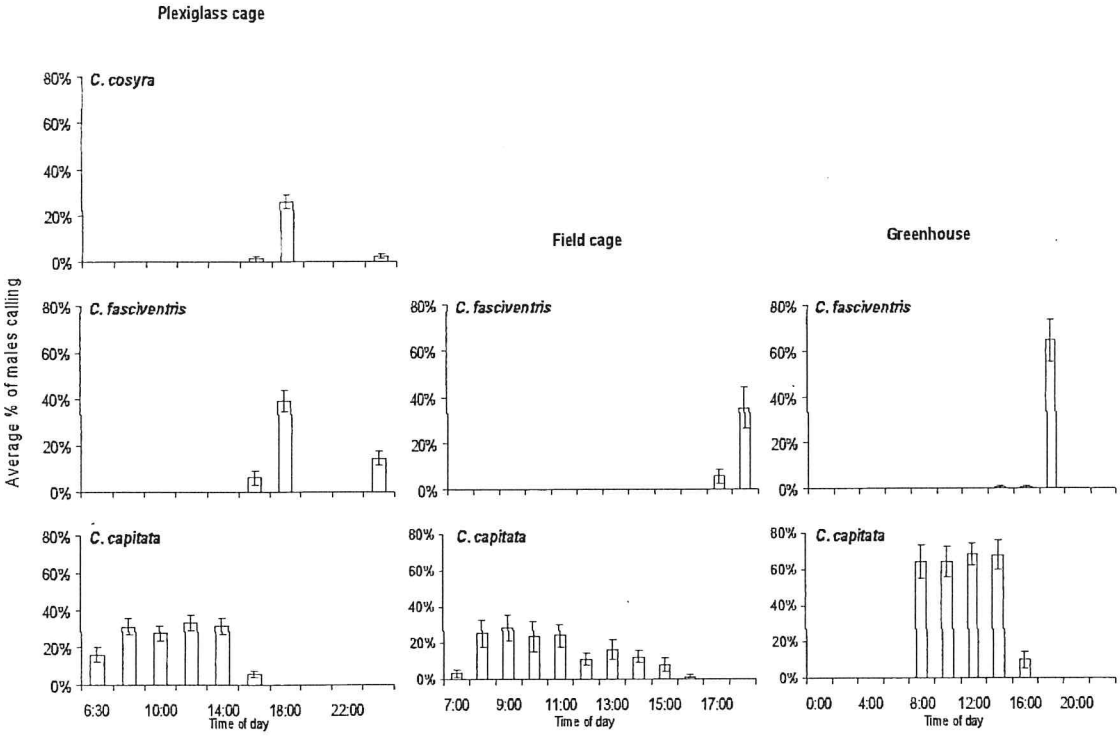

Fig. 8 . Hourly to 2- Hourly distribution of male *C. cosyra*, *C. fasciventris* and *C. capitata* calling activities in plexiglass cages, field cages and Greenhouse. Values represent means ± SE. Different set ups have different x- scales with respect to time of observations.

Mating. Mating of all species was observed in plexiglass and field cages (Fig. 9). In the greenhouse, no mating couples of *C. cosyra* were found.

In plexiglass and field cages, mating couples of *C. cosyra* and *C. fasciventris* were observed at dusk and for *C. fasciventris* the same observation was made in the greenhouse. Mating couples of both species were suspected to stay *in copula* overnight since they were found to disengage before dawn in the case of *C. cosyra* and after dawn in the case of *C. fasciventris*. In the greenhouse where observations were made over 24 hours, mating *C. fasciventris* couples were confirmed to stay *in copula* overnight to disengage in mating at dawn. Mating duration for both *C. cosyra* and *C. fasciventris* was estimated to be between 11 to 14 hours, mostly taking place during the night.

Mating of *C. capitata* occurred about 1 hour after start of calling and peaked between 10 00 to 14 00 hours in the plexiglass cage and in the greenhouse (Plexiglass cage: χ^2= 21.92, df=6, P<0.01; Greenhouse: χ^2= 12.67, df=6, P=0.05). While in the field cages, frequency of mating of *C. capitata* was at its highest over a longer period of time, starting from 10 00 hours and ending at 17 00 hours (χ^2= 21.12, df=11, P=0.03).

There were significant differences in frequency of mating activities due to set-ups for each species. Frequency of mating activities of *C. cosyra* and *C. fasciventris* were higher in the plexiglass cages while for *C. capitata* mating events were recorded in higher numbers in the field cages (*C. cosyra*: χ^2= 33.61, df=1, P<0.01; *C. fasciventris*: χ^2= 135.97, df=2, P<0.01; *C. capitata*: χ^2= 10.21, df=2, P<0.01).

