STUDIES ON NATIVE *TRICHOGRAMMA* (HYMENOPTERA: TRICHOGRAMMATIDAE) FOR BIOCONTROL OF SELECTED LEPIDOPTERAN PESTS IN KENYA

BY

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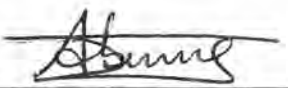
DECLARATIONS

This thesis is my original work and has not been presented for a degree in any other University or any other award.



28th May 2001

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DEDICATION

I dedicate this Ph.D. dissertation to

My wife, Admas Moges

My daughters, Eden and Bethlehem

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Abstract

Trichogramma species parasitise eggs of Lepidoptera and have been extensively used in augmentative biological control. Studies were conducted during 1997-2001 on the native egg parasitoids occurring in Kenya. The objectives of the study were (i) to identify the native egg parasitoids that attack *Helicoverpa armigera* and *Plutella xylostella,* (ii) to study the conventional and molecular taxonomy of the native *Trichogramma* species, (iii) to evaluate the response of two native *Trichogramma* species to different temperature regimes, (iv) to determine the life table parameters of the two *Trichogramma* species and (v) to determine the influence of host plants and host insects on parasitism of the two *Trichogramma* species.

In survey and field trials in Kenya, five native trichogrammatid egg parasitoids species: *Trichogramma bournieri* Pintureau & Babault, *Trichogramma* sp. nr. *mwanzai* Schulten & Feijen, *Trichogramma* sp. nr. *bruni* Nagaraja, *Trichogrammatoidea* sp. nr. *lutea* Girault and *Trichogrammatoidea* sp. were recovered from the eggs of *H. armigera*, *P. xylostella* and *Chilo partellus*. *Trichogramma bournieri* and *T.* sp. nr. *bruni*, recovered from *C. partellus,* are the first records for Kenya and probably the latter for Africa. The natural occurrence of *Trichogrammatoidea* sp. nr. *lutea* on *P. xylostella* eggs was also the first record for Kenya as well as for eastern Africa.

Trichogramma sp. nr. *mwanzai* was morphologically more similar to the Australasian *T. chilonis* Ishii than to *T. bournieri*. The genitalia of *T.* sp. nr. *mwanzai* was more similar to the Palaearctic species, *T. evanescens* Westwood,

than to the genitalia of *T. bournieri*. *Trichogramma bournieri* and *T.* sp. nr. *mwanzai* had 40% genetic similarity. The Kenyan species are more similar genetically than morphologically.

For all temperatures tested, *T.* sp. nr. *mwanzai* had the highest preimaginal survivorship. The developmental period for all the species decreased as the temperature increased to a maximum of 34°C. Sex ratio was female biased at all temperatures for *T. bournieri* and *T. chilonis*. In contrast, *T.* sp. nr. *mwanzai* sex ratio was males biased.

The life table study showed that *T.* sp. nr. *mwanzai* had a significantly higher fertility than *T. bournieri*. The respective proportions of female progenies were 52 and 72%. There was no significant difference in the intrinsic rate of natural increase and the net reproductive rate between the two native species at 26±1°C, 70±10% relative humidity and 16L: 8D photoperiod.

In the laboratory no-choice experiments, *T. bournieri* and *T.* sp. nr. *mwanzai* showed a high preference for *C. partellus* and *Corcyra cephalonica* compared to *H. armigera*. In the field cage experiment using *C. partellus* eggs, both species showed a stronger preference for maize plant than for tomatoes, in both choice and no choice tests. There was a strong host insect and host plant effect on the preference and suitability of the two native species.

Trichogramma sp. nr. *mwanzai* could be a candidate species for mass production and field releases due to its high fertility and tolerance to higher temperature regimes. This study has generated new information on egg parasitoids in Kenya and it is hoped that this study will foster more research on the biology and ecology of egg parasitism.

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CHAPTER 1

1.0 GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 GENERAL INTRODUCTION

1.1.1 Lepidopteran pests of vegetable crops in Kenya

Vegetable crop production is emerging as an income generating agricultural enterprise among smallholder farmers in Kenya, mainly due to the attractive prices offered in urban markets. Commonly grown vegetable crops are tomatoes and brassicas for the urban demand, while capsicums, French beans, snow peas and okra are for export especially to the European market. The area under vegetable crops in 1992 and 1994 was 82,414 and 91,970 hectares, respectively (HCDA, 1998). Pests and diseases are among the major production constraints in vegetable production in Kenya (Farrell *et al.*, 1995).

The African bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae), and the diamondback moth, *Plutella xylostella* Linnaeus (Lepidoptera: Yponomeutidae) are important pests of vegetables in Kenya and in different parts of the world (Hill, 1983; Ikin *et al.*, 1993; Farrell *et al.*, 1995). According to Van den Berg (1993), almost 300 different plant species have been recorded as hosts of the polyphagous *H. armigera*. The diamondback moth is a serious pest of crucifer species (Hill, 1983).

In addition to direct loss in yield, the cosmetic damage caused by larval feeding often results in reduced market prices. In Kenya, yield losses of up to 100% by *P. xylostella* on cabbage and 24% on tomato by *H. armigera* has been reported (ICIPE, 1997).

1.1.2 Control of *Helicoverpa armigera* and *Plutella xylostella*

Cultural practices such as manipulation of planting dates, stubble cultivation and destruction of crop residues, use of closed seasons and destruction of alternative hosts are some of the agronomic practices used in the management of *Helicoverpa* species. Microbial agents such as the bacterium *Bacillus thuriengiensis*, the fungi *Nomuraea rileyi* and *Entomophthora* spp., the protozoa *Nosema heliothidis* and *Vairimorpha necatrix*, and several viral pathogens including the nuclear polyhedrosis virus (NPV) are also used in controlling *H. armigera* (King and Coleman, 1989). Van den Berg (1993) catalogued 93 parasitoids as natural enemies of *H. armigera*.

The Diamondback moth, *P. xylostella,* is amenable to control by intercropping, use of sprinkler irrigation, trap cropping, rotation, and clean cultivation. There are over 90 parasites and predators recorded attacking the diamondback moth (Talekar and Shelton, 1993).

The indiscriminate use of synthetic pesticides for controlling these pests is undesirable because of pesticide residues and other adverse effects on the ecosystem. Pesticide resistance has also been reported in eastern Africa among populations of *H. armigera* and *P. xylostella* (Ikin *et al.*, 1993). In a regional workshop on pest management in vegetables in Africa, held in Senegal in 1992, the need for reversing the trend of unilateral dependence on pesticide use through Integrated Pest Management (IPM) strategies involving use of biological and traditional pest control techniques was emphasized (Ikin *et al.*, 1993). The use of biocontrol agents offers promise as an important

component of environmentally sustainable control system against *H. armigera* (King and Coleman, 1989) and *P. xylostella* (Talekar and Shelton, 1993). Among the potential biocontrol agents for these key pests are the egg parasitoids.

1.1.3 Biological control using egg parasitoids

The field of biological control includes importation (classical), augmentation, and conservation of beneficial organisms such as parasitoids, predators and pathogens for the regulation of population densities of other organisms (Waage and Greathead, 1986; Van Driesche and Bellows, 1996). According to Van Driesche and Bellows (1996) and Knutson (1998), classical biological control entails the importation and establishment of exotic natural enemies into a new environment based on the fact that many, if not most, agricultural pests have been accidentally introduced into the area concerned, while their indigenous natural enemies have been left behind.

Augmentation of natural enemies is the action taken to increase the populations or beneficial effects of natural enemies, whereas conservation of natural enemies refers to actions purposely taken to protect and maintain populations of natural enemies (Van Driesche and Bellows, 1996; Knutson, 1998). Overholt (1997) outlined the considerations involved in the selection, mass production, packaging, transport and evaluation of natural enemies used in biological control.

As a means of preventing larval damage, there is the need to explore the scope for increasing the egg stage mortality of lepidopteran pests. Egg parasitoids, particularly *Trichogramma* species, are good natural enemies of caterpillar pests because they parasitise and kill pests in the egg stage, before the crop is damaged (Wajnberg and Hassan, 1994). *Trichogramma* species have been reported as successful candidates for augmentative biological control of vegetable pests, from several countries such as Germany (Hassan and Wührer, 1997) and Japan (Hirai, 1990). Most of the research on egg parasitoids has been in cereal- sugarcane-and cotton- based systems (Wajnberg and Hassan, 1994).

1.1.4 Naturally occurring egg parasitoids

Bin (1994) reported that besides Trichogrammatidae, seven other families have been used for biological control, two of which are composed entirely of egg parasitoids (Scelionidae and Mymaridae), while five include scattered genera and species (Eulophidae, Encyrtidae, Eupelmidae, Platygastridae and Tetracampidae), a few of which, as immatures or as adults (host feeding), are also egg predators. According to Pinto and Stouthamer (1994), there are about 80 genera and over 600 species of insect egg parasitoids in the family Trichogrammatidae of the order Hymenoptera. To date, there are about 200 species of the oophagous *Trichogramma* and 25 species of *Trichogrammatoidea* worldwide (Nagaraja, 1987; Pinto, 1999).

The natural occurrences of trichogrammatid and scelionid egg parasitoids have been reported from several countries in Africa (Tables 1.1, 1.2 and 1.3). Tsankov *et al.* (1995) also reported the occurrence of the egg parasitoids, *Baryscapus servadeii* (Eulophidae) and *Ooencyrtus pityocampae* (Encyrtidae) on *Thaumetopoea pityocampae* in Algeria. About 40 species of egg parasitoids occurring in four families; Trichogrammatidae, Scelionidae, Eulophidae and Encyrtidae were recorded in Africa, most of which were recovered from *Chilo* species (Table 1.1). *Trichogrammatoidea lutea* Girault was found in many African countries like South Africa, Kenya, Mali, Ivory Coast, Ethiopia, Senegal and Mozambique (Table 1.2). Among the scelionids, *Telenomus bini* Polaszek & Kimani and *Telenomus busseolae* Gahan were common to many countries in Africa (Table 1.3).

Research on the two lepidopteran target pests in Kenya has so far revealed the occurrence of few species of egg parasitoids in two families: Trichogrammatidae and Scelionidae. Van den Berg (1993) recorded four species of *Trichogrammatoidea* attacking *H. armigera* eggs in Kenya: *T. armigera* Nagaraja, *T. eldanae* Viggiani, *T. lutea* Girault and *T. simmondsi* Nagaraja. Ochiel (1989) and Ngi-Song (1990) reported *Trichogramma* sp. nr. *exiguum* Pinto & Platner and *Trichogramma* sp. nr. *mwanzai* Schulten & Feijen as naturally occurring egg parasitoids of *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae) in Kenya, respectively.

Telenomus busseolae Gahan, *Telenomus bini* Polaszek & Kimani, *Telenomus nemesis* Polaszek & Kimani and *Telenomus thestor* Nixon and *Telenomus ullyetti* Nixon (Hymenoptera: Scelionidae) were reported as being native to Kenya (Van den Berg, 1993; Polaszek and Kimani, 1998). There are likely to be many more species occurring in this country, and additional surveys would be useful especially in unexplored geographical areas. While *Trichogramma* has been the predominant genus so far utilized, it is important to recognize that other genera may also show potential.

Table 1.1 *Trichogramma* egg parasitoids reported from Africa

Trichogramma species	Host Insect	Host Plant	Country	Reference
Trichogramma bourarachae Pintureau & Babault	*Helicoverpa armigera*	-	Morocco	Pintureau and Babault, 1988
Trichogramma bournieri Pintureau & Babault	*Chilo partellus*	-	Comoros	Pintureau and Babault, 1988
	C. partellus	Maize	Kenya	Abera *et al.*, 2000a
Trichogramma sp. nr. *bruni* Nagaraja	*C. partellus*	Sorghum	Kenya	Abera *et al.*, (unpublished)
Trichogramma cacoeciae Marchal	-	-	Morocco	Pintureau and Babault, 1988
Trichogramma chilonis Ishii	*Eldana saccharina*	Sugarcane	South Africa	Conlong and Hastings, 1984
	Busseola fusca and *C. partellus*	Maize and Sorghum	South Africa	Kfir, 1991
Trichogramma ethiopicum Risbec	-	-	Cameron	Pintureau and Babault, 1988
Trichogramma evanescens Westwood	*H. armigera*	-	Egypt	Zaki, 1985
Trichogramma sp. nr. *evanescens* Westwood	*H. armigera*	Cotton	Madagascar	Vaissayre, 1977
Trichogramma japonicum Ashmead	*C. partellus*	Rice	Malawi	Feijen and Schulten, 1981
Trichogramma kalkae Schulten & Feijen	*Diopsis macrophthalma*	Rice	Malawi	Schulten and Feijen, 1978
Trichogramma sp. nr. *kalkae* Schulten & Feijen	-	-	Zimbabwe	J. Pinto (unpublished)
Trichogramma kayo Risbec	-	-	Sudan	Pintureau and Babault, 1988
Trichogramma mandelai Pintureau & Babault	*Diparopsis watersi*	-	Chad	Pintureau and Babault, 1988
Trichogramma mwanzai Schulten & Feijen	*Chilo diffusilineus*	Rice	Malawi	Schulten and Feijen, 1982
Trichogramma sp. nr. *mwanzai* Schulten & Feijen	*C. partellus*	Sorghum	Kenya	Ngi-Song, 1990
Trichogramma ostriniae Pang & Chen	*B. fusca* and *C. partellus*	Maize and Sorghum	South Africa	Kfir, 1991
Trichogramma pinneyi Schulten & Feijen	*D. macrophthalma*	Rice	Malawi	Schulten and Feijen, 1978
Trichogramma sp. nr. *exiguum* Pinto & Platner	*C. partellus*	Sorghum	Kenya	Ochiel, 1989
Trichogramma vogelei Pintureau	-	-	Morocco	Mimouri, 1991

Table 1.2 *Trichogrammatoidea* egg parasitoids reported from Africa

Trichogrammatoidea species	Host Insect	Host Plant	Country	Reference
Trichogrammatoidea armigera Nagaraja	*H. armigera*	-	Kenya	Van den Berg, 1993
Trichogrammatoidea bactrae Nagaraja	*Pectinophora gossypiella*	-	Egypt	Ei-Hafez and El-Hafez, 1995
Trichogrammatoidea citri Risbec	-	-	Madagascar	Pintureau and Babault, 1988
Trichogrammatoidea combreti Risbec	-	-	Senegal	Pintureau and Babault, 1988
Trichogrammatoidea cryptophlebia Nagaraja	*E. saccharina*	Sugarcane	South Africa	Conlong and Hastings, 1984
	C. batrochopa and *C. leucotreta*	Macadamia trees	Malawi	Chambers *et al.*, 1995
Trichogrammatoidea eldanae Viggiani	*Sesamia calamists*	Maize	Nigeria	Bosque *et al.*, 1994
	H. armigera	-	Kenya	Van den Berg, 1993
Trichogrammatoidea lutea Girault	*H. armigera*	Cotton	South Africa	Van Hamburg and Kfir, 1990
	H. armigera	-	Kenya	Van den Berg, 1993
	-	-	Mali	Pintureau and Babault, 1988
	-	-	Ivory Coast, Ethiopia, Senegal, Mozambique	Nagaraja, 1978
Trichogrammatoidea sp. nr. *lutea* Girault	*Plutella xylostella*	Kale	Kenya	Abera *et al.*, 2000b
Trichogrammatoidea simmondsi Nagaraja	*H. armigera*	-	Kenya	Van den Berg, 1993
	C. partellus	Rice	Malawi	Feijen and Schulten, 1981
	Atherigona soccata	-	Burkina Faso	B. Pintureau (unpublished)
Trichogrammatoidea sp.	*H. armigera*	Pigeon pea	Kenya	Abera *et al.*, (unpublished)

Table 1.3 *Telenomus* egg parasitoids reported from Africa

Telenomus species	Host Insect	Host Plant	Country	Reference
Telenomus applanatus Bin & Johnson	*E. saccharina*	Sugarcane	South Africa	Conlong and Hastings, 1984
	E. saccharina	Sugarcane	Ivory Coast	Bin and Johnson, 1982
	Maliarpha separatella	Rice	Ivory Coast	Bin and Johnson, 1982
	E. saccharina	Maize	Nigeria	Bosque *et al.*, 1994
	M. separatella	-	Gabon, Ghana	Polaszek and Kimani, 1998
Telenomus bini Polaszek & Kimani	-	-	Ghana, Ivory Coast, Kenya, Malawi, Madagascar, Senegal, Tanzania	Polaszek and Kimani, 1998
Telenomus busseolae Gahan			Cameroon, Egypt, Ghana, Kenya, Mauritius, Niger, Nigeria, Uganda, Reunion, Senegal, South Africa	Polaszek and Kimani, 1998
Telenomus creusa Polaszek & Kimani	*C. diffusilineus*	-	Malawi	Polaszek and Kimani, 1998
Telenomus isis Polaszek	*S. calamistis*	Maize	Nigeria	Bosque *et al.*, 1994
	-	-	Benin, Cameroon, Ghana, Ivory Coast	Polaszek and Kimani, 1998
Telenomus nemesis Polaszek & Kimani	*Chilo* spp.	-	Kenya, Mozambique, Senegal, Ghana,	Polaszek and Kimani, 1998
Telenomus nephele Nixon	*Scirpophaga* spp.	-	Cameroon, Ghana, Ivory Coast, Malawi, Mali, Senegal	Polaszek and Kimani, 1998
Telenomus soudanensis Risbec	-	-	Mali, Senegal	Polaszek and Kimani, 1998
Telenomus thestor Nixon	-	-	Kenya, Uganda, Ivory Coast, Zaire, Senegal,	Polaszek and Kimani, 1998
Telenomus ullyetti Nixon	*H. armigera*	-	Kenya	Van den Berg, 1993
	H. armigera	Cotton	South Africa	Van Hamburg and Kfir, 1990
	C. diffusilineus	Rice	Malawi	Feijen and Schulten, 1981
Telenomus versicolor Bin & Johnson	-	-	Ghana, Ivory Coast, Malawi, Senegal	Polaszek and Kimani, 1998

Local species are generally preferred on the basis that they are likely to be better adapted to the ecological conditions such as the climate, habitat, and host conditions than exotic species (Hassan, 1994; Voegele, 1988; Smith, 1996). For example, six native species of *Trichogramma* have been used successfully around the world to control *Ostrinia* species, namely *T. nubilale* Ertle & Davis and *T. pretiosum* Riley in the United States, *T. ostriniae* Pang & Chen and *T. dendrolimi* Matsumura in China, and *T. evanescens* Westwood and *T. brassicae* (=*maidis*) Bezdenko in Europe (Smith, 1996). In Australia, Scholz (1990) reported that field releases of exotic egg parasitoids (the American species *T. pretiosum*) against *Helicoverpa* in cotton failed to prevent economic damage.