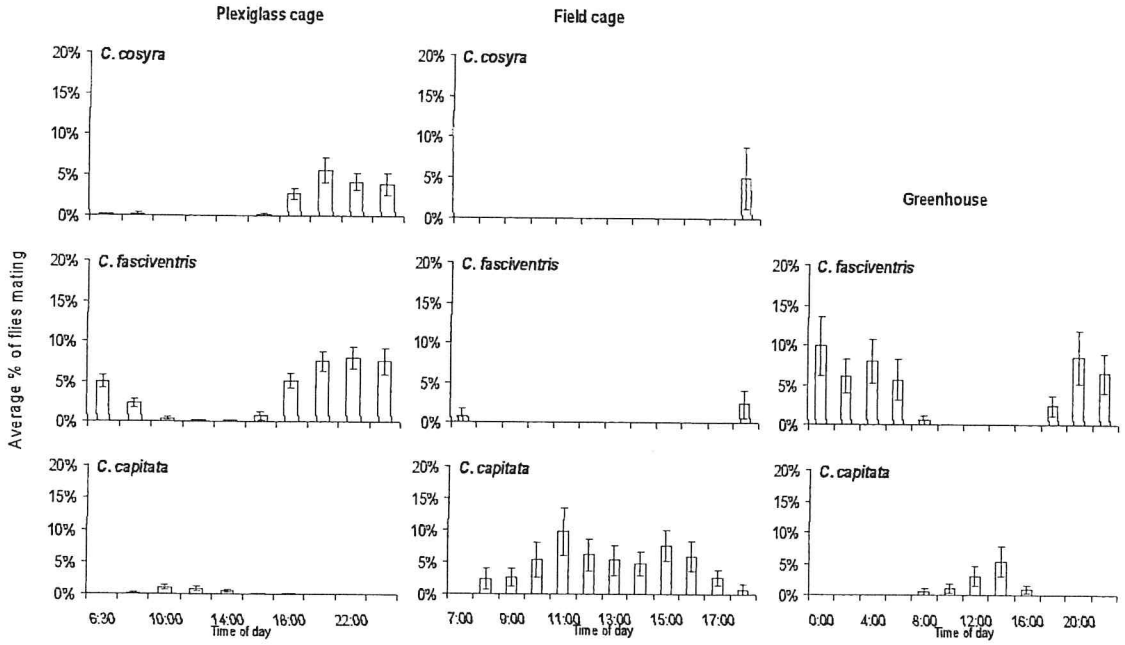

Fig. 9. Hourly to 2- Hourly Distribution of *C. cosyra*, *C. fasciventris* and *C. capitata* mating activities in plexiglass cages, field cages and in the greenhouse. Values represent means ± SE. Different set ups have different x- scales with respect to time of observations.

Oviposition. Oviposition activities by *C. cosyra* were only recorded in plexiglass cages and field cages. For *C. fasciventris* and *C. capitata*, on the other hand, oviposition activities were recorded under the three different set-ups (Fig. 10).

Oviposition peaked in late afternoon for all three species in plexiglass cages (*C. cosyra*: χ^2= 34.93, df=6, P<0.01; *C. fasciventris*: χ^2= 31.61, df=6, P<0.01; *C. capitata*: χ^2= 37.19, df=6, P<0.01). Some *C. fasciventris* females were found to oviposit at 22H30 in the presence of red light in the plexiglass cages.

In field cages, a higher percentage of ovipositing females of *C. cosyra* and *C. fasciventris* were recorded in late afternoon hours though the difference was not significant. There were no significant differences in frequency of oviposition by *C. capitata* females in field cages at different times of the day.

In the greenhouse, oviposition peaked in the morning and at dusk for *C. fasciventris* (χ^2= 20.59, df=6, P<0.01). There was no significant difference in percentages of ovipositing females at different times of the day for *C. capitata* in the greenhouse.

C. fasciventris and *C. capitata* females had significantly higher frequency of ovipositing in plexiglass cages compared to field cages and the greenhouse (*C. fasciventris*: χ^2= 30.02, df=2, P<0.01; *C. capitata*: χ^2= 7.52, df=2, P=0.02). For *C. cosyra*, there were no significant difference in frequency of oviposition between plexiglass cages and field cages.

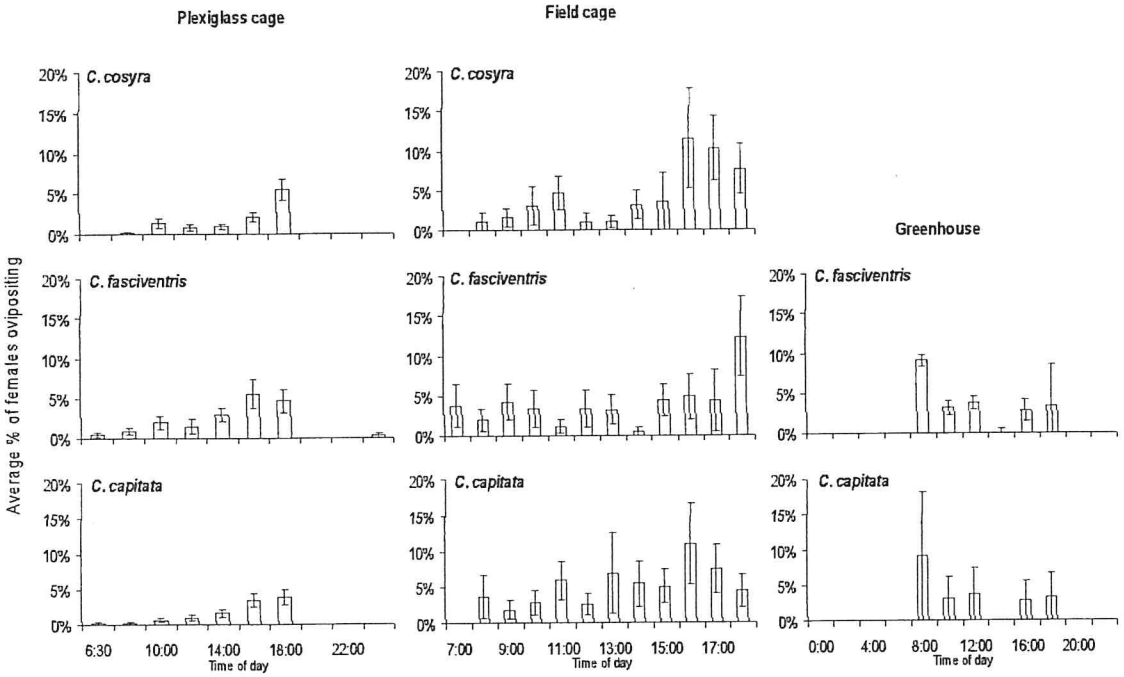

Fig. 10. Hourly to 2- Hourly Distribution of female *C. cosyra*, *C. fasciventris* and *C. capitata* ovipositing activities in plexiglass cages, field cages and in the greenhouse. Values represent means ± SE. Different set ups have different x- scales with respect to time of observations.

Resting. In all set ups, resting activities of all three fruit fly species were recorded throughout the day and also at night time in plexiglass cages and in the greenhouse.

In plexiglass cages, males and females of *C. cosyra* and *C. fasciventris* became significantly more active around dusk, period coinciding with the peak in their reproductive activities, and they were also highly active ,when the red lights were switched on in the night at 22 30 hours (*C. cosyra* females: χ^2= 65.84, df=9, P<0.01; males: χ^2= 80.87, df=9, P<0.01; *C. fasciventris* females: χ^2= 60.48, df=9, P<0.01; males: χ^2= 89.44, df=9, P<0.01). *C. capitata* females were significantly active around dusk and at 22 30 hours, as well as during mid morning hours, around 10 00 (χ^2= 33.12, df=9, P<0.01). For *C. capitata* males, significantly higher activity was recorded from 08 00 to 14 00, a period coinciding with their calling activities (χ^2= 47.92, df=9, P<0.01). Higher frequencies of resting activities

were recorded for males of *C. cosyra* and *C. fasciventris* compared to females (Wilcoxon signed rank test: P<0.01 for both *C. cosyra* and *C. fasciventris*). There were no significant difference in resting activities between females and males of *C. capitata*.

In field cages, similar to observations in plexiglass cages, males and females of *C. cosyra* and *C. fasciventris* were found to be significantly more active around dusk at 18 00 hours (*C. cosyra* females: χ^2= 23.81, df=11, P=0.01; males: χ^2= 22.81, df=11, P=0.02; *C. fasciventris* females: χ^2= 23.38, df=11, P=0.02; males: χ^2= 36.76, df=11, P<0.01). In contrast to observations in the plexiglass cages, for *C. capitata* males and females, there were no significant differences in resting activities in field cages throughout the day. There were no significant differences in frequency of resting activities in field cages between males and females of the three fruit fly species.

There were significant differences in resting activities of males and females of the three fruit fly species in the greenhouse (*C. cosyra* females: χ^2= 23.81, df=11, P=0.01; males: χ^2= 22.81, df=11, P=0.02; *C. fasciventris* females: χ^2= 23.38, df=11, P=0.02; males: χ^2= 36.76, df=11, P<0.01; *C. capitata* females: χ^2= 63.55, df=11, P<0.01; males: χ^2= 119.45, df=11, P<0.01). *C. cosyra* were more active during the day starting 08 00 in the morning to 16 00 in the afternoon. At 18 00 few flies were seen around, most moved to the ceiling which made observations difficult but the rest of the flies that stayed on the canopy were mostly resting then and throughout night time. Again, for *C. fasciventris* males and females, flies were significantly more active around dusk and for *C. capitata* males, a higher period of activity was recorded between early morning hours to afternoon hours. There were no significant difference in frequency of resting activities between males and females of *C. cosyra* and *C. fasciventris*. While for *C. capitata*, males were significantly more active than females (Wilcoxon signed rank test, P=0.03).

2.3.4 Distribution of activities among and within trees in field cages and greenhouse containing host and non-host trees

In field cages and in the greenhouse, feeding by males and females of all species occurred predominantly on host trees (Field cages: *C. cosyra*: females: χ^2= 23.54, df=3, P<0.01; males: χ^2= 29.28, df=3, P<0.01; *C. fasciventris*: females: χ^2= 27.71, df=3, P<0.01; males: χ^2= 25.84, df=3, P<0.01; *C. capitata*: females: χ^2= 13.41, df=3, P<0.01; males: χ^2= 39.85, df=3, P<0.01; Greenhouse: *C. cosyra*: females: χ^2= 16.82, df=2, P<0.01; males: χ^2= 15.28,

df=2, P<0.01; *C. fasciventris*: females: χ^2= 39.37, df=2, P<0.01; males: χ^2= 30.87, df=2, P<0.01; *C. capitata*: females: χ^2= 4.38, df=2, P=0.11; males: χ^2= 7.49, df=2, P=0.02). Among host trees in field cages, fewer feeding events were recorded on citrus trees compared to mango and guava trees. Very few feeding events took place on the non-host banana plant for all species in both the field cages and in the greenhouse.