Furthermore, national agencies tend to be often restrictive in issuing import permits for exotic species, which may have a detrimental side effect on autochthonous parasitoids and thus it is important that effective native species be identified, especially during the first screening (Michael and Woods, 1980; Wajnberg, 1994). Hence, an inventory of locally occurring egg parasitoids should be established first and introduction of exotic species should be considered only if there are no promising locally occurring species or if pre-introductory evaluations indicate advantages of exotic over local species (Smith, 1996).

The principles applied in the design of an augmentative biological control program using *Trichogramma* has been summarized by Smith (1996). The major principles to be considered in the design are selection of the

parasitoids, production systems for mass rearing, distribution of the parasitoids, and the release as well as impact assessment strategies to be applied in the field (Smith, 1996). Selection of species is based on different attributes reflected on life table parameters, temperature tolerance and host plant and host insect effects (Hassan, 1994; Smith, 1996). In this study, some aspects that are relevant for utilization of native *Trichogramma* egg parasitoids against *H. armigera* and *P. xylostella* in Kenya under the framework of IPM (FAO, 1968; Brader, 1979; Dent, 1991; Morse and Buhler, 1997) are reviewed.

1.2 LITERATURE REVIEW

1.2.1 Insect pest control using trichogrammatid egg parasitoids

Trichogramma species are the most widely used insect natural enemies in the world, partly because they are easy to mass rear and they attack many important crop insect pests (Li, 1994). *Trichogramma* wasps occur naturally in almost every terrestrial habitat, and some aquatic habitats as well (Knutson, 1998). According to Hoffmann and Frodsham (1993), no other group of parasitoids has been used worldwide as extensively as *Trichogramma* for direct control of pests.

Trichogramma is usually associated with attacking eggs of Lepidoptera. However, they are also reported from eggs of Diptera, Coleoptera, Hemiptera, Homoptera, Hymenoptera, and Neuroptera (Wajnberg and Hassan, 1994). Globally, several *Trichogramma* egg parasitoid species have been reported as useful for biocontrol of *H. armigera* such as *T. evanescens* (Abbas, 1998), *T. pretiosum* (Krishnamoorthy and Mani, 1996; Knutson, 1998), *T. chilonis* Ishii (Romeis *et al.*, 1998), *T. dendrolimi*, *T. pintoi* Voegele, *T. carverae* Oatman & Pinto, and *T. rhenana* Voegele & Pintureau (Li, 1994). *Trichogramma papilionis* Viggiani and *T. chilonis* were also used for biocontrol of *P. xylostella* (Hirai, 1990).

Inundative release of mass-reared *Trichogramma* egg parasitoids is regarded as a promising method to reduce the egg hatching and the subsequent crop damage due to larval feeding (Wajnberg and Hassan, 1994; Smith, 1996). In Egypt, for example, Abbas (1998) released the native *T. evanescens* (50,000

adult parasitoids/hectare at two week intervals) against *H. armigera* and obtained 73.7% parasitism. According to Li (1994), nine species of *Trichogramma* are reared in private or government-owned insectaries around the world and released annually on an estimated 32 million hectares of agricultural crops and forests in 30 countries. The countries of the former Soviet Union lead in *Trichogramma* production, followed by China and Mexico (Li, 1994).

Trichogramma controls different lepidopteran pests that attack various crops such as maize, sorghum, rice, wheat, sugarcane, cotton, tomato, cabbage, fruit trees and pines (Li, 1994). Moreover, *Trichogramma* can be an effective way of pest control compared with other approaches in many parts of the world. Parasitoid releases in China, Switzerland, Canada, and the former USSR have all shown consistently high levels of parasitism (60-80%), with reduction in pest damage by 77- 92% on such crops as sugarcane, wheat, corn, and cole (Li, 1994).

Romeis *et al.* (1998) reported on constraints in using *T. chilonis* for the control of *H. armigera* on cotton in India. Low product quality has been recognised as one of the most important reasons for the failure of these biocontrol agents. According to Knutson (1998), effective pest control using *Trichogramma* is determined by many factors including, the species, the quality and fitness of the parasite product, the numbers and the timing of the release, the release method as well as the complex interactions among the parasite, the target pest, the crop and the environmental conditions.

In most crop production systems, the number of caterpillar eggs destroyed by native populations of *Trichogramma* is not sufficient to prevent the pest from reaching damaging levels. This necessitates inundative releases of *Trichogramma* to control the pest from reaching damaging levels (Smith, 1996; Knutson, 1998).

1.2.2 Taxonomy of *Trichogramma*

The genus *Trichogramma* is one of the 80 genera in the family Trichogrammatidae. All members of this family are parasites of insect eggs (Richards and Davies, 1977; Pinto and Stouthamer, 1994). Reliable identification of the species is a pre-requisite for successful biocontrol programmes with *Trichogramma*. Taxonomy of *Trichogramma* species has been a great problem due to their small size and uniform morphological characters (Pinto and Stouthamer, 1994).

The male genitalia have been widely used as the main character in distinguishing species of *Trichogramma*, but body colour, wing venation and features of the antennae serve as supporting characteristics (Nagarkatti and Nagaraja, 1971; Pinto and Stouthamer, 1994). The conventional taxonomy of *Trichogramma* is entirely based on male genitalia and useful only for arrhenotokous species. However, this classification does not include *Trichogramma* species that are thelytokous (100% female offspring) (Stouthamer *et al.*, 1990; Pinto and Stouthamer, 1994).

DNA-based species-specific markers are unaffected by the sex or life stage of *Trichogramma* (Vanlerberghe, 1994). All organisms have ribosomal DNA (rDNA), which is composed of several regions (genes and spacers), and there are two internal transcribed spacer regions (ITS-1 and ITS-2) (Hoy, 1994). Internally transcribed spacer 2 (ITS-2) of the nuclear ribosomal gene (rDNA) complex is useful in providing taxonomic characters for identification of *Trichogramma* species (Stouthamer *et al.*, 1999; Silva, 1999). Individual wasps could be also identified by amplification (RAPD -Random Amplified Polymorphic DNA) of their ITS-2 with general primers (Stouthamer *et al.*, 1999). Silva (1999) used molecular techniques for differentiation of five *Trichogramma* species from Portugal; *T. turkestanica* Meyer, *T. bourarachae* Pintureau & Babault, *T. cordubensis* Vargas & Cabello, *T. pintoi* and *T. evanescens*.

1.2.3 Biology of *Trichogramma*

Trichogramma (Hymenoptera: Trichogrammatidae) are extremely tiny wasps (ca. 0.5 mm in length and 8μg in weight) that lay their eggs inside the eggs of other insects, primarily lepidopterous insects (Richards and Davies, 1977; Wajnberg and Hassan, 1994; Silva, 1999). At 26°C, they complete their development from egg to adult in about ten days. The number of wasp eggs laid in a host egg increases with host size and thus, development is usually gregarious except in very small hosts where only one wasp develops in one host egg (Waage and Ming, 1984; Wajnberg and Hassan, 1994).

There are five phases in the oviposition behaviour of *Trichogramma*: contact, drumming, drilling, oviposition and host feeding (Pak, 1988). Ruberson and Kring (1993) also demonstrated the process of parasitism; that once a female finds a bollworm egg, it drills a hole through the chorion (egg shell) and inserts two to three eggs into the bollworm egg. The internal pressure of the bollworm egg forces a small drop of yolk out of the oviposition hole. Females feed on this yolk, which increases their longevity.

Under laboratory conditions, a female parasitises from one to ten bollworm eggs per day or from 10 to 190 during her life. Large females parasitise more eggs than smaller females. They also reported that females provided with honey and young bollworm eggs live on average 11 days, while females receiving only honey live for 3 days. Young bollworm eggs are preferred for parasitism to older eggs (Ruberson and Kring, 1993).

After oviposition, *Trichogramma* eggs hatch in about 24 hours and the parasite larvae develop very quickly. There are three larval instars in *Trichogramma* (Strand, 1986). The bollworm egg turns black during the third instar (3 to 4 days after the host egg was parasitised) as a result of dark melanin granules deposited on the inner surface of the egg chorion. According to Strand (1986), the black layer inside the chorion and the exit hole are evidence of parasitism by *Trichogramma*. Larvae then transform to the inactive pupal stage.

After about 4 to 5 days, the adult wasps emerge from the pupae and escape the bollworm egg by chewing a circular hole in the eggshell (Strand, 1986). Waage and Ming (1984) reported that where more than one *Trichogramma* develops in an egg or an egg mass, mating occurs on the hosts, with adult males emerging from eggs shortly before females. Hence, *Trichogramma* females have mature eggs at the time of emergence and they begin egg laying within a few hours of emergence (Knutson, 1998). The life cycle of the egg parasitoid *Trichogramma* is shown in Fig. 1.1.

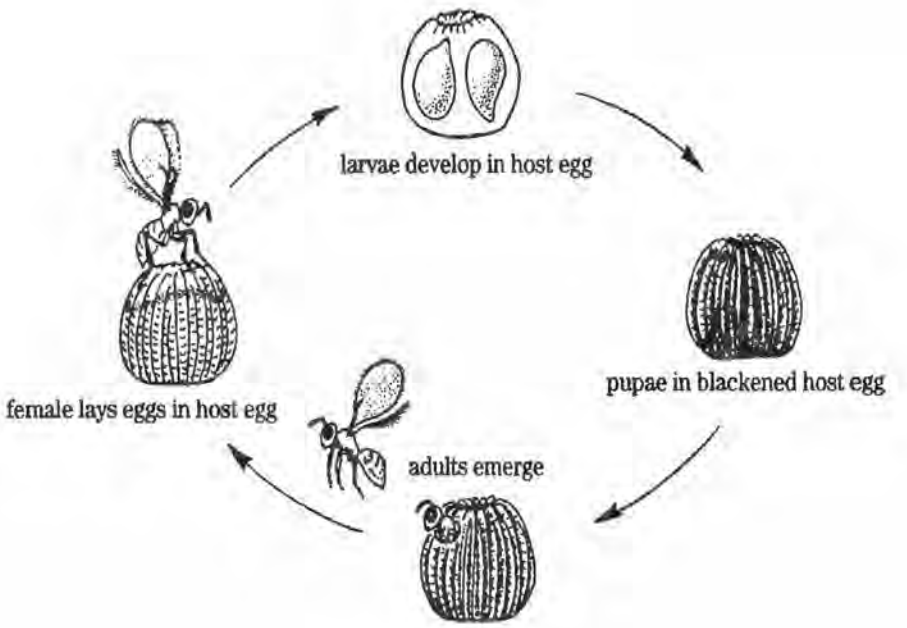


Fig. 1.1 Life cycle of the egg parasitoid *Trichogramma* species

(Hoffmann and Frodsham, 1993)

Parthenogenesis is characteristic of reproduction in *Trichogramma*, and most commonly arrhenotoky (sex ratio offspring, 50-75% female), however, thelytokous (100% female offspring) and deuterotokous (almost all female offspring) reproduction also occur (Stouthamer *et al.*, 1990; Pinto and Stouthamer, 1994). According to Pinto and Stouthamer (1994), some species are entirely thelytokous while others consist of both thelytokous and arrhenotokous populations. Arrhenotoky is the common mode of reproduction in *Trichogramma* whereby unfertilised eggs produce haploid males and fertilised eggs produce diploid females. In contrast, thelytokous populations consist of only females that produce female offspring without mating (Stouthamer *et al.*, 1990; Pinto and Stouthamer 1994).

According to Stouthamer and Kazmer (1994), two forms of thelytoky are recognised in *Trichogramma*, revertible or microbe-associated thelytoky and non-reversible thelytoky. Microbes are absent in non-reversible thelytoky, where as bacteria of the genus *Wolbachia* cause revertible thelytoky. The latter can be reverted to arrhenotoky by treatment with several specific antibiotics or elevated rearing temperatures (Stouthamer *et al.*, 1990).

1.2.3.1 Effect of temperature

Temperature is one of the main environmental factors affecting the developmental rate, fecundity, longevity, and sex ratio of *Trichogramma* (Cabello and Vargas, 1988). Forsse *et al.* (1992) observed that under laboratory conditions, lower temperatures resulted in lower levels of

parasitism. This has also been verified in field studies (Parker *et al.*, 1971). In Kenya, Ochiel (1989) observed significant effect of temperature on progeny production of *T.* sp. nr. *exiguum* when tested at 18 and 30°C. He, however, reported no significant difference in the proportion of female offspring at all temperatures tested. Pavlik (1990) studied the effect of three different temperatures on five *Trichogramma* species: *T. maidis, T. evanescens, T. dendrolimi, T. pintoi* and *T. ostriniae* and observed significant differences in parasitisation capacity.

According to Sharpe and DeMichele (1977), the development of a poikilothermic organism is driven by a rate-determining enzyme or enzyme complex, which has three basic reversible energy states: inactive at cold temperature, active at median temperature and inactive at hot temperature. Hassan (1994) stressed the importance of tolerance of prospective *Trichogramma* strains to extreme weather conditions in the target area. Temperature is also critical in mass rearing and release of *Trichogramma* parasitoids (Hassan, 1993; Smith, 1996).

1.2.3.2 Life table parameters

Life table analysis helps in understanding the dynamics of a population (Southwood, 1978). The basic population parameters like the intrinsic rate of natural increase (r_m), the net reproductive rate (R_o) and finite population growth rates (λ) are useful in the selection of the promising species (Southwood, 1978). According to Southwood (1978) and Dent and Walton

(1997), there are two types of life tables; the age-specific (cohort, horizontal) and the time-specific (static, vertical). The time-specific life tables consider census data taken on a single occasion, when it is assumed that all generations are completely overlapping and hence, all age classes are present simultaneously. By contrast, age-specific life tables involve repeated counting of a single cohort of similarly aged individuals over time, or mortalities are determined over the course of time for each stage of the species. Age-specific life tables are commonly used in entomology than time-specific life tables (Southwood, 1978; Dent and Walton, 1997).

The intrinsic rate of natural increase was used as a criterion for differentiating species of *T. pretiosum* and *T. retorridum* Girault (Orphanides and Gonzalez, 1971). Hassan and Guo (1991) conducted extensive experiments and selected *T. evanescens* for the control of the European corn borer, *Ostrinia nubilalis* Hübner on the basis of its high fertility. Adult longevity has also been used for comparing *T. chilonis* and *T. ostriniae* (Hirashima *et al.*, 1990). Life table studies and evaluation of fitness parameters of species of egg parasitoids that show potential for parasitising the target pests are also useful. However, these parameters have to be defined and should be able to be assessed in the laboratory.

1.2.3.3 Effect of host plants and host insects

The role of host plants and host insects on parasitism by different *Trichogramma* species could be determined by laboratory studies under choice

and no choice situations (Guang and Oloo, 1990; Bjorksten and Hoffmann, 1998) and field tests (Romeis *et al.*, 1997, 1998, 1999). Biological and behavioural selection on the basis of high fecundity, progeny production, sex ratio, longevity, host preference for the target species, host-searching activity, and tolerance to local conditions are useful (Hassan, 1994; Smith, 1996; Romeis *et al.*, 1997).

In a laboratory host preference and suitability study, Guang and Oloo (1990) found that *T.* sp. nr. *mwanzai* Schulten & Feijen showed no significant difference in progeny production on *C. partellus*, *Busseola fusca* Fuller, and *Sitotroga cerealella* Oliver while on *Eldana saccharina* Walker, it produced significantly fewer progeny. Further, the parasitoids did not accept eggs of the silk worm, *Bombyx mori* Linnaeus even under no choice situation. Ngi-Song (1990) provided a follow up study on this parasitoid and worked out a model of parasitism on the sorghum stalk borer, *C. partellus* in Kenya.

To locate a bollworm egg, the adult female wasp uses chemical and visual cues. The chemical cues, called kairomones, are on the moth scales left near the eggs by the female moth during oviposition (Nordlund, 1994). Ruberson and Kring (1993) and Romeis *et al.* (1998) reported egg shape and colour might also be visual cues to the wasp. Plant volatiles produced when the host plants are attacked also influence searching behaviour and parasitism of *Trichogramma* species (Romeis *et al.*, 1997; Bjorksten and Hoffmann, 1998).

1.2.4 Rearing of *Trichogramma*

According to Smith (1996), many systems of mass rearing have been developed. Rearing of *Trichogramma* using eggs of the rice meal moth, *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae), the Angoumois grain moth, *Sitotroga cerealella* Oliver (Lepidoptera: Gelechidae) and the Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lepidoptera: Pyralidae) are common in different parts of the world (Hassan, 1993; Singh and Jalali, 1994; Smith, 1996). *Trichogramma* can also be reared on artificial diets (Grenier, 1994). About 18 species of egg parasitoids (most of them Trichogrammatidae) were reared using artificial eggshell in vitro. Some *Trichogramma* species produced under artificial conditions were released in the fields and successfully controlled pests (Grenier, 1994). The optimum rearing condition for mass production of *Trichogramma* species is 27°C temperature and 70% relative humidity (Hassan, 1993).

Most of the above studies concentrated on cereal- sugarcane and cotton- based systems. Very little is known about the natural occurrence of egg parasitoids and their biology particularly in eastern Africa. Thus studies on their occurrence, biology, temperature response, life table parameters and effects of host plants and host insects were regarded as priority areas. The present research project was therefore undertaken with the following hypotheses and objectives.

1.3 HYPOTHESES

The three main hypotheses were (i) the egg parasitoids species occurrence is wider than what is known so far in Kenya, (ii) *Trichogramma* species show preference to host plants and host insects and (iii) differential tolerance exists in local *Trichogramma* species to extremes of temperature.

1.4 OBJECTIVES OF THE STUDY

1.4.1 General objective

The general objective was to determine the existing egg parasitoids species occurrence and assess the potential for *Trichogramma* in enhancing egg mortality on the two major lepidopteran pests (*Helicoverpa armigera* and *Plutella xylostella*) on vegetable crops in Kenya.

1.4.2 Specific objectives

The specific objectives of the study were as follows:

(i) To identify the native egg parasitoids that attack *Helicoverpa armigera* and *Plutella xylostella*

(ii) To study the conventional and molecular taxonomy of the native *Trichogramma* species.

(iii) To evaluate the response of two native *Trichogramma* species to different temperature regimes.

(iv) To determine the life table parameters of two native *Trichogramma* species.

(v) To determine the influence of host plants and host insects on parasitism of two native *Trichogramma* species.