Within trees in both field cages and in the greenhouse, the main site of feeding for all species was leaf surfaces (Tables 3 & 4). Feeding occurred mainly on the upper leaf surfaces. In the field cages, upper leaf surfaces contained accumulated dust, some bird's droppings and unidentified food sources. In the greenhouse containing host trees, leaves of guava and citrus trees were heavily infested with aphids, scales and mealy bugs and as a result most of the upper leaf surfaces were covered with honeydew.

There were significant species related differences in feeding events with respect to some feeding surfaces in the field cages. For instance, *C. cosyra* females and males had a higher frequency of feeding on the upper leaf surfaces than did females and males of *C. fasciventris* and *C. capitata* (Females: χ^2= 20.79, df=2, P<0.01; Males: χ^2= 21.24, df=2, P<0.01). Whilst on sugar, C. *capitata* females fed more frequently than females of *C. cosyra* and *C. fasciventris* (χ^2= 12.39, df=2, P<0.01). In the same set-up, feeding events of *C. cosyra* males on yeast were significantly less compared to feeding events of males *C. fasciventris* and *C. capitata* on yeast (χ^2= 9.57, df=2, P<0.01).

In the greenhouse, the major species difference which occurred in feeding events with respect to surfaces was on yeast. *C. cosyra* females fed the least on yeast compared to the two other species (χ^2= 18.16, df=2, P<0.01).

Differences between sexes in their feeding frequencies on different surfaces were also observed in the field cages. For both *C. cosyra* and *C. fasciventris*, feeding events on yeast were significantly more for females than for males (*C. cosyra*: Wilcoxon test: P=0.01; *C. fasciventris*: Wilcoxon test, P=0.04). With respect to other feeding surfaces, there were no significant differences in feeding frequency between females and males of both *C. cosyra* and *C. fasciventris*. For *C. capitata*, there was no significant difference between females and males in their feeding frequencies on yeast. While on sugar, *C. capitata* females fed more frequently than males (Wilcoxon test, P=0.01). On fruit and leaf surfaces there were no significant differences between males and females in their feeding frequencies.

In the greenhouse, there were no significant differences in feeding frequencies on different surfaces between females and males of *C. cosyra*. *C. fasciventris* females had a

significantly higher frequency of feeding on the upper leaf surfaces and on yeast compared to males of this species (Wilcoxon signed rank tests for both surfaces: P=0.02). Similarly, *C. capitata* females had a significantly higher frequency of feeding on upper leaf surfaces compared to *C. capitata* males (Wilcoxon signed rank test, P=0.03).

Calling events for *C. fasciventris* and *C. capitata* as well as mating events for the two species took place also predominantly on host trees in the field cages and in the greenhouse (Field cages: Calling: *C. fasciventris*: χ^2= 8.86, df=3, P=0.03; *C. capitata*: χ^2= 13.26, df=3, P<0.01; Mating: *C. fasciventris*: χ^2= 8.53, df=3, P=0.04; *C. capitata*: χ^2= 12.57, df=3, P<0.01; Greenhouse: Calling: *C. fasciventris*: χ^2= 27.15, df=2, P<0.01; *C. capitata*: χ^2= 13.51, df=2, P<0.01; Mating: *C. fasciventris*: χ^2= 13.55, df=2, P<0.01; *C. capitata*: χ^2= 4.89, df=2, P=0.09). In field cages, the preferred site for calling and mating activities of both *C. fasciventris* and *C. capitata* was mango trees while in the greenhouse, guava trees were the preferred site for the sexual activities. Considerable mating events for *C. capitata* were however observed in citrus trees in both field cages and in the greenhouse. The only two mating events of *C. cosyra* in the field cages occurred, on both citrus and mango host trees.

Calling by *C. fasciventris* and *C. capitata* males occurred mostly on lower leaf surfaces of host trees (95% - *C. fasciventris* and 98% - *C. capitata*, in field cages; 97% - *C. fasciventris* and 99% - *C. capitata*, in field cages).

Mating couples of *C. cosyra* in field cages were found exclusively on the lower leaf surfaces of host trees. Similarly for *C. fasciventris*, most mating couples in both field cages and in the greenhouse were found on the lower leaf surfaces (100% in field cages and 95% in the greenhouse. Mating by *C. capitata* occurred on both lower and upper leaf surfaces, with a higher frequency on the lower leaf surfaces (59% -Lower leaf surface, 37%-Upper leaf surface in the field cages; 64% - Lower leaf surface and 36%- Upper leaf surface in the greenhouse). A few mating events for *C. capitata* were recorded on fruits in the field cages and none were recorded in the greenhouse.

In field cages, there were no significant differences in frequency of oviposition of *C. cosyra* and *C. fasciventris* on different host fruits (mango, citrus and guava). On the other hand, in the same set up, citrus was the preferred oviposition site for *C. capitata* females (χ^2= 19.20, df=2, P<0.01). In the greenhouses, ovipositing *C. fasciventris* females were recorded on both citrus and guava trees and there were no significant differences in the frequency of oviposition between these fruits. Whilst for *C. capitata* females in the greenhouse, similar

to observations made in the field cages, oviposition was recorded mostly on citrus trees (Wilcoxon signed rank test, P=0.01).