CHAPTER 2

2.0 NATIVE EGG PARASITOIDS OF *HELICOVERPA ARMIGERA* AND *PLUTELLA XYLOSTELLA*

2.1 INTRODUCTION

Inundative release of mass-reared trichogrammatid egg parasitoids (Hymenoptera: Trichogrammatidae) is regarded as a promising method to control several lepidopteran pests (Wajnberg and Hassan, 1994; Smith, 1996). Egg parasitoids of the genus *Trichogramma* are the most widely used insect natural enemies in the world, partly because they are easy to mass rear and they attack many important crop insect pests (Li, 1994). *Trichogramma* are particularly good natural enemies of caterpillar pests because they parasitise and kill pests in the egg stage, before the crop is damaged (Wajnberg and Hassan, 1994).

Parasitoid releases in China, Switzerland, Canada, and the former USSR have all shown consistently high levels (60-80%) of parasitism, with reduction in pest damage by 77-92% on such crops as sugarcane, wheat, corn, and cole (Li, 1994). Globally, over 32 million hectares of agriculture and forestry has been treated annually with *Trichogramma* species for controlling different insect pests (Li, 1994). According to Hoffmann and Frodsham (1993), no other group of parasitoids has been used worldwide as extensively as *Trichogramma* for direct control of pests.

Naturally occurring egg parasitoids on the polyphagous noctuid *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) have been reported from several countries such as Egypt (Abbas, 1998), South Africa (Van Hamburg and Kfir, 1990), the former USSR (Nikonov *et al.*, 1990), China (Fucheng and Zhang, 1990), Portugal (Meierrose *et al.*, 1990), Australia (Scholz, 1990), USA (Knutson, 1998) and India (Rawat and Pawar, 1993; Romeis and Shanower, 1996; Monje *et al.*, 1998). Keinmeesuke *et al.* (1990) in Thailand and Wührer and Hassan (1993) in Germany have also reported the natural occurrence of egg parasitoids on *Plutella xylostella* Linnaeus (Lepidoptera: Yponomeutidae). To date, there are about 200 species of the oophagous *Trichogramma* and 25 species of *Trichogrammatoidea* worldwide (Nagaraja, 1987; Pinto, 1999).

There were no reports on the natural occurrence of egg parasitoids of *P. xylostella* in Kenya (Waiganjo, 1996). However, Van den Berg (1993) recorded four species of *Trichogrammatoidea* affecting *H. armigera* eggs in Kenya: *T. armigera* Nagaraja, *T. eldanae* Viggiani, *T. lutea* Girault and *T. simmondsi* Nagaraja. Ochiel (1989) and Ngi-Song (1990) reported *Trichogramma* sp. nr. *exiguum* Pinto & Platner and *Trichogramma* sp. nr. *mwanzai* Schulten & Feijen, respectively as naturally occurring egg parasitoids of *Chilo partellus* Swinhoe (Lepidoptera: Pyralidae) in Kenya. *Telenomus busseolae* Gahan, *Telenomus bini* Polaszek & Kimani, *Telenomus nemesis* Polaszek & Kimani and *Telenomus thestor* Nixon and *Telenomus ullyetti* Nixon (Hymenoptera: Scelionidae) were also reported as egg

parasitoids which are native to Kenya (Van den Berg, 1993; Polaszek and Kimani, 1998).

Hassan (1994) and Voegele (1988) observed that the local species are generally selected for inundative releases on the basis that they tend to be better adapted to the climate, habitat, and host conditions. In Australia, Scholz (1990) reported that field releases of exotic egg parasitoids (the American species *Trichogramma pretiosum* Riley) against *Helicoverpa* in cotton failed to prevent economic damage. Furthermore, the recent move of national agencies in some countries to restrict the importation of organisms for biological control makes it important that effective native species be identified, especially during the first screening (Michael and Woods, 1980; Wajnberg, 1994).

According to Overholt (1997), the decision whether to select a native or an exotic natural enemy for an augmentation programme depends on the type of the pest whether it is native or exotic. If the pest is native, then it is most likely that the best-adapted natural enemies can be found in the same environment. If the pest is exotic, the approach of choice should be classical biological control through the importation of 'old association' natural enemies (Overholt, 1997). Apart from isolated reports of occurrence of some egg parasitoids, no systematic studies have been conducted on native species of Africa. Therefore, survey and identification of native egg parasitoids of *H. armigera* and *P. xylostella* was considered a priority area in the International Centre of Insect Physiology and Ecology (ICIPE) programmes.

Several researchers have studied the natural mortality of eggs in the field. In Kenya, Oloo (1989) and Bonhof (2000) have also studied the role of predation and disappearance on eggs of *Chilo* spp. on maize and sorghum. Van den Berg (1993) has also studied the role of predation on *H. armigera* eggs on sunflower. Andow (1990) showed the role of predation on egg masses of the European corn borer *Ostrinia nubilalis* Hübner. However, studies on the natural mortality factors of *P. xylostella* eggs were not available, particularly in Africa.

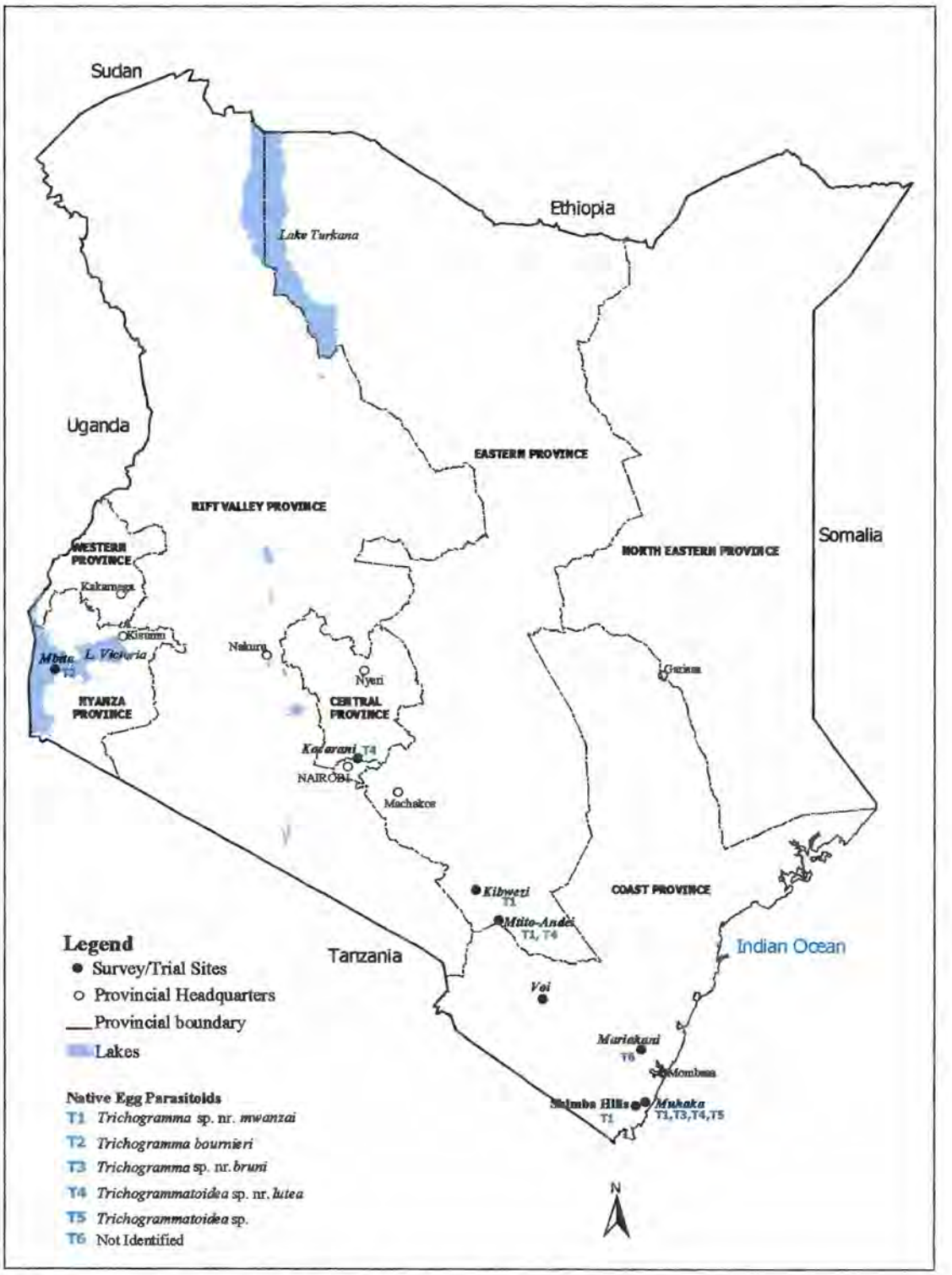
Therefore, the objectives of this study were (i) to assess the natural occurrence of egg parasitoids and their parasitism levels at different sites and habitats, and (ii) to identify the major sources of mortality of *P. xylostella* eggs under natural conditions in the field.

2.2 MATERIALS AND METHODS

2.2.1 Survey of egg parasitoids

Survey of egg parasitoids was conducted at seven sites along the Nairobi-Mombasa highway in Kenya with the aim of determining species occurrence in different habitats and locations. Except Kasarani (the head quarters of ICIPE), the profile of the survey was at an altitudinal difference of about 200 meters above sea level (Thorpe, 1984; Cagan *et al.*, 1998). The seven sites were Kasarani (01° 13' 06.2" S, 36° 53' 41.9" E and 1600 m altitude), Kibwezi (02° 24' 19.3" S, 37° 58' 9.5" E and 976 m), Mtito Andei (02° 40' 45.9" S, 38° 10' 18.5" E and 700 m), Voi (03° 23' 24.5" S, 38° 34' 37.3" E and 510 m), Mariakani (03° 51' 20.6" S, 39° 27' 58.8" E and 263 m), Shimba Hills (04° 21' 23.7" S, 39° 25' 17.2" E and 250 m) and Muhaka (04° 19' 18.1" S, 39° 30' 24.3" E and 40 m) (Map 1). Location of the sites was determined using the Geo Positioning System (GPS).

To assess parasitism, egg cards containing freshly laid eggs of *Corcyra cephalonica* Stainton (Lepidoptera: Pyralidae) (ca. 150 eggs/card) were used. The method of egg card preparation is outlined in section 2.2.5. To prevent hatching, before parasitisation, eggs were treated with ultraviolet rays (15 V lamp) for six minutes at a distance of 15 cm (Singh *et al.*, 1994). Sterilisation of host eggs was necessary to prevent caterpillars from emerging from unparasitised eggs and feeding on the parasitised eggs.



Map1 Egg parasitoids field trials and survey sites in Kenya

In every site, eight plant species (four crops and four weeds) were randomly selected among a group of nine crops and weeds. Plants used for the study were banana (*Musa acuminata* Collar), kale (*Brassica oleracea* L.), maize (*Zea mays* L.), okra (*Hibiscus esculentus* L.), pigeon pea (*Cajanus cajan* Mill.), sorghum (*Sorghum bicolor* L.), sugarcane (*Saccharum officinarum* L.), sunflower (*Helianthus annuus* L.), and tomato (*Lycopersicon esculentum* Mill.). The weeds were *Amaranthus gangeticus* L., *Argemone mexicana* L., *Bidens pilosa* L., *Commelina benghalensis* L., *Cynodon dactylon* L., *Cyperus esculentus* L., *Cyperus papyrus* L., *Tridax procumbens* L. and *Pennisetum purpureum* Schum.

Four egg cards were placed on four plants of the particular species (one egg card per plant) using fine staples (Thorpe, 1984; Scholz, 1990). In a single survey, a total of 32 egg cards (ca. 4800 eggs) were exposed per site. The study was conducted three times in every two months (in October and December 1999 and February 2000). After three days of exposure, recovered egg cards were placed in separate glass vials (50 mm long x 12 mm in diameter) and brought into the laboratory for observations of parasitism and parasitoids emergence. Species composition, percentage parasitism and emergence, and sex ratio were recorded. The identification of the emerged parasitoids was undertaken with the same protocol as described in section 2.2.2.3. Eggs were considered parasitised if they turned black (Hassan, 1990).

2.2.2 Field trials

2.2.2.1 Study sites

Field trials were conducted at the International Centre of Insect Physiology and Ecology's Muhaka field station located at latitude 04° 19' 18.1" S and longitude 39° 30' 24.3" E at the southern coastal area of Kenya. The site is located at an elevation of 40 meters above sea level and receives approximately 1200 millimetres rainfall annually. In many parts of Kenya, including Muhaka field station there are two rainy seasons, the long rainy season (April-August) and the short rainy season (October-February) (Van den Berg, 1993). The soils in the study area are sandy with very low fertility. Earlier, similar field trials were conducted in June and November 1998 at Mbita ICIPE's field station (latitude 0° 25' S, longitude 34° 10' E and altitude 1170 m) at the eastern shores of Lake Victoria, western Kenya (Map 1). However, due to very low level of parasitism, no recovery was made at Mbita.

2.2.2.2 Experimental design and management

Field trials were conducted for two seasons to assess the occurrence of native egg parasitoids and determine their natural parasitism levels on three major lepidopteran pests: *H. armigera*, *P. xylostella* and *C. partellus*. Due to previous records of native egg parasitoids in the country (Ochiel, 1989; Ngi-Song, 1990), *C. partellus* was included for comparison. The field experiment was laid out in a 6 x 6 Latin-square design. The plot size was 5.6 m x 5.6 m (31.36 m^2). Six crops; tomato, okra, kale, pigeon pea, sunflower and sorghum

were used for the trial. Tomato, okra, pigeon pea, sunflower and sorghum were host plants for *H. armigera,* while kale and sorghum were host plants for *P. xylostella* and *C. partellus*, respectively. The field layout is shown in Appendix 1.

Tomato and kale seedlings were raised at the nursery and planted at the same time with the other crops. Planting was done in June (first season) and November 1999 (second season). Sorghum seeds were treated with Gaucho FS 490gm ai/100kg seed to control the shoot fly, while Karate 1.75% EC (1.0 litre/ha) was applied at early seedling stage of kale to control grasshopper infestations. Fertilizer application involved Di-Ammonium Phosphate (125 kg/ha) at planting and Calcium Ammonium Nitrate top dressing (125 kg/ha) at three weeks after planting. Sprinkler irrigation was applied during dry spells, and weeding was done by hand.

2.2.2.3 Sampling and identification

Each plot was divided into four subplots, and four plants per subplot (16 plants per plot) were tagged randomly. The last row was excluded from sampling so as to avoid border effect. Sampling of eggs was conducted by examining all parts of the individually tagged plants twice a week (every Monday and Thursday). Sampling was conducted starting from five weeks after planting so as to synchronise the presence of eggs of the three target hosts. This was continued for ten weeks making a total of 20 samplings per season. All sampling was conducted during morning hours.

Density of eggs of *H. armigera*, *P. xylostella* and *C. partellus* were counted per plant using a hand lens (magnification 10x). During each sampling period, a maximum of 30 eggs of *H. armigera* and *P. xylostella,* and 1 egg mass (30-40 eggs/egg mass) of *C. partellus* were collected from each plant and placed in glass vials (50 mm long x 12 mm diameter). The samples were brought into the laboratory to determine the parasitism levels and identify the egg parasitoids species.

Pupae of *Trichogramma* (confirmed by the blackening of the egg chorion of the host) were counted within the host eggs using binocular microscope (magnification 16x) to determine the number of parasitised eggs (Hassan, 1990). Emergence of adult parasitoids was noted and sex ratios recorded. Antennal characters were used to distinguish females from the males (Pinto *et al.*, 1978). Percentage parasitism and emergence was calculated as recommended by Van Driesche (1983). The rearing was conducted at ambient temperatures of 24-29°C, 50-80% relative humidity and a photoperiod of 12L: 12D. Species identification was based on male genitalia characters (Nagarkatti and Nagaraja, 1971; Pinto and Stouthamer, 1994) whereby male trichogrammatid species were slide-mounted in Canada balsam (Platner *et al.*, 1999) and examined under compound microscope (magnification 40x). Identification of the species was conducted at the Institute of Phytomedicine, University of Hohenheim in Germany. Voucher specimens are also deposited at the University of Hohenheim.

2.2.3 Mortality of *Plutella xylostella* eggs in the field

The study was conducted on kale (*Brassica oleracea*), at Muhaka for two seasons (August-October 1999 and January-March 2000), concurrently with the main field trial mentioned in 2.2.1. Six plots each 5.6 m x 5.6 m (31.36 m^2) were used for the study. In each plot 12 plants were tagged randomly (excluding those tagged for the main trial) and all the eggs laid were removed prior to the experiment. Ten singly laid *P. xylostella* eggs were randomly marked per plant. Using a hand lens (magnification 10x) eggs were monitored daily until the fate of all eggs (parasitised, predated or disappeared) was known.

According to Van den Berg (1993) and Bonhof (2000), 'predation' refers to when at least part of the chorion is present, but the contents of the egg have vanished, while 'disappearance' refers to the case where both the contents and the chorion of the egg have vanished. Disappearance was regarded as a separate mortality factor; however, the causes are not determined in this study. The observations were conducted monthly and repeated three times per season. The 1st season was in August-October 1999, whereas the 2nd season was in January-March 2000. Due to low population of *H. armigera* eggs in the trials plot, motality experiment was not conducted.

2.2.4 Rearing of egg parasitoids

The parent generation of egg parasitoids emerging from the field-collected samples and surveys were offered freshly laid *C. cephalonica* eggs to parasitise and fed with a mixture of honey (66%), gelatine (1%) and distilled water (33%). Thin stripes of the diet were prepared on A4 size paper (80 g/m^2) using a sterile syringe. Smaller pieces were cut using sterile scissors and each piece was inserted into the rearing glass vials using fine forceps. The diet was placed on opposite side to the fresh host eggs so as to avoid contact of the honey with the eggs.

On the fifth day of exposure, the parasitised eggs were transferred into clean rearing glass vials (75 mm long x 25 mm in diameter) (Hassan, 1990) and cotton plugs used as stoppers for the vials. Upon emergence, parasitoids were offered freshly laid *C. cephalonica* eggs and feeding continued to maintain the live cultures (Singh and Jalali, 1994). Every parent sample was given a rearing code, indicating the date of sampling, host insect, host plant, number of eggs collected, number of parasitised eggs, number of emerged adults and sex ratio.

According to Stouthamer *et al.* (1990), a single egg parasitoid female was isolated from every arrhenotokous and thelytokous samples to start isofemale lines. Arrhenotoky is the common mode of reproduction in Hymenoptera whereby males are produced from unfertilised eggs and females from fertilised eggs. In contrast, thelytokous populations consist of only females that produce female offsprings without mating (Stouthamer *et al.*,

1990). A total of 160 isofemales were established and maintained as live cultures (Appendix 2). *Trichogramma bournieri* parasitising eggs of the Angoumois grain moth, *Sitotroga cerealella* Oliver (Lepidoptera: Gelechidae) in the laboratory is shown in Plate 1.