Resting by flies of all three species in both field cages and greenhouses occurred predominantly on host trees (Field cages: *C. cosyra*: females: χ^2= 22.94, df=3, P<0.01; males: χ^2= 25.07, df=3, P<0.01; *C. fasciventris*: females: χ^2= 35.91, df=3, P<0.01; males: χ^2= 30.95, df=3, P<0.01; *C. capitata*: females: χ^2= 22.09, df=3, P<0.01; males: χ^2= 30.12, df=3, P<0.01; Greenhouse: *C. cosyra*: females: χ^2= 11.92, df=2, P<0.01; males: χ^2= 7.75, df=2, P=0.02; *C. fasciventris*: females: χ^2= 17.28, df=2, P<0.01; males: χ^2= 10.11, df=2, P<0.01; *C. capitata*: females: χ^2= 12.95, df=2, P<0.01; males: χ^2= 13.44, df=2, P<0.01). Male and female resting of all three species in both field cages and in the greenhouse occurred predominantly on the undersides of leaves (Field cages: *C. cosyra*: χ^2= 303.36, df=5, P<0.01; *C. fasciventris*: χ^2= 539.27, df=5, P<0.01; *C. capitata*: χ^2= 414.81, df=6, P<0.01; Greenhouse: *C. cosyra*: χ^2= 231.96, df=5, P<0.01; *C. fasciventris*: χ^2= 441.09, df=5, P<0.01; *C. capitata*: χ^2= 230.04, df=5, P<0.01).

Table 3: Percentages within-tree location of *C. cosyra*, *C. fasciventris* and *C. capitata* feeding activities in field cages containing host and non-host trees

Species	Sex	N[a]	Lower leaf	Upper leaf	Fruit	Sugar	Yeast	Water	Kruskal Wallis Test
C. cosyra	Female	565	20	56	7	10	4	3	P<0.01
	Male	351	22	63	6	6	2	1	P<0.01
C. fasciventris	Female	290	25	28	14	10	19	4	P<0.01
	Male	309	30	31	14	9	11	5	P<0.01
C.capitata	Female	329	18	24	16	19	16	7	P<0.01
	Male	395	18	46	10	8	11	7	P<0.01

[a] Total number of sightings of flies on trees

Table 4: Percentages within-tree location of *C. cosyra*, *C. fasciventris* and *C. capitata* feeding activities in greenhouse containing host and non-host trees

Species	Sex	N[a]	Lower leaf	Upper leaf	Fruit	Sugar	Yeast	Water	Kruskal Wallis Test
C. cosyra	Female	132	20	76	2	0	2	1	P<0.01
	Male	91	18	77	1	0	4	0	P<0.01
C. fasciventris	Female	165	21	46	2	2	28	1	P<0.01
	Male	113	27	46	9	0	18	0	P<0.01
C.capitata	Female	40	5	70	0	3	23	0	P<0.01
	Male	25	4	52	0	0	40	4	P<0.01

[a] Total number of sightings of flies on trees

2.3.5 Weekly Pattern of activities

Survival of flies in plexiglass cages was higher than in field cages and in the greenhouse, as a result of higher protection from high temperatures and low humidity and more importantly from predators. Therefore, fly activities in plexiglass cages was studied for a longer period of time, 4 weeks, whilst in the field cages and in the greenhouse, flies could be studied for only 1 - 2 weeks.

Feeding on leaf surfaces in all set ups and for all fly species decreased one or two weeks after fly emergence (Fig. 11 to 13). Feeding on fruits, which was more important in field cages compared to the two other set ups increased significantly in the second week for *C. fasciventris* (Wilcoxon signed rank test: females: P<0.01 and males: P=0.04) while no significant increase in fruit feeding was observed for *C. cosyra* and *C. capitata* .

Sugar and yeast feeding were higher in plexiglass cages compared to field cages and the greenhouse. In plexiglass cages, after 1-2 weeks, there was a decrease in frequency of feeding on sugar by females of *C. fasciventris* and *C. capitata* (*C. fasciventris*: χ^2= 11.85, df=4, P=0.02; *C. capitata*: χ^2= 23.87, df=4, P<0.01) and also by males of *C. cosyra* and *C. capitata* (*C. cosyra*: χ^2= 18.07, df=4, P<0.01; *C. capitata*: χ^2= 25.62, df=4, P<0.01). No significant change in frequency of feeding on sugar was observed for females of *C. cosyra*

and males of *C. fasciventris*. There was a significant variation on feeding frequency of *C. cosyra* females on yeast in plexiglass cages, across the weeks (χ^2= 10.12, df=4, P=0.04). There was a significant decrease in feeding frequency on yeast after the first week for males of *C. fasciventris* and *C. capitata* (*C. fasciventris* males: χ^2= 10.22, df=4, P=0.04; *C. capitata* males: χ^2= 11.06, df=4, P=0.03), while no significant change in yeast feeding was observed for *C. cosyra* males and females of *C. fasciventris* and *C. capitata*.

In plexiglass cages, calling by *C. capitata* males was significantly higher in the third and fourth weeks (χ^2= 57.54, df=4, P<0.01), while no significant changes in frequency of calling was observed across the ages for males of *C. cosyra* and *C. fasciventris* (Fig. 14). Similarly in field cages, *C. capitata* males peaked in calling in the second week (χ^2= 33.53, df=1, P<0.01). In field cages, in contrast with the plexiglass cages, *C. fasciventris* peaked in calling in the second week (χ^2= 7.01, df=2, P=0.03). In the greenhouse, however, there were no significant changes in frequency of calling activities for *C. fasciventris* and *C. capitata*.