At the ICIPE, the egg parasitoids rearing was done at ambient temperatures of 24-29°C, 50-80% relative humidity and a photoperiod of LD 12:12, using the methods adopted from the Institute for Biological Pest Control, Darmstadt, Germany (Hassan, 1993).

Plate 1 *Trichogramma bournieri* Pintureau & Babault

parasitising eggs of *Sitotroga cerealella* in the laboratory

2.2.5 Rearing of hosts

Adult moths of *C. cephalonica* were collected from the National Cereals and Produce Board stores in Nairobi in April 1998. A circular oviposition tin chamber (28 cm height and 29 cm diameter) with iron mesh at the bottom was used for rearing moths. There was an opening (3 cm height and 3 cm diameter) on top of the chamber for moth entrance. Rearing of *C. cephalonica* was done following the methods described by Singh and Jalali (1994).

A solution of 20% sucrose and 80% distilled water was used to feed the adult moths. Feeding of moths was done by soaking cotton in the solution and suspending it inside the oviposition chamber.

A plastic basin (16 cm height and 40 cm diameter) was placed at the bottom of the oviposition chamber so as to collect eggs passing through the mesh. Eggs were collected daily in Petri dishes (4.5 cm diameter) and used for preparing egg cards and maintaining the *C. cephalonica* culture. Maize flour (2.5kg) was mixed with 3ml of *C. cephalonica* eggs in plastic food containers (28 cm height and 27 cm diameter) and covered with fine cloth.

Moths usually emerged beginning from 30 days after infestation and were collected every two days using aspirators and then transferred into the oviposition chambers. Moth emergence reduces after 100 days of the initial infestation and plastic containers were re-used after cleaning. A4 size paper ($80g/m^2$) was used for egg card preparations. Egg cards were prepared by sprinkling host eggs on Traganth glued paper (Merk, Darmstadt, Germany).

After the card had dried, the eggs were adhered to the paper. At the ICIPE, the rearing was done at ambient temperatures of 22-27°C, 60-80% relative humidity.

2.2.6 Data analysis

Percentage parasitism and emergence was calculated based on the methods outlined by Van Driesche (1983). Percentage parasitism being the ratio of parasitised eggs to the total egg density, whereas percentage emergence is the ratio of progenies produced to the total parasitised eggs (Van Driesche, 1983). Insect counts were transformed into $\log_{10}$ (x+1) while percentage data were transformed using the arcsine function (Sokal and Rohlf, 1981).

The density, parasitism and emergence data were subjected to repeated measures of analysis of variance (ANOVA) for a Latin-square design using the General Linear Models (GLM) procedure (SAS Institute, 1996). When F-values showed significance at P=0.05, means were separated using Student-Newman-Keuls (SNK) test. All frequency data were analysed using the Chi-square (χ^2) tests (SAS Institute, 1996).

2.3 RESULTS

2.3.1 Survey

The results on the occurrence of trichogrammatid egg parasitoids at the different locations are given in Table 2.1. Apart from Voi location, egg parasitism was observed in all locations ranging from altitudes 40m to 1600m above sea level. *Trichogramma* sp. nr. *mwanzai* Schulten & Feijen and *Trichogrammatoidea* sp. nr. *lutea* Girault were recovered from parasitised *C. cephalonica* egg cards. *Trichogramma* sp. nr. *mwanzai* was recovered from maize plant (Kibwezi, Mtito Andei and Shimba Hills locations) and *Amaranthus* plants (Kibwezi). The host plants for *Trichogrammatoidea* sp. nr. *lutea* includes pigeon pea and tomato (Kasarani) and kale (Mtito Andei). Both genera were found together at Mtito Andei location. In general, percentage parasitism of eggs was low (<10%). A total of 448 egg cards (67% of the number exposed) were recovered and the highest parasitism recorded was 2.8% in Mtito Andei (Table 2.2).

2.3.2 Field trials

2.3.2.1 Density and parasitism

Egg density and parasitism of *H. armigera*, *P. xylostella* and *C. partellus* at Muhaka, coastal Kenya are presented in Tables 2.3-2.4 and Figs. 2.1-2.5. Density of eggs was not significantly different between the seasons for *H. armigera* ($F=1.34$; $df=1,50$; $P=0.2521$) and *P. xylostella* ($F=0.41$; $df=1,50$; $P=0.5265$). However, density of *C. partellus* eggs was significantly

different between the seasons (F=102.27; df=1,50; P=0.0001). There was significant difference in % parasitism between the seasons for *H. armigera* (F=227.17; df=1,20; P=0.0001), but not for *P. xylostella* (F=1.26; df=1,5; P=0.3133) and *C. partellus* (F=0.39; df=1,12; P=0.5431).

Density of *P. xylostella* eggs was first recorded on the first week of sampling and reached a peak at 6.4 ± 0.8 eggs per plant (Mean ± SE) one week after. This, however, dropped to 1.8 ± 0.3 eggs per plant in the third week, rising thereafter to a maximum of 9.5 ± 1.3 eggs per plant in the seventh week. This also represented the maximum level of egg density per plant during the first season. Parasitism was oscillating with the maximum peak being 38% in the third week. The highest density and percentage parasitism of *P. xylostella* eggs per plant on kale for the second season was 9.0 ± 1.1 (in January) and 37.8 ± 6.9 (in March) (Fig. 2.1).

The highest density and percentage parasitism of *C. partellus* eggs per sorghum plant was 4.7 ± 1.2 (in September) and 100 (in October) for the 1st season, and 4.7 ± 1.3 (in January and March) and 75.0 ± 25.0 (in February) for the 2nd season (Fig. 2.2). The occurrence of *C. partellus* eggs was not reported on kale earlier probably because it is a non-host plant. However, 23 instances of oviposition on kale were recorded (Fig. 2.3). Density (F=2380.50; df=5,50; P=0.0001) and parasitism (F=11.96; df=1,12; P=0.0047) of *C. partellus* eggs laid on sorghum and kale was significantly different. More *C. partellus* eggs were laid on kale in the 1st season compared to 2nd season (Fig. 2.3).

Helicoverpa armigera eggs were found in all crops in the trial except kale in lower densities and parasitism levels. The highest density and percentage parasitism per plant recorded in both seasons were 0.4 ± 0.1 and 22.2 ± 14.7 on pigeon pea and 0.2 ± 0.1 and 9.1 ± 9.1 on okra, respectively (Figs. 2.4 and 2.5). Density and parasitism of *H. armigera* eggs were generally very low in sunflower, sorghum and tomato plants.

The weather in the 1st season appeared to favour eggs density of *P. xylostella* and parasitism on *C. partellus*. Temperature was high in the 2nd season, whereas there was relatively high rainfall in the 1st season (Figs. 2.6 and 2.7).

2.3.2.2 Native egg parasitoids

Progenies produced from *H. armigera*, *P. xylostella* and *C. partellus* parasitised eggs were used for identification (Table 2.3). A list of identified egg parasitoids recovered from the three major lepidopteran pests is presented in Table 2.4. All thelytokous and a few arrehenotokous samples are still not yet identified (Appendix 2).

Five native species of trichogrammatid egg parasitoids belonging to the genus *Trichogramma* and *Trichogrammatoidea* were recovered. The species were *Trichogramma bournier* Pintureau & Babault, *Trichogramma* sp. nr. *bruni* Nagaraja, *Trichogramma* sp. nr. *mwanzai* Schulten & Feijen, *Trichogrammatoidea* sp. nr. *lutea* Girault and *Trichogrammatoidea* sp. The proportion of each species in the egg parasitoids fauna collected is presented

in Fig. 2.8. All the above mentioned egg parasitoids were recovered from Muhaka field trial except *T. bournieri*, which was recovered from a preliminary survey at Mbita using tagged *C. partellus* egg batches. The detailed information on host plant, host insect, collection dates, place of collections and identification names are presented in Appendix 2.

Eggs of *P. xylostella* were parasitised by a single species, *Trichogrammatoidea* sp. nr. *lutea*, whereas *H. armigera* and *C. partellus* eggs were found to be parasitised by more than one species (Table 2.2). Most of the samples (53%) were recovered from *P. xylostella* eggs (Fig. 2.9). The natural occurrence of trichogrammatid egg parasitoids (*Trichogrammatoidea* sp. nr. *lutea*) on *P. xylostella* eggs is the first record for Kenya as well as for eastern Africa. The egg parasitoids, *T. bournier* and *T.* sp. nr. *bruni*, recovered from *C. partellus* are also the first records for Kenya. Furthermore, the latter species might be the first record for the African continent as a whole.

Trichogramma sp. nr. *mwanzai* and *Trichogrammatoidea* sp. nr. *lutea*, the two most common species (Fig. 2.8) were further studied to investigate the effects of host insects and host plants on egg parasitism under natural conditions in the field. There was significant difference in parasitism of *C. partellus* eggs laid on sorghum and kale by *T.* sp. nr. *mwanzai* (χ^2=93.429; df=1; p=0.001) and *Trichogrammatoidea* sp. nr. *lutea* (χ^2=54.201; df=1; p=0.001) (Fig. 2.10). *Trichogrammatoidea* sp. nr. *lutea* parasitised significantly higher *P. xylostella* eggs than *C. partellus* on Kale (χ^2=28.927; df=1; p=0.001) (Fig. 2.11). *Trichogramma* sp. nr. *mwanzai* failed to parasitise

eggs of *P. xylostella* in both seasons (Fig. 2.12). There was significant difference in percentage emergence (F=15.51; df=1,124; P=0.0001) and proportion of females (F=12.68; df=1,124; P=0.0005) between the two genera, *T.* sp. nr. *mwanzai* and *Trichogrammatoidea* sp. nr. *lutea*.

2.3.3 Mortality of *Plutella xylostella* eggs

To study the role of natural mortality factors on *P. xylostella* eggs in the field, a total of 1151 eggs (538 for the 1st season and 613 for 2nd season) were marked. In the 2nd season, parasitism increased as disappearance decreased. Parasitism (χ^2=70.087; df=1; P=0.001) and disappearance (χ^2=14.102; df=1; P=0.001) were significantly different between the seasons.

There was also significant difference among the sampling months in parasitism (χ^2=15.244; df=2; P=0.001 for 1st season; χ^2=66.596; df=2; P=0.001 for 2nd season) and disappearance (χ^2=17.839; df=2; P=0.001 for 1st season; χ^2=18.248; df=2; P=0.001 for 2nd season). The highest and lowest percentage parasitism recorded were 63% (March, 2nd season) and 12% (September, 1st season), respectively.

On the other hand, the lowest and highest percentages of disappearance were recorded in March (37%) and September (86%), respectively. The percentage mortality of *P. xylostella* eggs caused by different factors is shown in Fig. 2.13. Overall, disappearance accounts for 60% (n=695) of the mortality, followed by parasitism 39% (n=447). Predation was less important, accounting for only 1% (n=9) mortality.

2.4 DISCUSSION

2.4.1 Survey

The survey shows the occurrence of native egg parasitoids in different altitudes and host habitats. Despite 67% recovery of *C. cephalonica* egg cards, percentage parasitism was very low (2.7%, n=18). Thorpe (1984) placed gamma sterilised *Heliothis virescens* (F.) egg cloths in the field in 1981 and 1982 and found 8% and 14% parasitism, respectively. In Europe, Cagan *et al.* (1998) collected egg clusters of *O. nubilalis* from maize fields and found no parasitism in two successive years (1993 and 1994). The relatively low level of parasitism in our survey is similar to the low natural recoveries in the above reports.

Among many factors, one of the reasons for low parasitism of the exposed *C. cephalonica* egg cards at different transect sites could be that parasitoids did not prefer artificially exposed *C. cephalonica* eggs. In addition to this, volatiles emitted from the different host plants might have attributed to this low parasitism (Romeis *et al.* 1997). The indiscriminate use of insecticides particularly in many vegetable growing areas could have hampered the fauna of egg parasitoids (Kibata, 1996). The low parasitism could also be attributed to the absence of natural association of host plants, as *C. cephalonica* is a storage pest.

2.4.2 Field trials

In 1998/99, no egg parasitoids were recorded from the field trials at Mbita apart from *T. bournieri*, which was recovered only from the exposed *C. partellus* egg batches on Maize. In the 1999/2000 field trials at Muhaka, coastal Kenya, quite a number of native egg parasitoids species from *H. armigera*, *P. xylostella* and *C. partellus* were recovered. Egg parasitoids were also recovered from the exposed *C. cephalonica* egg cards at seven sites along the Nairobi-Mombasa highway in Kenya. Except for *H. armigera*, as the density or total number of eggs decreased, percentage parasitism increased in *P. xylostella* (13.4% to 17.9%) and *C. partellus* (20.0% to 38.9%). This could be attributed to the overall low number of egg parasitoids locally available.

Van den Berg (1993) reported that the average egg parasitism of *H. armigera* by *Trichogrammatoidea* spp. and *Telenomus ullyetti* was low at about 10%. He also reported the occurrence of more than one egg parasitoid species concurrently at the same site. This is in agreement with the low percent parasitism recorded for *H. armigera* in our studies (<5%) and the occurrence of four trichogrammatid egg parasitoids species (*Trichogrammatoidea* sp. nr. *lutea*, *Trichogrammatoidea* sp., *Trichogramma* sp. nr. *mwanzai* and *Trichogramma* sp. nr. *bruni*) concurrently at Muhaka field trial site in coastal Kenya.

In South Africa, Van Hamburg and Kfir (1990) reported that the parasitism rates of *H. armigera* eggs by two indigenous hymenopteran parasitoids, *Trichogrammatoidea lutea* Girault and *Telenomus ullyetti* on

cotton varied greatly between seasons and also between localities. Kfir (2000) also recorded egg parasitoids from *Busseola fusca* Fuller on maize and sorghum. Abbas (1998) found no egg parasitism on *H. armigera* around lake Nasser in Aswan, Egypt. However, after releasing the native *Trichogramma evanescens* Westwood (50,000 adult parasitoids/hectare at 2 weeks interval) a high percentage parasitism was reached (73.7%).

In China (Yuncheng area), the natural parasitism rate of *H. armigera* by the indigenous *Trichogramma pintoi* Voegele on cotton was only 3% (Fucheng and Zhang, 1990). Monje *et al.* (1998) reported six species of *Trichogramma* parasitising *H. armigera* eggs on pigeon pea and three of them on sorghum in India. He further reported that *Trichogramma chilonis* Ishii was the predominant species on both crops. *Trichogramma achaeae* Nagaraja and Nagarkatti accounted for about 10% of the total parasitism on pigeon pea, but it was virtually absent on sorghum (Monje *et al.*, 1998). The low percentage parasitism recorded on *H. armigera* in this study agrees with the several reports mentioned above.

Trichogrammatoidea bactrae Nagaraja was found for the first time attacking eggs of *P. xylostella* in a naturally infested plot in Thailand (Keinmeesuke *et al.*, 1990), with a parasitism of 16.2 to 45.2%. In semi-field experiments, Wührer and Hassan (1993) observed parasitism rates of 55.8%, 40.3% and 28.7% of *P. xylostella* eggs by *Trichogrammatoidea bactrae*, *Trichogramma ostriniae* Pang & Chen and *T. chilonis*, respectively. In another field cage experiment, Klemm *et al.* (1990) observed 34.1%

parasitism of *P. xylostella* eggs by *T. pretiosum*. The parasitism level recorded in these field studies on *P. xylostella* by *Trichogrammatoidea* sp. nr. *lutea* (13.4% and 17.9%) was closer to the range reported by Keinmeesuke *et al.* (1990).

Parasitism (2 to 11%) by *T.* sp. nr. *mwanzai* has been also reported by offering various densities of *C. partellus* eggs in the field experiment (Chacko and Dwumfour, 1990). Ogol (1996) recorded over 77% parasitism of stem borer eggs by *Trichogramma* species in coastal Kenya. Egg parasitism of *C. partellus* by trichogrammatid species in general and *T.* sp. nr. *mwanzai* in particular is higher in these field trials than reported earlier by Ngi-Song (1990) and Chacko and Dwumfour (1990). The main reasons for this higher parasitism could be the location of trials being in warmer climates compared to Mbita where they conducted their studies.

Trichogramma sp. nr. *mwanzai* parasitised *C. partellus* eggs on kale but failed to parasitise eggs of *P. xylostella* under natural conditions. Furthermore, a strong association was observed between *T.* sp. nr. *mwanzai* and sorghum/maize plants both in the field trials as well as in the survey studies. On the other hand, *P. xylostella* eggs were solely parasitised by a single trichogrammatid species, *Trichogrammatoidea* sp. nr. *lutea*. This study shows that the host plants influenced parasitism, and this is in agreement with the work of Romeis *et al.* (1997).

2.4.3 Mortality of *Plutella xylostella* eggs

Oloo (1989) reported disappearance of eggs as the major mortality factors in the field, with up to 97% *C. partellus* eggs on sorghum, 93% on maize and 85% on maize/sorghum intercrop in western Kenya. In the Kenyan coast, Bonhof (2000) reported that egg mortality of *Chilo* spp. ranged from 18 to 78% on maize, with parasitism being the most important factor. Van den Berg (1993) observed 73-78% predation of *H. armigera* eggs on sunflower in Kenya. The results on disappearance in this study, agree with the observations reported by Oloo (1989). However, further studies on this aspect in the field are of paramount importance because egg parasitoids kill pests in the egg stage, before the crop is damaged (Wajnberg and Hassan, 1994). Furthermore, the information obtained from the field mortality could also partly provide a rational and predictive basis for planning of efficient pest control programmes (Southwood, 1978).

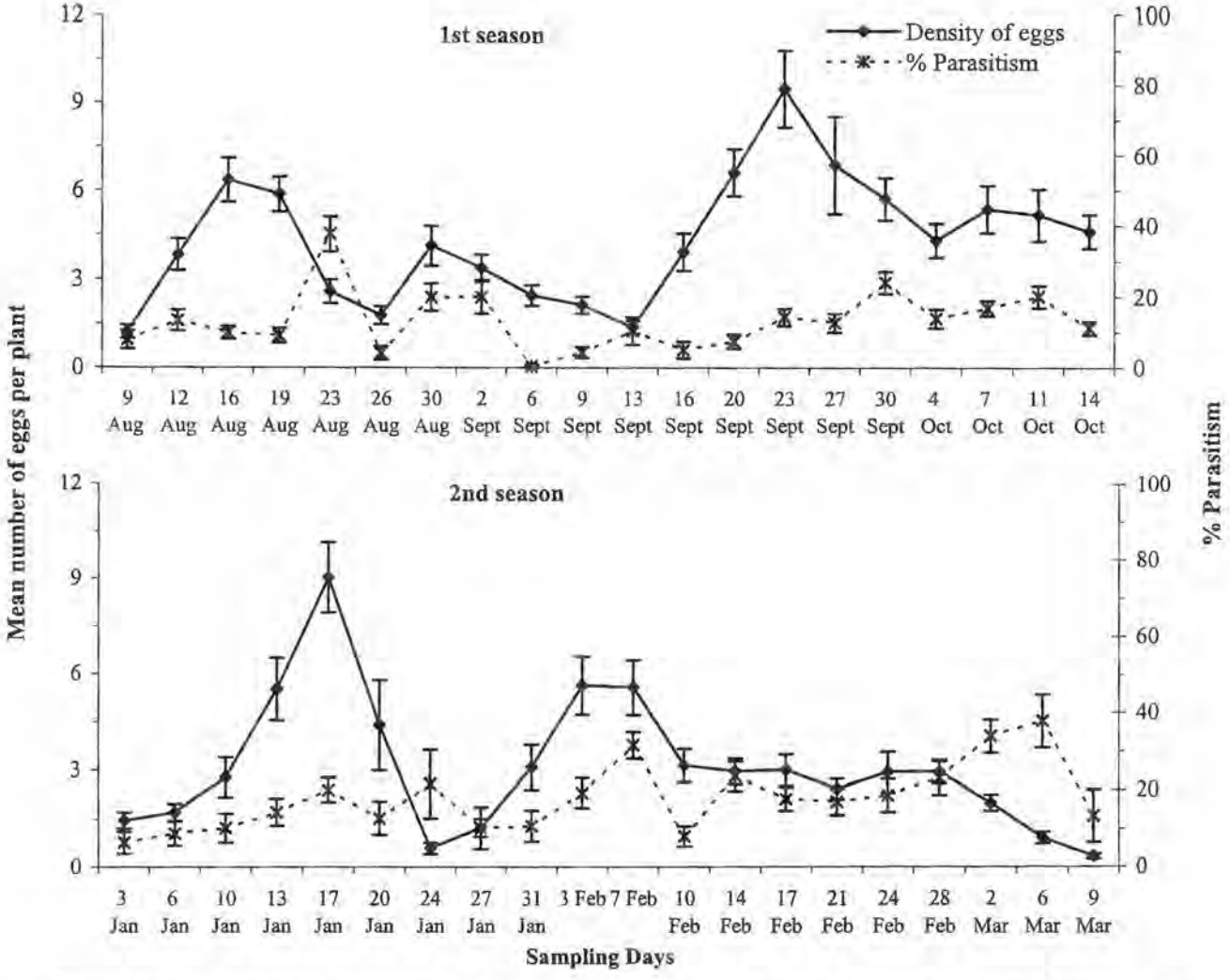


Figure 2.1 Population dynamics of *Plutella xylostella* on kale at Muhaka, Coastal Kenya (1999/2000)

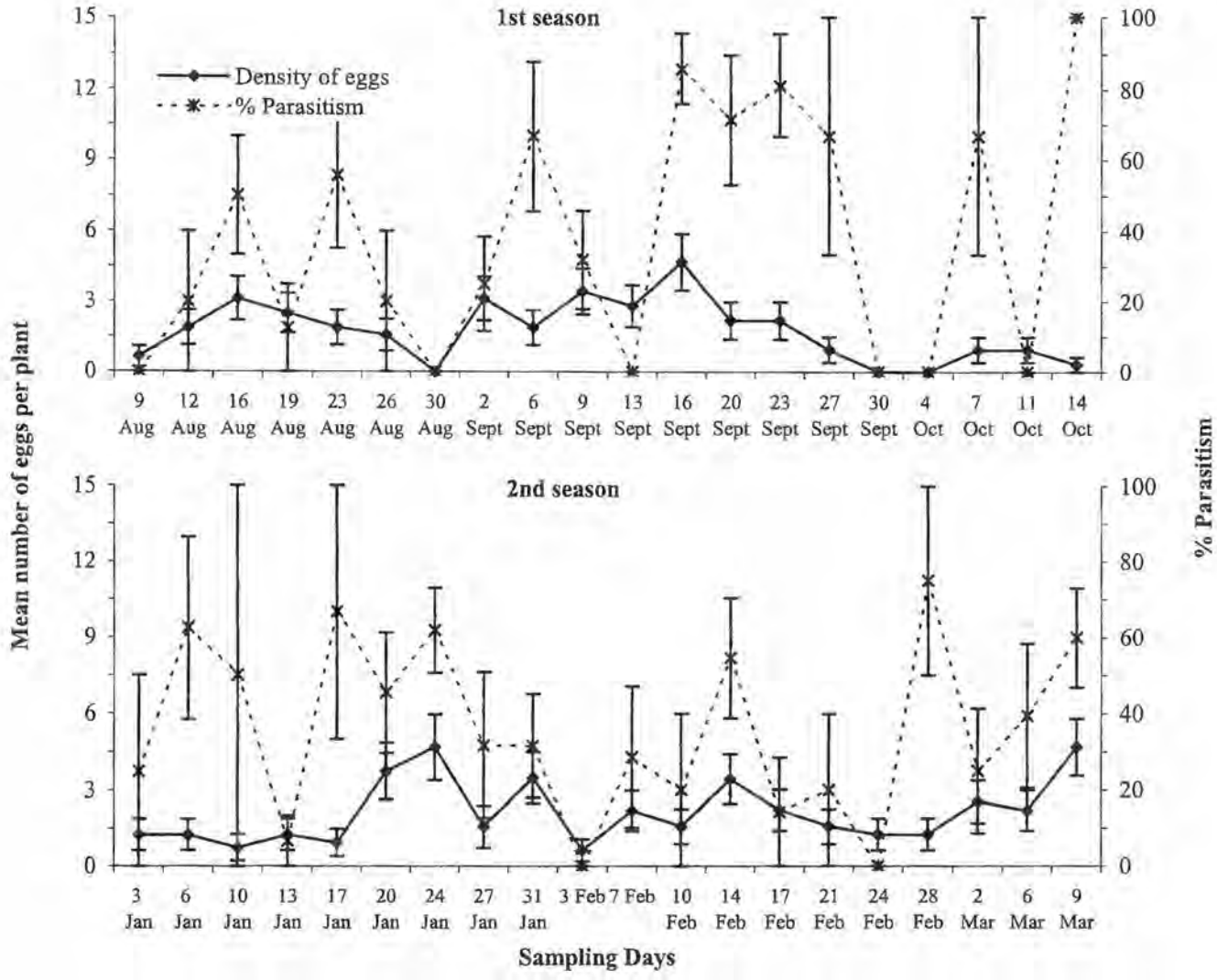


Figure 2.2 Population dynamics of *Chilo partellus* on sorghum at Muhaka, Coastal Kenya (1999/2000)

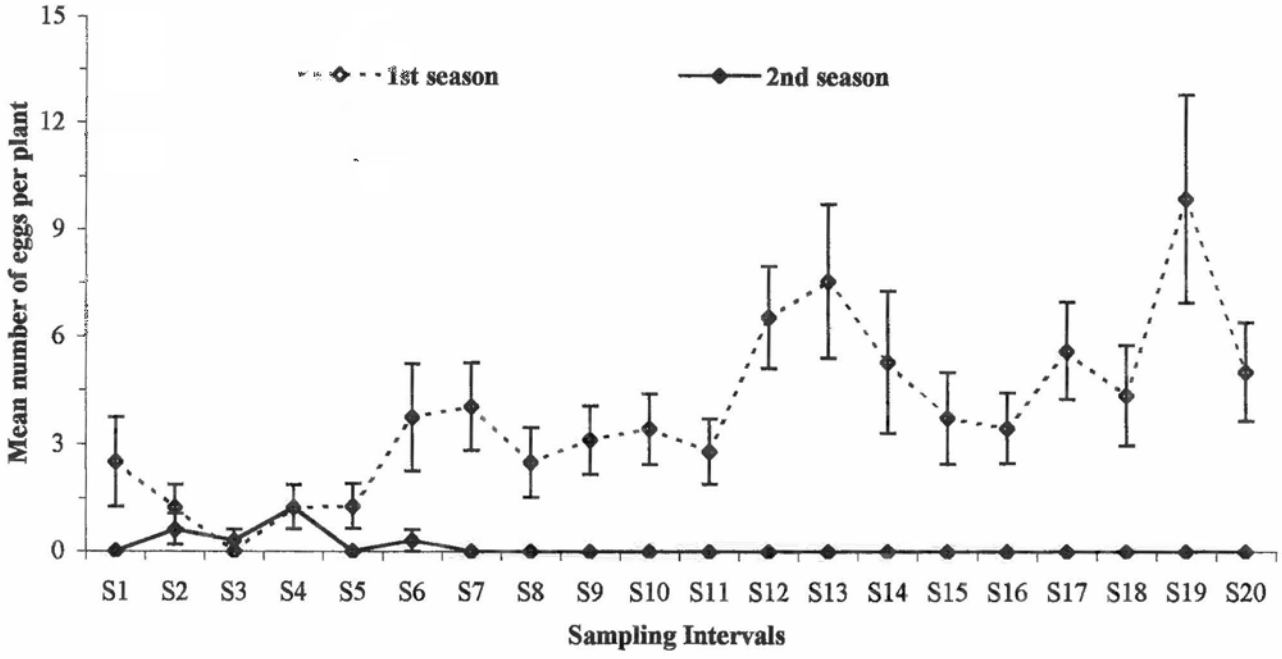


Figure 2.3 Density of *Chilo partellus* eggs on kale at Muhaka, Coastal Kenya (1999/2000)

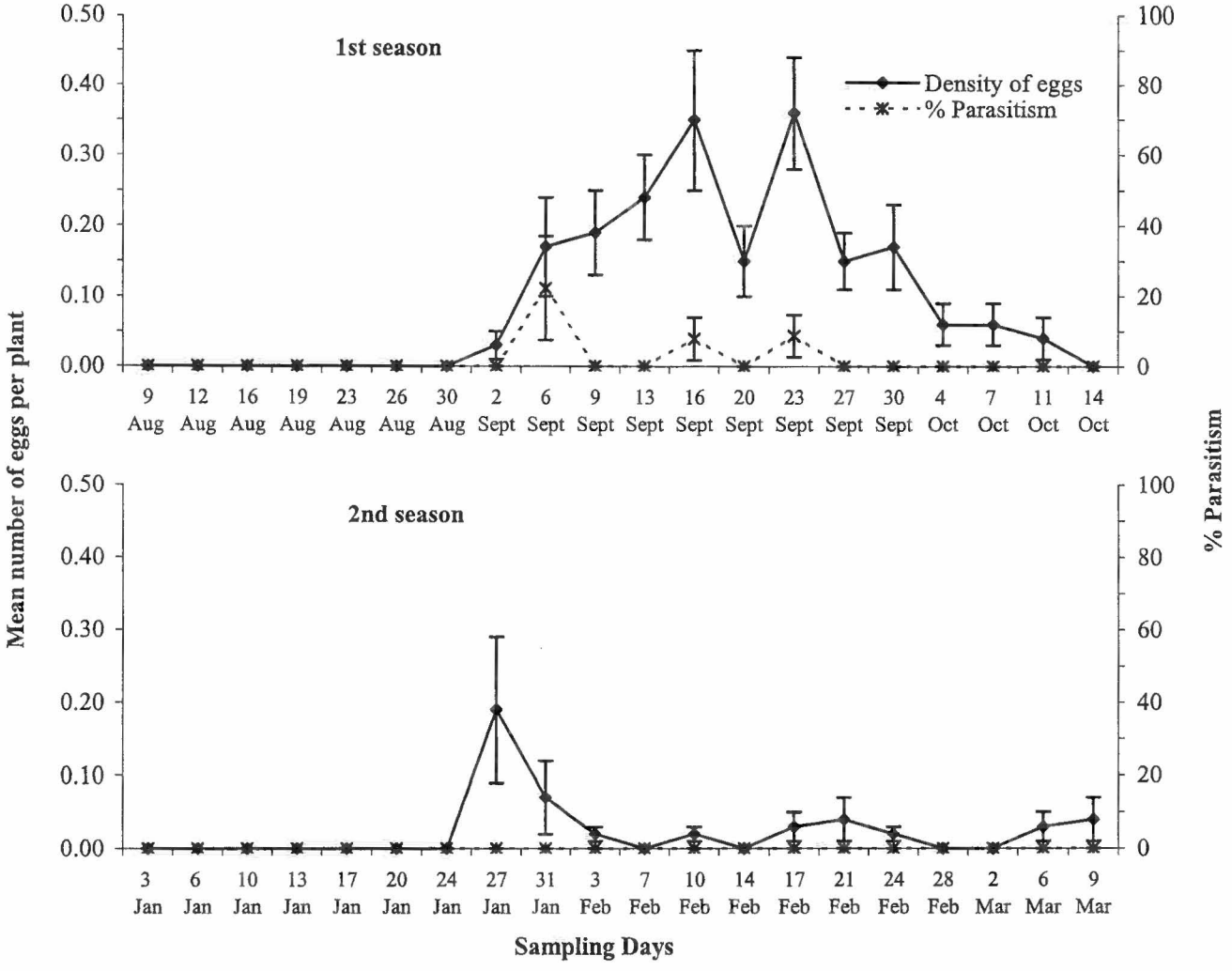


Figure 2.4 Population dynamics of *Helicoverpa armigera* on pigeon pea at Muhaka, Coastal Kenya (1999/2000)

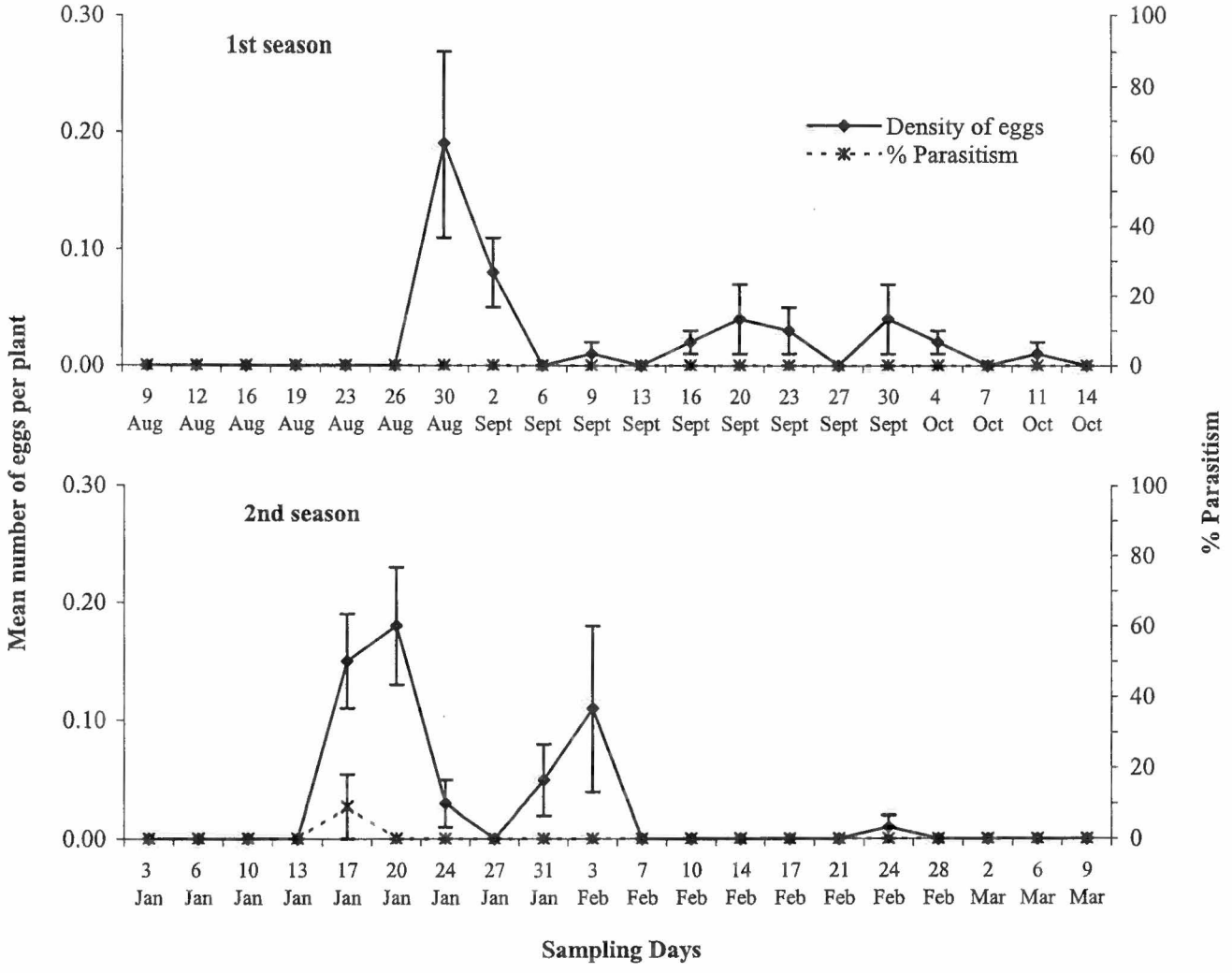


Figure 2.5 Population dynamics of *Helicoverpa armigera* on okra at Muhaka, Coastal Kenya (1999/2000)

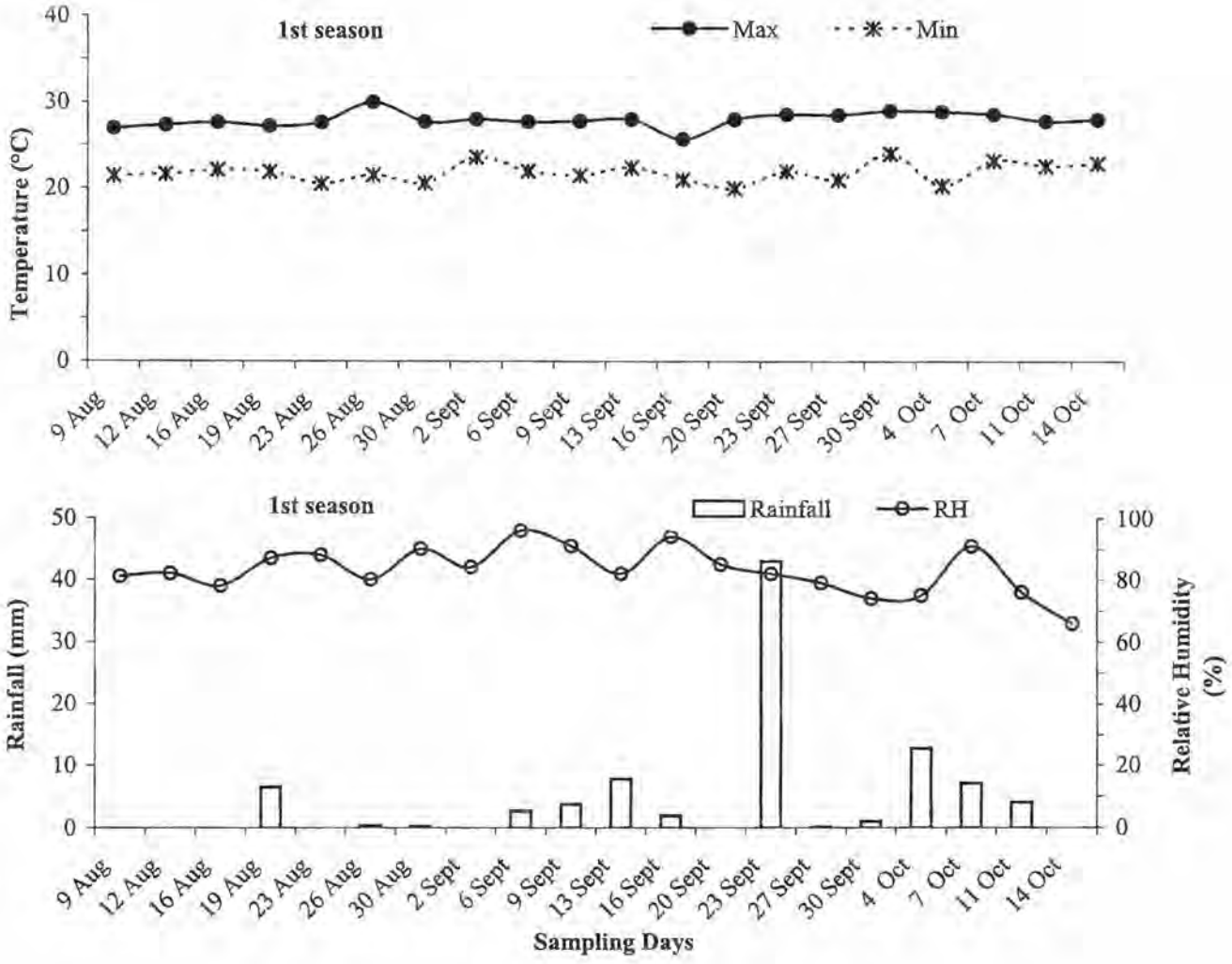


Figure 2.6 Temperature, rainfall and relative humidity patterns at coastal Kenya (1st season). Data obtained from the Kenyan Agricultural Research Institute (KARI) in Mtwapa.