Matings of all fly species in plexiglass cages peaked in weeks 2 and 3 (Fig. 15) (*C. cosyra*: χ^2= 11.21, df=4, P=0.02; *C. fasciventris*: χ^2= 25.82, df=4, P<0.01; *C. capitata*: χ^2= 29.15, df=4, P<0.01). In field cages, there was no significant change in mating frequency for all fly species across the weeks. In the greenhouse, there was a significant increase in mating frequency for *C. fasciventris* in the second and third week (χ^2= 31.86, df=3, P<0.01), while no significant change in mating frequency was observed for *C. capitata* across the weeks.

In plexiglass cages and field cages, frequency of oviposition for all species increased significantly in the second week (Fig. 16) (*C. cosyra*: χ^2= 34.90, df=4, P<0.01; *C. fasciventris*: χ^2= 12.09, df=4, P<0.01; *C. capitata*: χ^2= 16.10, df=4, P<0.01). Similarly in greenhouse, oviposition by *C. fasciventris* females peaked in the second week (χ^2= 12.10, df=3, P<0.01) while no significant change in frequency of oviposition was observed for females of *C. cosyra* and *C. capitata*.

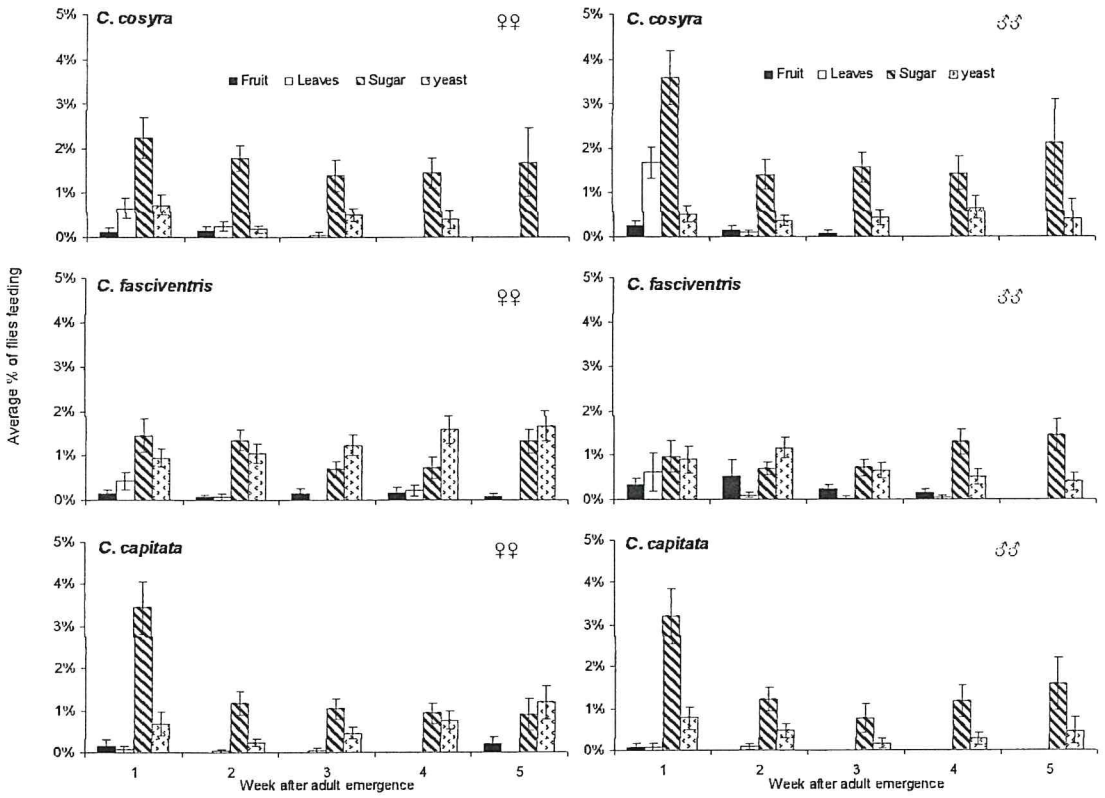

Fig. 11. Weekly feeding activities in plexiglass cages for females and males of *C. cosyra*, *C. fasciventris* and *C. capitata*. Values represent means ± SE.