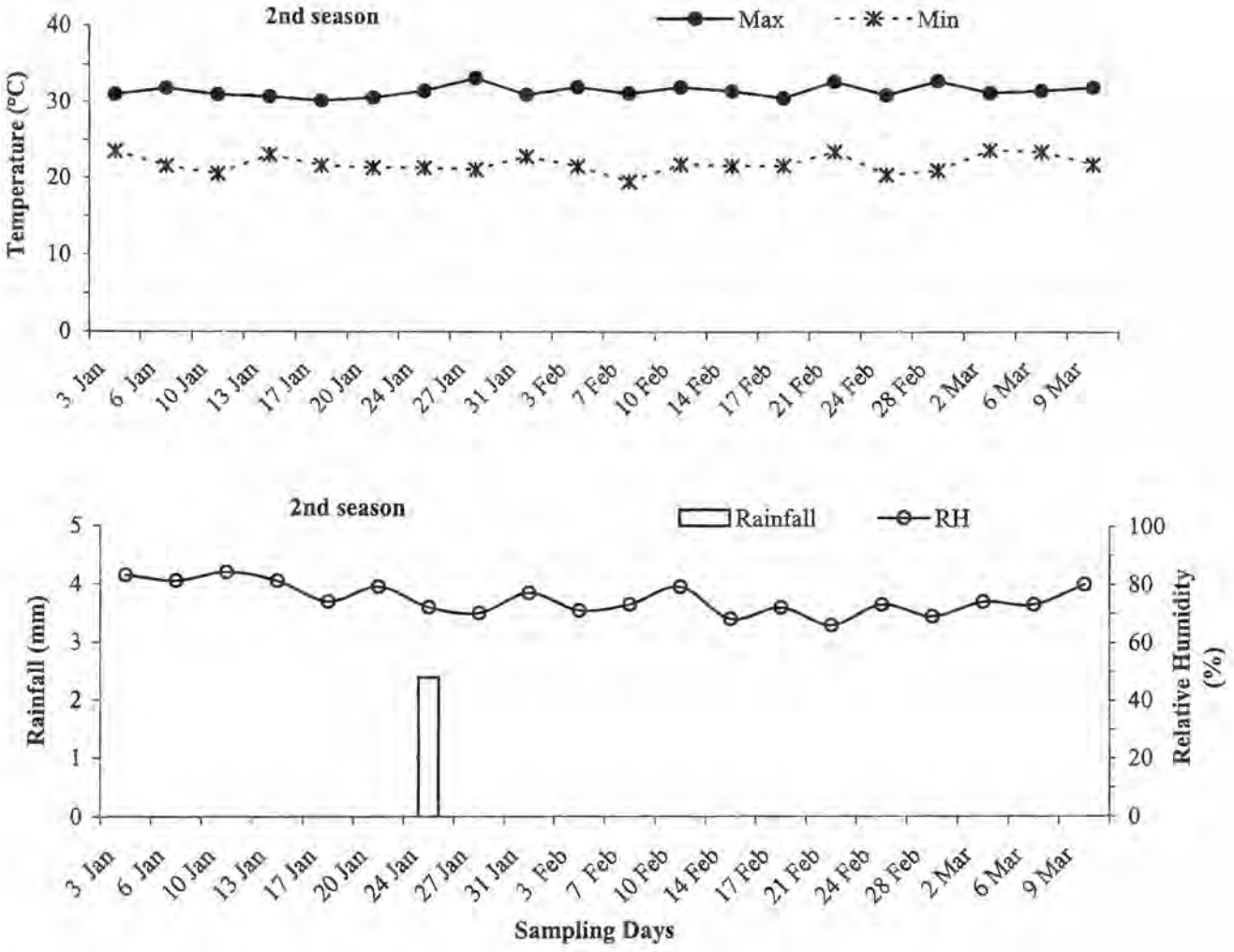


Figure 2.7 Temperature, rainfall and relative humidity patterns at coastal Kenya (2nd season). Data obtained from the Kenyan Agricultural Research Institute (KARI) in Mtwapa.

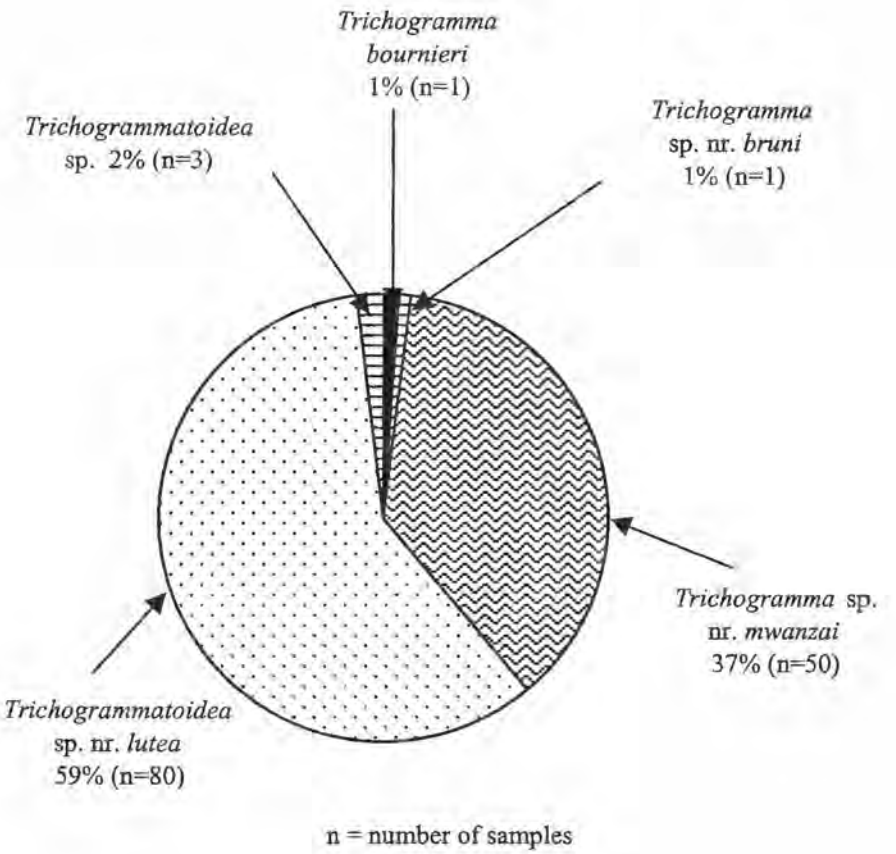


Figure 2.8 Natural occurrences of trichogrammatid egg parasitoids on *Helicoverpa armigera* , *Plutella xylostella* and *Chilo partellus* in selected sites in Kenya

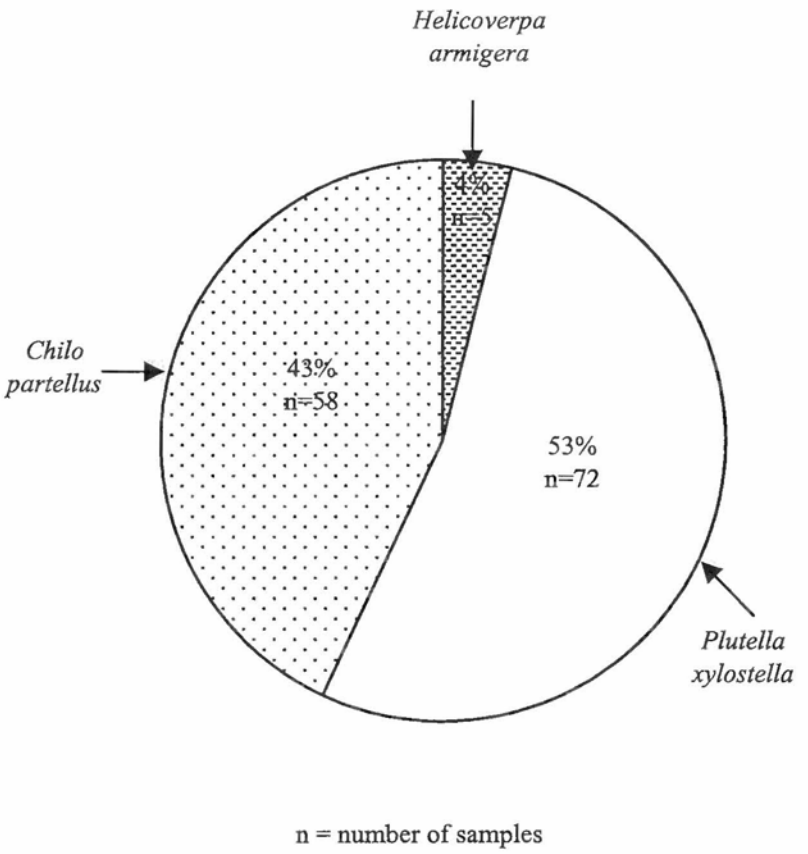


Figure 2.9 Proportion of trichogrammatid egg parasitoids on three lepidopteran hosts in selected sites in Kenya

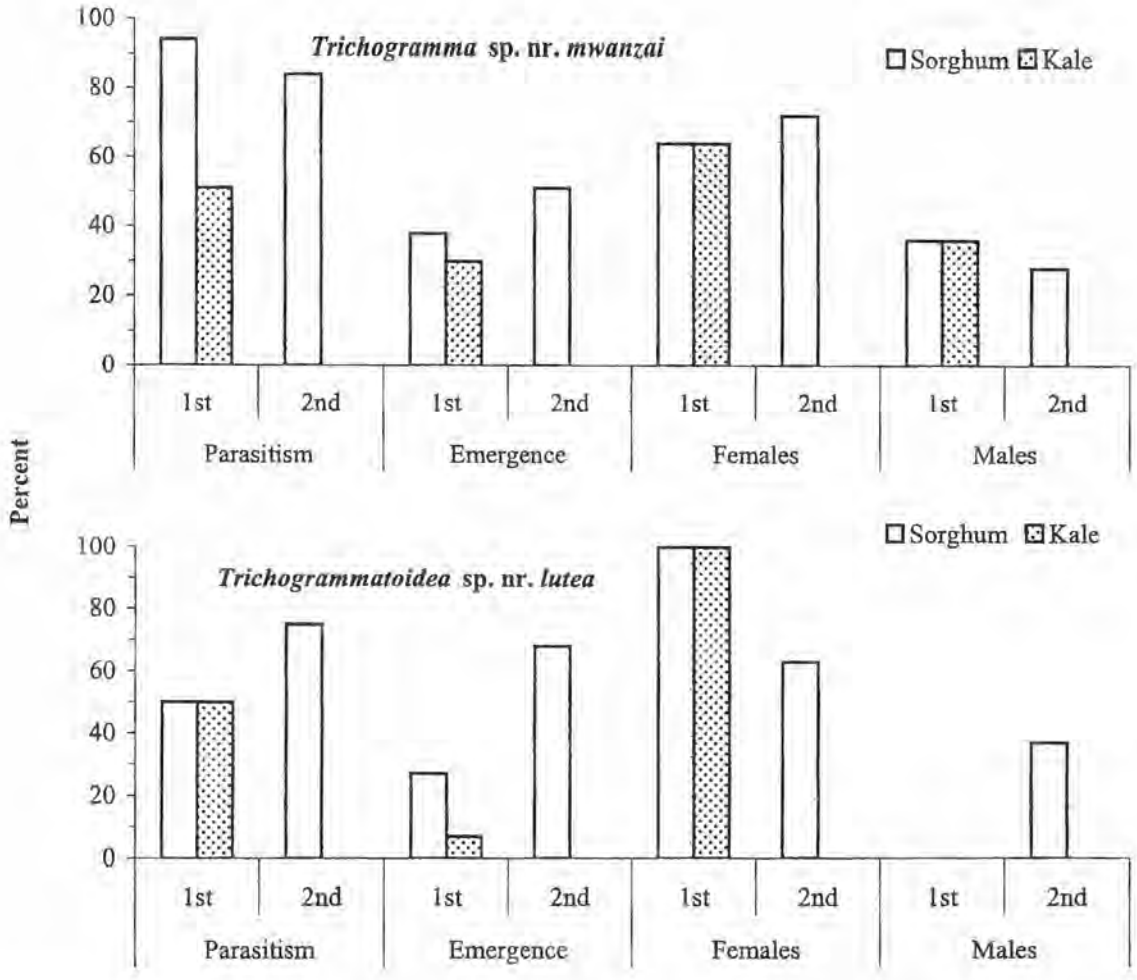


Figure 2.10 *Trichogramma* sp. nr. *mwanzai* and *Trichogrammatoidea* sp. nr. *lutea* parasitising eggs of *Chilo partellus* on sorghum and kale at Muhaka, Coastal Kenya

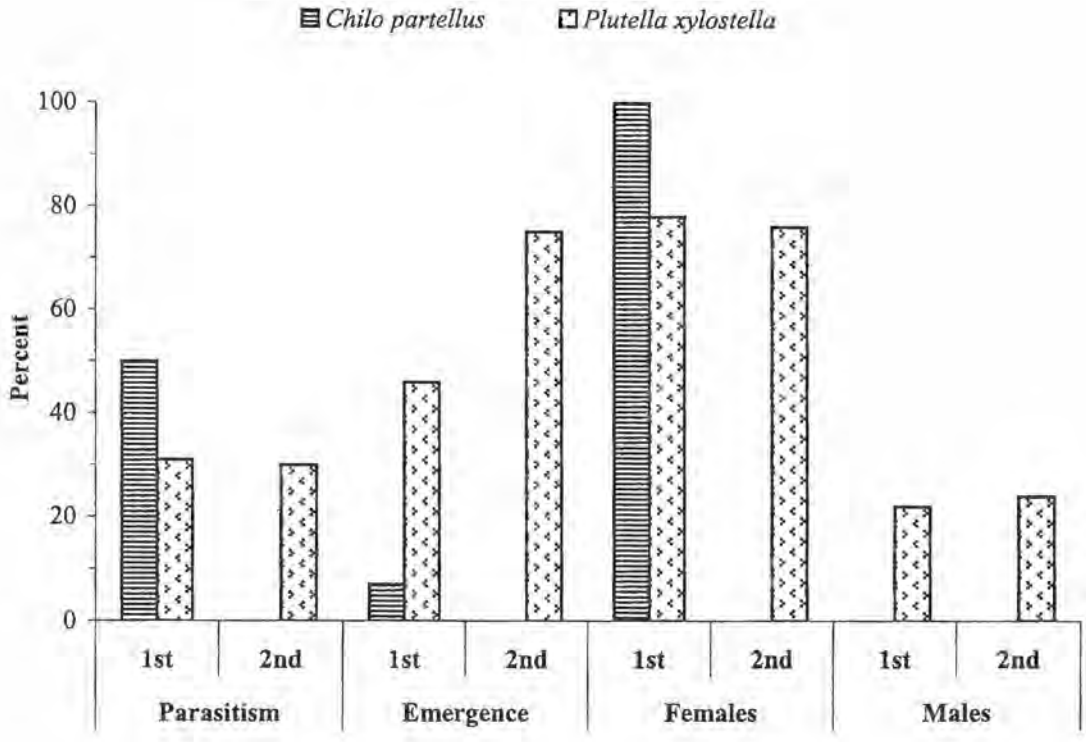


Figure 2.11 *Trichogrammatoidea* sp. nr. *lutea* parasitising eggs of *Chilo partellus* and *Plutella xylostella* laid on kale at Muhaka, Coastal Kenya

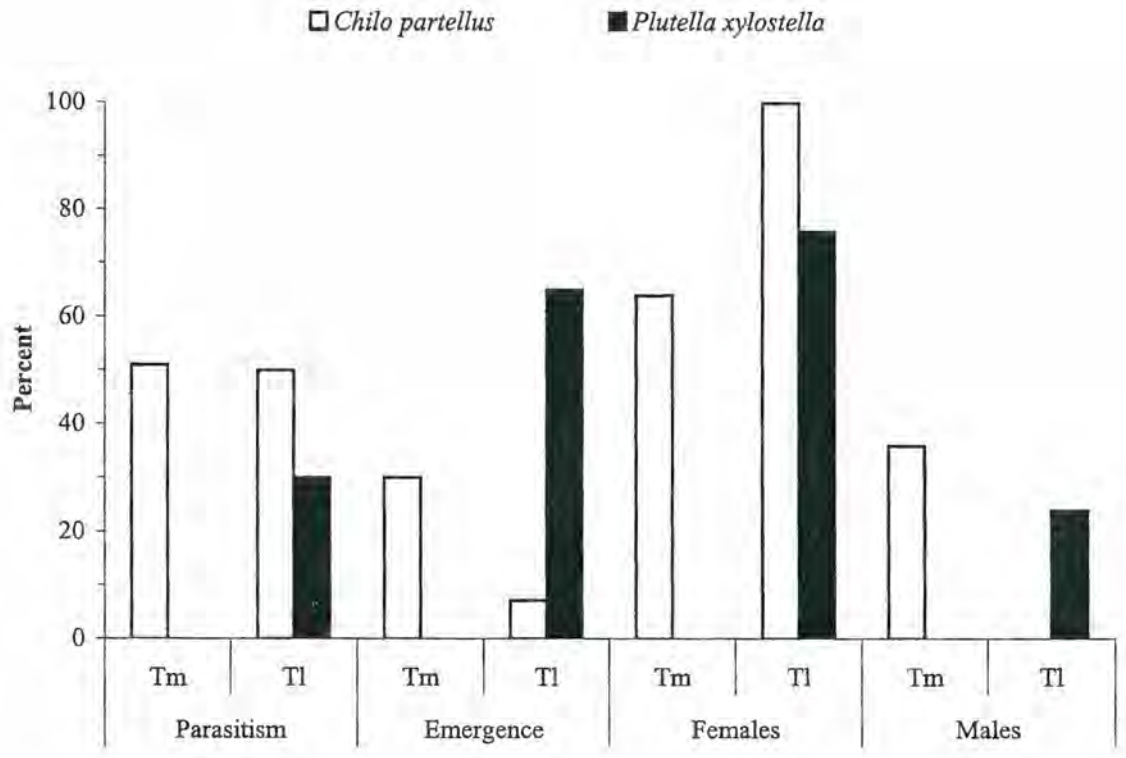


Figure 2.12 Comparison of *Trichogramma* sp. nr. *mwanzai* (Tm) and *Trichogrammatoidea* sp. nr. *lutea* (Tl) in natural parasitism of eggs of *Chilo partellus* and *Plutella xylostella* on kale at Muhaka, Coastal Kenya

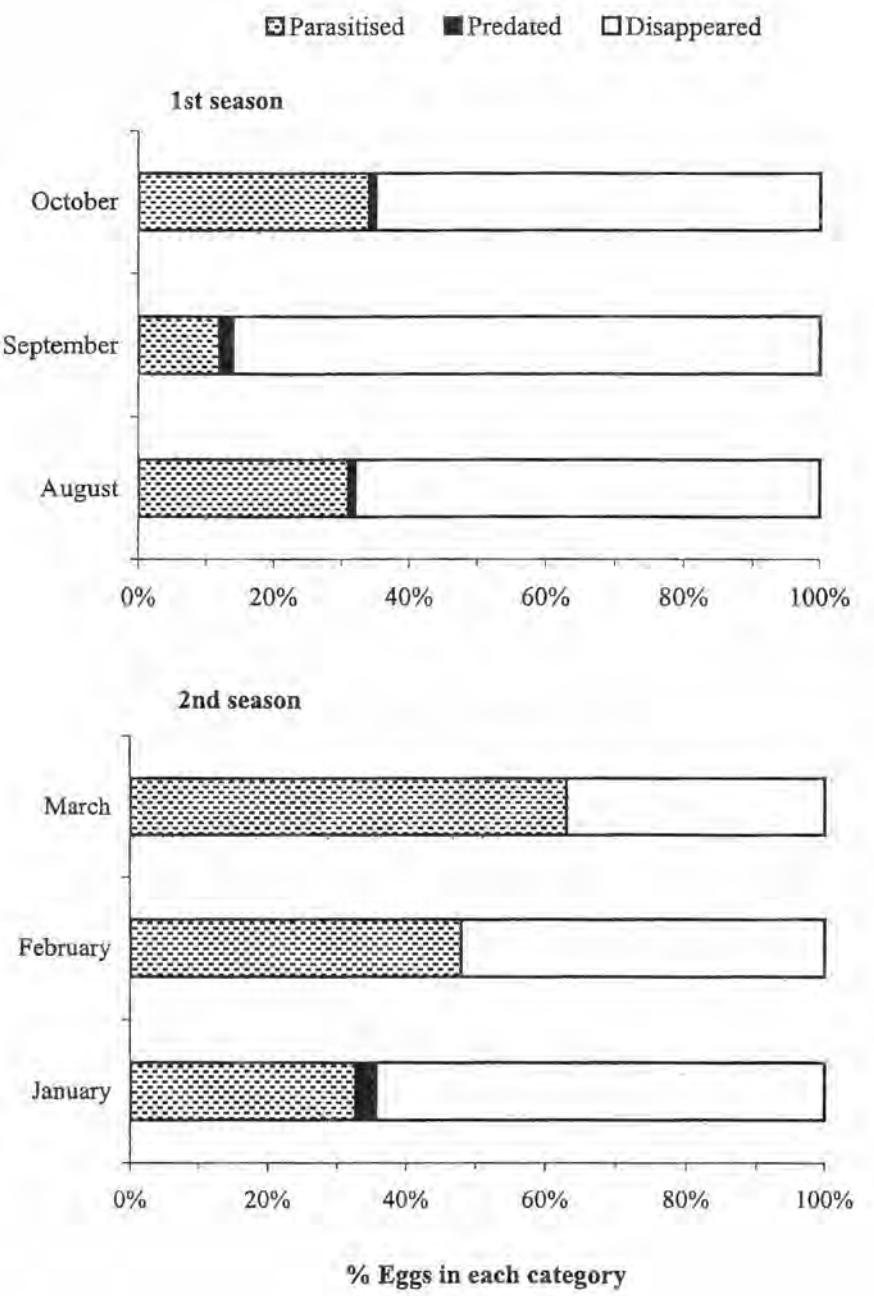


Figure 2.13 Fate of *Plutella xylostella* eggs on kale at Muhaka, coastal Kenya

Table 2.1 Egg parasitoids recovered from the exposed *Corcyra cephalonica* egg cards at seven survey sites in Kenya (1999/2000)

Survey Site	Egg Parasitoid Species	Host Plant	% Parasitism	% Emergence
Kasarani (1600m)	*Trichogrammatoidea* sp. nr. *lutea*	*Cajanus cajan*	11 (n=1)	25
		Lycopersicon esculentum	3 (n=1)	60
Kibwezi (976m)	*Trichogramma* sp. nr. *mwanzai*	*Amaranthus gangeticus*	17 (n=1)	8
		Zea mays	19 (n=1)	45
	Not identified	*Zea mays*	3 (n=1)	20
		Cyperus papyrus	7 (n=1)	64
Mtitoandei (700m)	*Trichogrammatoidea* sp. nr. *lutea*	*Brassica oleracea*	1 (n=1)	100
	Trichogramma sp. nr. *mwanzai*	*Zea mays*	34 (n=5)	60
Voi (510m)	—	—	—	—
Mariakani (263m)	Not identified	*Brassica oleracea*	3 (n=1)	50
Shimba Hills (250m)	*Trichogramma* sp. nr. *mwanzai*	*Zea mays*	15 (n=2)	64
	Not identified	*Zea mays*	37 (n=1)	55
Muhaka (40m)	Not identified	*Brassica oleracea*	5 (n=2)	19

- n = number of parasitised *Corcyra cephalonica* egg cards.

Table 2.2 Parasitism of egg parasitoids in seven sites in Kenya (1999/2000)

Site	Survey	Number of *Corcyra cephalonica* Egg Cards (ca. 150 eggs/card)			Parasitised Eggs (No.)	% Parasitism Per Site*
		Exposed	Recovered	Parasitised		
Kasarani (1600m)	1	32	32	0	0	
	2	32	30	1	5	
	3	32	20	1	16	
	Total	**96**	**82**	**2**	**21**	**0.2**
Kibwezi (976m)	1	32	19	0	0	
	2	32	23	3	45	
	3	32	23	1	25	
	Total	**96**	**65**	**4**	**70**	**0.7**
Mtitoandei (700m)	1	32	21	1	11	
	2	32	18	5	248	
	3	32	23	0	0	
	Total	**96**	**62**	**6**	**259**	**2.8**
Voi (510m)	1	32	21	0	0	
	2	32	15	0	0	
	3	32	23	0	0	
	Total	**96**	**59**	**0**	**0**	-
Mariakani (263m)	1	32	12	0	0	
	2	32	13	1	4	
	3	32	21	0	0	
	Total	**96**	**46**	**1**	**4**	**0.1**
Shimba Hills (250m)	1	32	31	0	0	
	2	32	32	3	101	
	3	32	0	0	0	
	Total	**96**	**63**	**3**	**101**	**1.1**
Muhaka (40m)	1	32	31	0	0	
	2	32	30	1	4	
	3	32	10	1	12	
	Total	**96**	**71**	**2**	**16**	**0.2**

* Percent parasitism was calculated based on the actual number of parasitised eggs from the recovered egg cards.

Table 2.3 Density and parasitism of *Helicoverpa armigera*, *Plutella xylostella* and *Chilo partellus* eggs at Muhaka, coastal Kenya in 1999/2000

Sampling Season	Host Insect	Density		Parasitism	
		Eggs per plant (Mean ± SE)	Total eggs (No.)	Collected eggs (No.)	% Parasitism‡ (Mean ± SE)
First	*Helicoverpa armigera* (n=11512)†	0.023 ± 0.002	263	263	4.90 ± 1.64
	Plutella xylostella (n=11507)†	0.722 ± 0.030	8304	8008	13.41 ± 0.67
	Chilo partellus (n=11510)†	0.936 ± 0.059	10770	9210	19.96 ± 2.24
Second	*Helicoverpa armigera* (n=11514)†	0.013 ± 0.003	151	151	1.25 ± 1.25
	Plutella xylostella (n=11511)†	0.514 ± 0.027	5918	5608	17.90 ± 0.93
	Chilo partellus (n=11518)†	0.374 ± 0.032	4306	4170	38.90 ± 3.98

† Numbers in parenthesis indicate the total number of plants observed in twenty-repeated samplings.

‡ Egg parasitism per plant both by identified and unidentified egg parasitoids per plant.

Table 2.4 Native egg parasitoids recovered from *Helicoverpa armigera*, *Plutella xylostella* and *Chilo partellus* eggs in Kenya

Host Insect	Identified Scientific Name	Host Plant	Sample (n)	Place
Helicoverpa armigera (n = 5)	*Trichogramma* sp. nr. *mwanzai* Schulten & Feijen	*Cajanus cajan*	1	Muhaka
	Trichogrammatoidea sp. nr. *lutea* Girault	*Cajanus cajan*	1	Muhaka
	Trichogrammatoidea sp.	*Cajanus cajan*	2	Muhaka
	Trichogrammatoidea sp.	*Hibiscus esculentus*	1	Muhaka
Plutella xylostella (n = 72)	*Trichogrammatoidea* sp. nr. *lutea* Girault	*Brassica oleracea*	72	Muhaka
Chilo partellus (n = 58)	*Trichogramma bournieri* Pintureau & Babault	*Zea mays*	1	Mbita
	Trichogramma sp. nr. *mwanzai* Schulten & Feijen	*Sorghum bicolor*	46	Muhaka
	Trichogramma sp. nr. *mwanzai* Schulten & Feijen	*Brassica oleracea*	3	Muhaka
	Trichogramma sp. nr. *bruni* Nagaraja	*Sorghum bicolor*	1	Muhaka
	Trichogrammatoidea sp. nr. *lutea* Girault	*Sorghum bicolor*	6	Muhaka
	Trichogrammatoidea sp. nr. *lutea* Girault	*Brassica oleracea*	1	Muhaka
Total			135	

CHAPTER 3

3.0 MORPHOMETRIC AND MOLECULAR STUDIES OF *TRICHOGRAMMA* SPECIES

3.1 INTRODUCTION

Trichogramma (Hymenoptera: Trichogrammatidae), which is represented by about 200 nominal species, is the largest genus in the family Trichogrammatidae (Richards and Davies, 1977; Pinto, 1999). Taxonomy of *Trichogramma* species has been problematic due to their small size and morphological homogeneity (Richards and Davies, 1977; Pinto and Stouthamer, 1994; Pinto, 1999; Silva, 1999). However, reliable species identification is a prerequisite for successful biocontrol programmes.

According to Davis (1983), taxonomy should be based upon genotypic relationships, but taxonomists usually work with phenotypes. Pinto *et al.* (1989) also emphasized that highly plastic characters (e.g. size, colour and wing setation) in which environmental influence obscures genetic relationships, should be identified and should subsequently contribute only minimally to taxonomic decisions. Correct identification of *Trichogramma* species is vital for the appropriate selection of species for mass rearing and field releases against several lepidopteran pests (Hassan, 1990, 1993, 1994; Smith, 1996).

The male genitalia have been widely used as basic morphological traits in distinguishing species of *Trichogramma*, but body colour, wing venation and features of the antennae serve as supporting characteristics (Nagarkatti

and Nagaraja, 1971, 1977; Pinto *et al.*, 1989; Pinto and Stouthamer, 1994). According to Nagarkatti and Nagaraja (1977), wing pigmentation and antennal characters are unstable and can be influenced either by temperature or by the host on which the insects were reared. The effect of temperature on the size and morphology of *Trichogramma* has also been reported by Gross (1988) and Consoli and Parra (1995a&b).

In addition to linear morphological characteristics, ratios of measurements have also been used in the taxonomy of *Trichogramma* (Nagaraja and Nagarkatti, 1973; Pinto *et al.*, 1986; Pinto, 1992). Pinto *et al.* (1989) reported that ratios are more reliable than simple characters. Nagaraja and Nagarkatti (1973) also used ratios of the longest fringe seta on fore wing to width (FWFL/FWW) to distinguish species. However, Pinto *et al.* (1989) indicated that the ratio FWFL/FWW is a particularly poor character because fringe length usually is negatively correlated with wing width. Ratio ranges have been used to support the ratio characters in separating the species (Pinto *et al.*, 1989).

The conventional taxonomy of *Trichogramma* is entirely based on male genitalia and therefore useful only for arrhenotokous species. Therefore, this classification does not include *Trichogramma* species that are thelytokous (Stouthamer *et al.*, 1990; Pinto and Stouthamer, 1994). Several researchers have used morphometric and molecular techniques for differentiation of *Trichogramma* species such as Nagarkatti and Nagaraja (1971, 1977), Oatman

et al. (1982), Pinto *et al.* (1986, 1989, 1997), Pinto (1992), Landry *et al.* (1993), Sappal *et al.* (1995) and Stouthamer *et al.* (2000).

DNA (deoxyribonucleic acid) based molecular traits are unaffected by the sex or life stage of *Trichogramma* (Vanlerberghe, 1994; Stouthamer *et al.*, 1999). The use of Polymerase Chain Reaction (PCR) based techniques has made it possible to develop fast and exact identification tools for some species in this genus (Stouthamer *et al.*, 1999). PCR is a method for amplifying DNA by means of DNA polymerases such as Taq polymerase (Hoy, 1994). PCR fundamentally involves denaturing double-stranded DNA, adding dNTPs, DNA polymerase, and primers (Hoy, 1994).

Williams *et al.* (1990) and Vanlerberghe (1994) used the Random Amplified Polymorphic DNA (RAPD) for rapid identification of *Trichogramma* species. Reineke and Zebitz (1999) also used PCR-based approaches for identification of different genotypes of gypsy moth (*Lymantria dispar* L.). The ribosomal DNA (rDNA) is composed of tandem units; each of them comprises several regions (coding genes and spacers). The two internal transcribed spacers (ITS1 and ITS2) separated the coding regions (Hoy, 1994). Since the spacers evolve faster than the highly conserved coding regions, they may be variable enough to permit species separation. According to Stouthamer *et al.* (1999), the DNA sequence of the internally transcribed spacer (ITS2) of *Trichogramma* wasps could be used for species identification.

Morphometric and molecular techniques have been used for differentiation of *T. pretiosum* Riley and *T. deion* Pinto & Oatman (Pinto *et al.* 1986; Stouthamer *et al.*, 1999). Silva (1999) also used molecular techniques for differentiation of five *Trichogramma* species occurring in Portugal: *T. turkestanica* Meyer, *T. bourarachae* Pintureau & Babault, *T. cordubensis* Vargas & Cabello, *T. pintoi* Voegele and *T. evanescens* Westwood.

Reliable species identification is a prerequisite for successful biological control measures. Therefore, the objective of this study was to identify and characterise the native *Trichogramma* species and to compare the morphological and molecular relationships with related species. Based on morphological relationships, *T. chilonis* Ishii was chosen as an out-group species to obtain a higher resolution of both morphometric and molecular analysis.

3.2 MATERIALS AND METHODS

3.2.1 Cultures

The native species from Kenya, *T. bournieri* Pintureau & Babault, collected in Lake Victoria area (Mbita), from maize and *T.* sp. nr. *mwanzai* Schulten & Feijen, collected in coastal area (Muhaka), from sorghum plants (Abera *et al.*, 2000a) were used for the study. For comparison, the Palaearctic species, *T. evanescens* (Germany), the nearctic species, *T. platneri* Nagarkatti (USA) and the Australasian species, *T. chilonis* (China) were obtained from the Federal Biological Research Centre for Agriculture and Forestry (BBA), Institute for Biological Pest Control, Darmstadt, Germany. Morphometric and molecular studies were conducted using the above five species at the Institute of Phytomedicine, University of Hohenheim in Germany. All the five species used in this study were arrhenotokous (Stouthamer *et al.*, 1990; Pinto and Stouthamer, 1994).

Each species of *Trichogramma* was reared in glass vials (75 mm long x 25 mm in diameter) and fed with a mixture of honey (66%), gelatine (1%) and distilled water (33%) and kept in an incubator at 26 ± 1°C with 70 ± 10% relative humidity and a photoperiod of 16L: 8D. Cotton plugs were used as stopper for the vials. Eggs of the Angoumois grain moth, *Sitotroga cerealella* Oliver (Lepidoptera: Gelechidae) were used for rearing the parasitoids throughout the study (Hassan, 1993). Rearing of the parasitoids was conducted at BBA, Darmstadt.