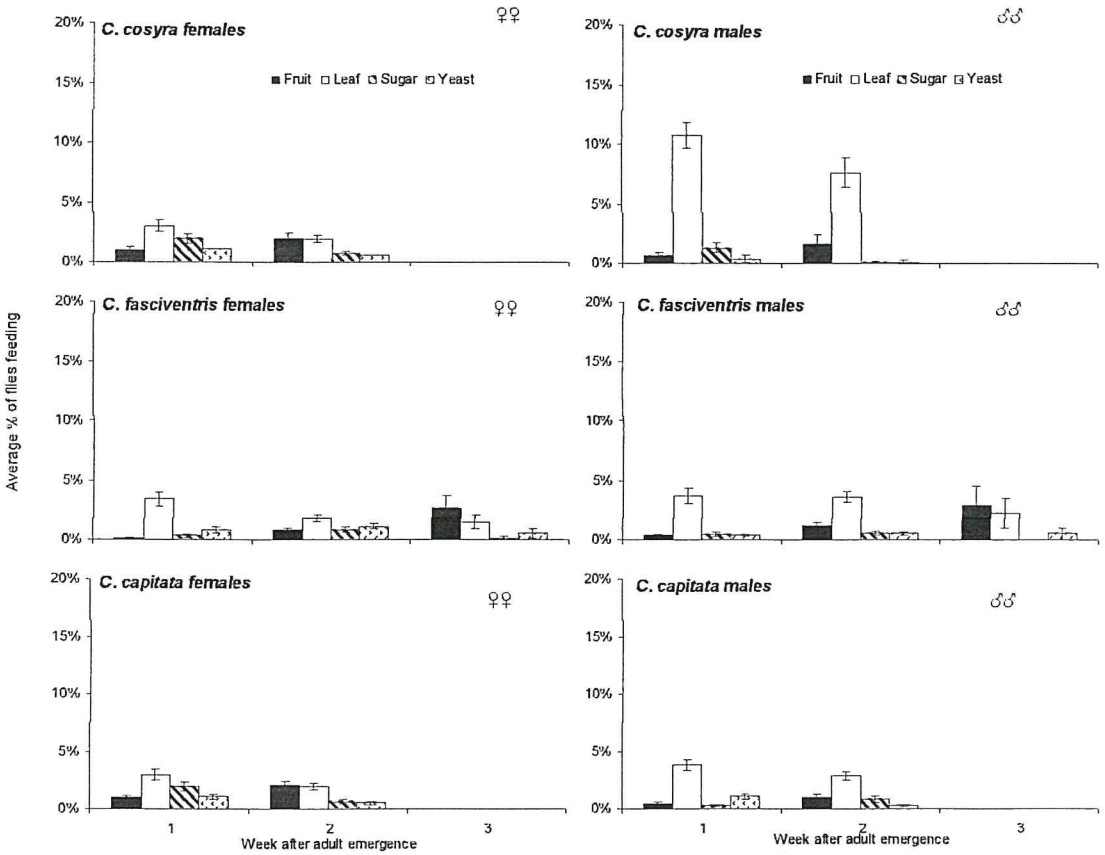

Fig. 12. Weekly feeding activities in field cages for females and males of *C. cosyra*, *C. fasciventris* and *C. capitata*. Values represent means ± SE.

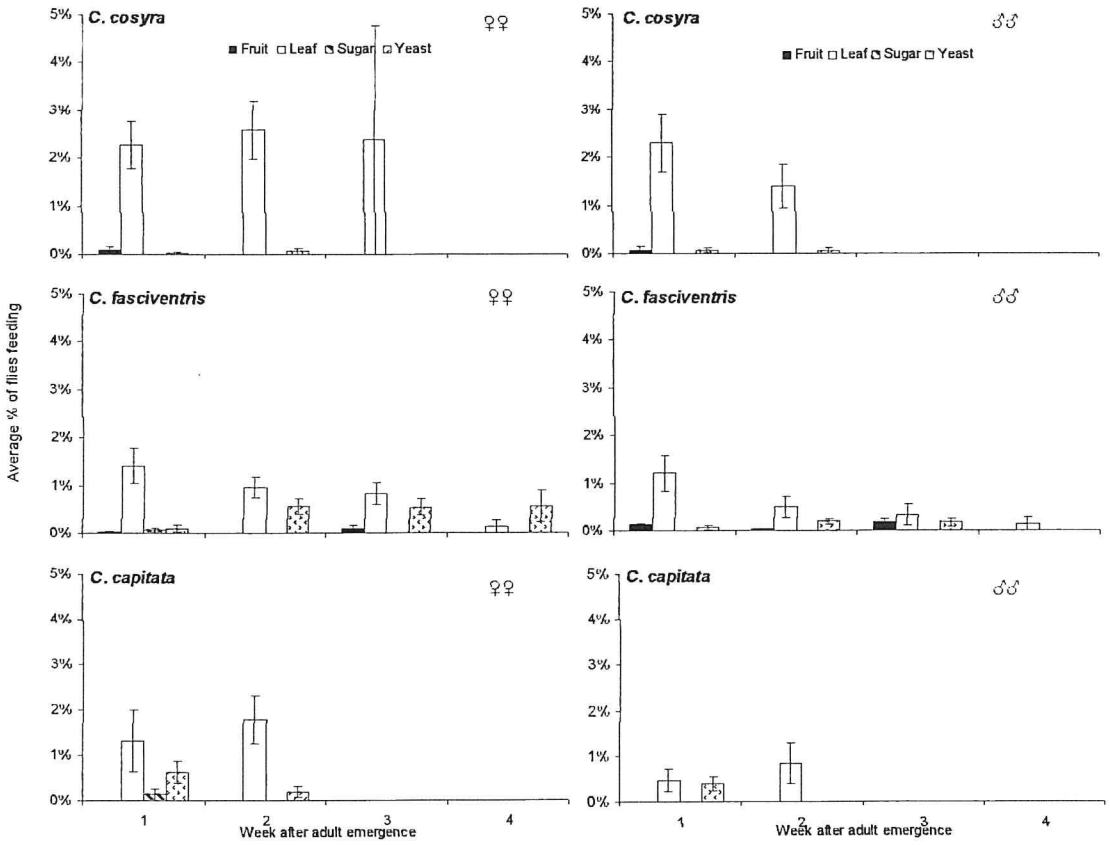

Fig. 13. Weekly feeding activities in the greenhouse for females and males of *C. cosyra*, *C. fasciventris* and *C. capitata*. Values represent means ± SE.

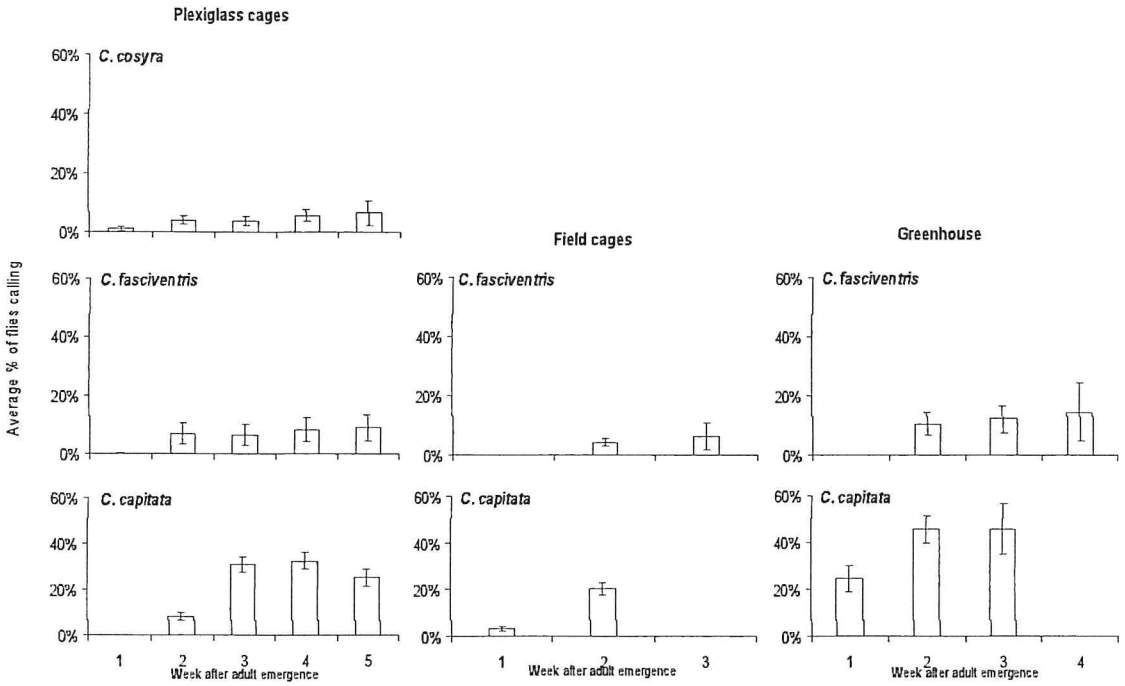

Fig. 14. Weekly calling activities in plexiglass cages, field cages and in the greenhouse for males of *C. cosyra*, *C. fasciventris* and *C. capitata*. Values represent means ± SE.

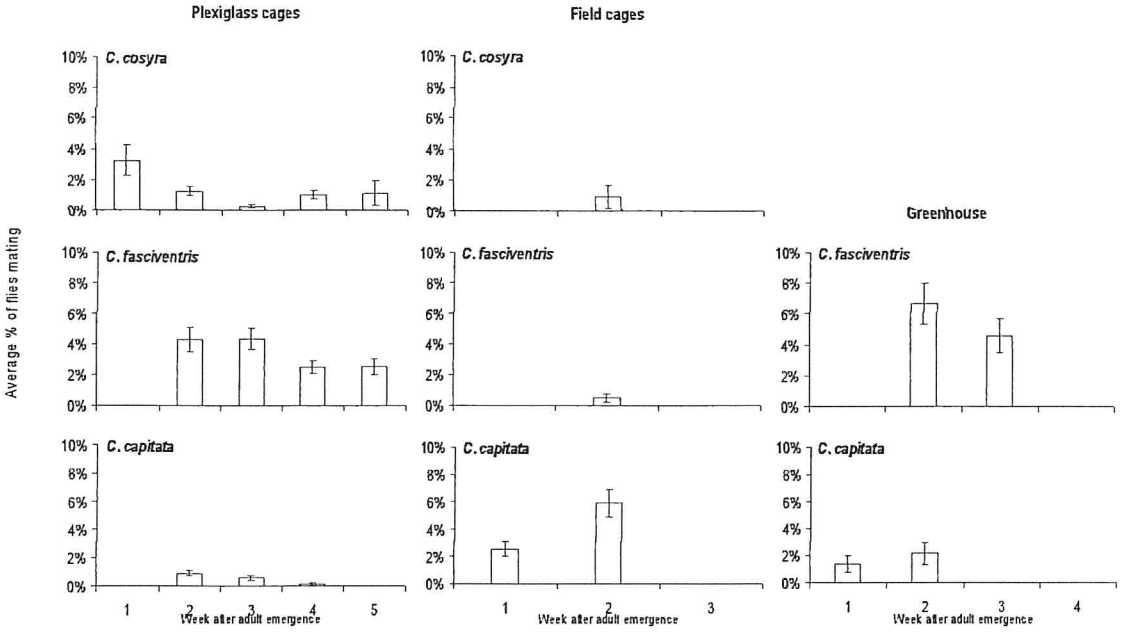

Fig. 15. Weekly mating activities in plexiglass cages, field cages and in the greenhouse for *C. cosyra*, *C. fasciventris* and *C. capitata*. Values represent means ± SE.