3.2.2 Morphological characters and slide preparation

For each species, ten males were slide-mounted into Canada balsam for microscopic study following the method described by Platner *et al.* (1999). Antennal characters were used to distinguish the female *Trichogramma* from the male (Pinto *et al.*, 1978). A total of 23 morphological characters which included antenna (5), wings (6), hind tibia (2) and genitalia characters (10) were either measured with a micrometer plate (μm) or counted under a compound microscope (magnification 40x) (Monje, 1996). Ratios of morphometric characters were also compared among the species. The terms length and width are maximum values obtained by rotating the specimen. Acronyms of morphological characters and ratios are presented in Table 3.1. Antennal and genitalia characters are shown in Fig. 3.1. Morphological terms and ratios follow Nagarkatti and Nagaraja (1971, 1977), Miller (1972), Nagaraja (1978), Pinto *et al.* (1978), Oatman *et al.* (1982) and Pinto and Oatman (1985) methods.

Table 3.1 Acronyms, morphological terms and ratios

(Nagarkatti and Nagaraja, 1971, 1977; Miller, 1972; Nagaraja, 1978; Pinto *et al.*, 1978, 1989; Oatman *et al.,* 1982; Pinto and Oatman, 1985)

	Morphological Character	Acronyms
Antenna	Length of flagellum of antenna	FL
	Width of flagellum as measured at base	FW
	Length of longest seta on flagellum	FSL
	Number of setae on flagellum (only those from the base to the second flagellar constriction were counted)	FSNO
	Number of basiconic peg sensilla (these pegs occur at five positions along the male flagellum and they can be paired, single, or absent at each position)	BCPS
Wing	Fore wing length	FWL
	Fore wing width	FWW
	Longest fringe seta on fore wing	FWFL
	Number of setae in anterior tract of hind wing	AT
	Number of setae in middle tract of hind wing	MT
	Number of setae in posterior tract of hind wing	PT
Tibia	Hind tibial width	HTW
	Hind tibial length	HTL
Genitalia	Genital capsule length	GL
	Genital capsule width	GW
	Aedeagus length including apodemes	AL
	Dorsal lamina length	DLA
	Intervolsellar process length	IVP
	Volsellar digiti length	VS
	Ventral ridge length	VR
	Apical distance of genital capsule (from base of volsellae and IVP to apex of paramers, PM)	AD
	Basal distance of genital capsule (GL minus AD)	BD
	Constriction as measured at the base of DLA	CDLA
Ratios	Length of flagellum to hind tibial length	FL/HTL
	Flagellum length to width	FL/FW
	Longest flagellar seta to flagellum width	FSL/FW
	Genital capsule width to length	GW/GL
	Aedeagus length to hind tibial length	AL/HTL
	Longest fringe seta on fore wing to width	FWFL/FWW
	Longest fringe seta on fore wing to hind tibial width	FWFL/HTW

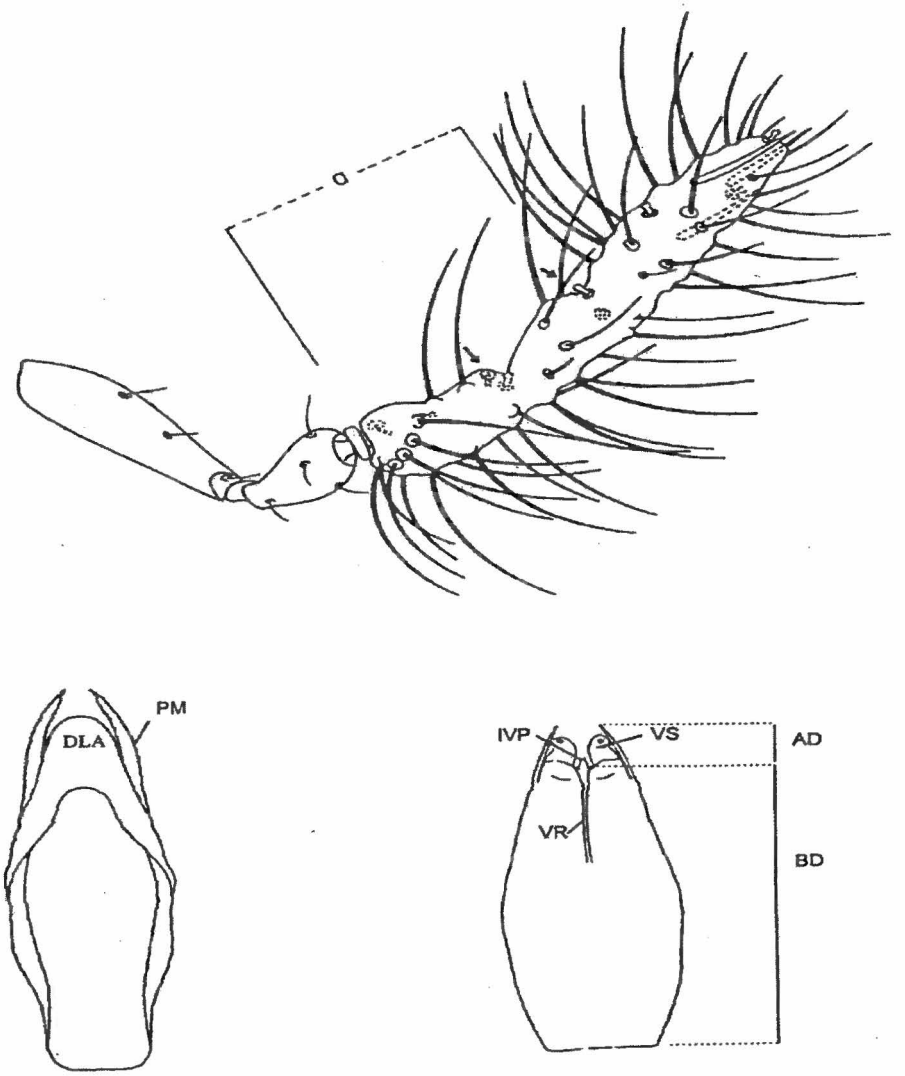


Figure 3.1 Above: Antenna of a male *Trichogramma* (Adapted from Pinto *et al.*, 1989). Arrows indicate the second and third positions of basiconic peg sensilla (BCPS) and "a" shows the area in which setae (FSNO) were counted.

Below: Dorsal (left) and ventral (right) view of a male genitalia of *Trichogramma* (Adapted from Pinto, 1992).

3.2.3 DNA extraction and PCR

DNA was extracted from males (n=20) of five *Trichogramma* species: *T. bournieri, T.* sp. nr. *mwanzai, T. evanescens, T. chilonis* and *T. platneri* following a modified cetyltrimethylammonium bromide (CTAB) protocol; with an additional polyethylene glycol precipitation (Moeller *et al.*, 1992; Reineke *et al.*, 1998). Twenty frozen males of each species were manually crushed in a 1.5 ml microfuge tube in liquid nitrogen using a Teflon-coated steel rod. The rods were washed and sterilized between each use. The powder was suspended in 100 µl TES (100 mM Tris-HCl, pH 8.0, 10 mM EDTA, 2% SDS). Proteinase K (20 mg/ml) was added and the solution was incubated for 1 hour at 55 -60°C with occasional gentle mixing. The salt concentration was adjusted to 1.4 M with 5M NaCl, 1/10 volume of 10% CTAB (Sigma, St. Louis, MO) and added, then the samples were incubated for 10 minutes at 65°C. After adding an equal volume of chloroform to isoamylalchol ratio of 24:1, the tubes were gently mixed, incubated for 30 minutes on ice and centrifuged for 15 minutes at 4°C and 14,000 rpm.

The supernatant was then transferred to a new tube with a Pasteur pipette and 45 µl of 5 M ammonium acetate was added. The tubes were then mixed gently and placed on ice for 30 minutes. After centrifugation for 20 minutes, the upper phase was transferred to a new tube and 0.25 volumes of 30% polyethylene glycol 6000 (Sigma, St. Louis, MO) was added. The samples were incubated on ice for 1 hour and centrifuged for 20 min to precipitate the DNA. The supernatant was discarded and the DNA pellet was

washed twice with cold (4°C) ethanol 70%, dried and suspended in 100µl TE (100mM Tris-HCl, 1 mM EDTA, pH 8.0) over night. RAPD-PCR and PCR of the internal transcribed spacer 2 region (ITS2) of the ribosomal DNA (rDNA) were performed.

PCR reactions were performed in 25 µl reaction mixtures containing 1 µl DNA template, 2.5 µl dNTP's (each in a 10mM concentration), 2 µl $MgCl_2$ (in a 25 mM concentration), 0.5 µl BSA, 0.21 µl Taq DNA polymerase (0.625U) (MBI Fermentas, Vilnius, Lithuania), 1 µl primer (0.2mM) in 2.5 µl (10x) PCR-buffer and 16 µl sterile water. The random Operon (Operon Technologies, Alameda, California) primer OPDO2 (5' GGA CCC AAC C 3') was used to obtain RAPD banding patterns; whereas the specific primer: forwards 5'-TGT CAA CTG CAG GAC ACA TG- 3' and reverse 5'-GTC TTG CCT GCT CTG AG- 3' were used to amplify the ITS-2 region of rDNA.

The PTC-100 thermocycler (Watertown, MA) was programmed for PCR as follows: initial denaturation at 94°C for 2 minutes followed by 45 cycles of 1 min. at 94°C, 1 min. at 36°C and 2 min. at 72°C, with 1 min. at 72°C after the last cycle (A. Reineke personal communication). After amplification, 7 µl "PCR-Blue Juice" was added and 5 µl of the total mixture was applied to a 1.2 % agarose gel in TBE-buffer. A 100 bp (base pairs) DNA ladder was used as a size marker (MBI Fermentals). After the electrophoresis run at 50V for 120 minutes, the gel was stained with ethidium bromide (0.5 µg/ml) and DNA was visualised in ultraviolet (UV) light. Negative control,

which consists of all reaction components, except template DNA was included to detect contamination.

3.2.4 Data analysis

All morphometric measurements and counting (except BCPS) were subjected to the analysis of variance (ANOVA) using the General Linear Models (GLM) procedure. Ratios of characters were subjected to ANOVA. When F-values showed significance at P=0.05, means were separated using Student-Newman-Keuls (SNK) test (SAS Institute, 2000).

To compare the degree of morphological similarity of the five *Trichogramma* species, hierarchical centroid cluster analysis based on the normalised minimum distance was conducted using all morphological characters (except BCPS) and genitalia traits separately. The pseudo F, pseudo T^2 and the cubic clustering criterion (CCC) were used to determine the optimum number of clusters (SAS Institute, 2000). Quantitative data represent the Mean ± SE; sample size is 10 in all cases except for ventral ridge length (VR).

The RAPD-PCR products were analysed using GelCompar 4.0 software (Applied Maths, 1996). The formula of Dice (1945) was used to estimate the genetic similarities between the species. For each species, a binary matrix reflecting the specific PCR-band presence (1) or absence (0) was generated. GelCompar assigned bands automatically to each track using a band search filter of 0.5% (bands with an area smaller than 0.5% of the total

area of the pattern were disregarded). Based on genetic similarities, a dendrogram was constructed using the unweighted pair group method of arithmetic averages (UPGMA) (Sneath and Sokal, 1973).

3.3 RESULTS

3.3.1 Identification of native species

Based on the shape of the male genitalia, the egg parasitoid specimen collected from coastal areas of Kenya was found to be close to *Trichogramma mwanzai* Schulten & Feijen, reported initially from Malawi (Schulten and Feijen, 1982). General shape of male genitalia in both species places them within the *exiguum* section, a large group of species that represents most of the known *Trichogramma* species at present (Pinto, 1999).

Antennal characters also placed them within species related to *T. minutum* Riley (FSL/FW <3) (Monje, 1996). Therefore, the egg parasitoid specimen collected from coastal areas of Kenya is identified as *Trichogramma* species near *mwanzai* Schulten & Feijen. Similarly, the second egg parasitoid specimen collected from Lake Victoria area was identified as *Trichogramma bournieri* Pintureau & Babault. This species was earlier reported from Comoros (Pintureau and Babault, 1988).

3.3.2 Morphometric analysis

Morphological characters of the five *Trichogramma* species are presented in Tables 3.2-3.7. There were significant differences among the species based on antennal characters: FL (F=9.67; df=4,36; P=0.0001), FW (F=3.26; df=4,36; P=0.0223), FSL (F=45.61; df=4,36; P=0.0001) and FSNO (F=22.51; df=4,36; P=0.0001) (Table 3.2). *Trichogramma bournieri* and *T. platneri* had the same formula (1-1-1-1-1) for basiconic capitate peg sensilla

(BCPS) on flagellum of the antennae. *Trichogramma* sp. nr. *mwanzai*, *T. evanescens* and *T. chilonis* had paired BCPS at the second and third positions (Table 3.3). The highest number of setae on flagellum of the antennae (Mean ± SE) was recorded in *T.* sp. nr. *mwanzai* (25.0 ± 0.45) (Table 3.2).

The longest fore wing length and width recorded in μm were 547.3 ± 7.01 and 289.5 ± 3.47 in *T. platneri*, respectively (Table 3.4). Significant differences were also observed in the length (F=9.23; df=4,36; P=0.0001) and width (F=18.67; df=4,36; P=0.0001) of the forewing among the five *Trichogramma* species (Table 3.4). *Trichogramma* sp. nr. *mwanzai* has the longest hind tibial length (182.1 ± 3.87), which is commonly used to estimate the relative body size in *Trichogramma* (Table 3.5).

Based on genitalia characters, *T. bournieri* was separated from the rest in having long IVP (26.2 ± 0.49) and AL (167.8 ± 2.70) and short VR (29.5 ± 1.50). The shortest IVP recorded was 17.1 ± 0.46 and 17.7 ± 0.30 in *T. platneri* and *T. chilonis*, respectively. The relatively longer VS separate the two native species, *T. bournieri* (28.0 ± 0.58) and *T.* sp. nr. *mwanzai* (26.6 ± 0.60) from the rest (Table 3.6).

Overall, the analysis of variance reveals significant differences among the species in genitalia characters: GL (F=10.37; df=4,36; P=0.0001), GW (F=15.50; df=4,36; P=0.0001), AD (F=9.23; df=4,36; P=0.0001), BD (F=14.60; df=4,36; P=0.0001), IVP (F=42.06; df=4,36; P=0.0001), VS (F=13.90; df=4,36; P=0.0001), DLA (F=35.50; df=4,36; P=0.0001), VR (F=69.18; df=4,30; P=0.0001), AL (F=15.30; df=4,36; P=0.0001) and CDLA

(F=30.64; df=4,36; P=0.0001). The higher ratios of FL/HTL (1.12 ± 0.02), FL/FW (6.35 ± 0.14) and FSL/FW (3.32 ± 0.09) separate *T. evanescens* from the other four species (Table 3.7).

Cluster analysis using all morphological characters (except BCPS) did not show much separation (Fig. 3.2). However, cluster analysis of genitalia characters showed that *T.* sp. nr. *mwanzai* was very close to *T. evanescens* at 0.61 normalized centroid distance (ncd), whereas the two native species, *T. bournieri* and *T.* sp. nr. *mwanzai* were closer at 0.88 ncd (Fig. 3.3).

3.3.3 Molecular analysis

The PCR-products using random primers (RAPD banding patterns) and specific primers (to amplify ITS2 regions) are shown in Figs. 3.4 and 3.5, respectively. The random primers resulted in different PCR product sizes among the species: *T. bournieri* (ca. 550 bp), *T.* sp. nr. *mwanzai* (530 bp), *T. evanescens* (ca. 620 bp), *T. platneri* (ca. 610 bp) and *T. chilonis* (ca. 580 bp) (Fig. 3.4). The PCR products of the ITS2 rDNA show little variations among the species and resulted in ca. 540 bp for *T. bournieri* and *T.* sp. nr. *mwanzai* (Fig. 3.5). The percent genetic similarity among the five species of *Trichogramma*, based on UPGMA cluster analysis of RAPD banding patterns using GelCompar computer software is shown in Fig. 3.6.

3.4 DISCUSSION

Phenotypic (morphometric) and genetic (molecular) characters of the five *Trichogramma* species were studied and the results are compared. Following combination of characters, the two native species could be separated: distinctly longer genital capsule and intervolsellar process with distinctly shorter dorsal lamina and ventral ridge in *T. bournieri* compared to *T.* sp. nr. *mwanzai.*

Additionally, differences in the number of antennal peg sensilla could be used to separate the two native species in that *T. bournieri* has single sensilla in all the five positions compared with paired sensilla at the second and the third positions in *T.* sp. nr. *mwanzai.* Pinto *et al.* (1986) separated the nearctic *T. deion* from *T. pretiosum* by the number of basiconic capitate peg sensilla (BCPS) on the antennae and by minor differences in the male genitalia. The nearctic species, *T. platneri* and the African species, *T. bournieri* have similar formula for basiconic capitate peg sensilla on the flagellum = 1-1-1-1-1 same as *T. deion;* whereas three specimens of *T.* sp. nr. *mwanzai* have the formula 1-2-2-1-1, similar to *T. pretiosum* (Pinto *et al.*, 1986). *Trichogramma bournieri* and *T.* sp. nr. *mwanzai* had longer hind tibia compared to the Latin American (*T. lachesis, T. clotho* and *T. atropos*) and Australian species (*T. primaevum*), reported by Pinto (1992).

The ratios FL/HTL and FSL/FW obtained for *T. bournieri* and *T.* sp. nr. *mwanzai* were higher than *T. deion* and *T. pretiosum.* However, the ratio GW/GL was lower than *T. deion* and similar to *T. pretiosum* (Pinto *et al.*,

1986). The ratio of FWFL/FWW obtained for *T. bournieri* and *T.* sp. nr. *mwanzai* was higher than *T. californicum* (Nagaraja and Nagarkatti, 1973). The range of the ratio FWFL/FWW was wider in *T. bournieri* than *T.* sp. nr. *mwanzai.*

Based on all morphological traits (except BCPS), the Kenyan species *T.* sp. nr. *mwanzai* was closer to the oriental *T. chilonis* at 0.82 normalized centroid distance (ncd) than the other Kenyan species, *T. bournieri* (0.83 ncd) (Fig. 3.2). However, based on genitalia characters, *T.* sp. nr. *mwanzai* was closer to the Palaearctic species, *T. evanescens* at 0.61 ncd than *T. bournieri* (0.88 ncd), while *T. chilonis* were distinctly separated from the four species (1.0 ncd) (Fig. 3.3).

The size of PCR products for ITS-2 rDNA obtained for *T. bournieri* and *T.* sp. nr. *mwanzai* (ca. 540 bp) was similar to 529 bp of *T. cordubensis,* 542-551 bp of *T. evanescens* (Silva, 1999) and 510-520 bp of *T. deion* (Stouthamer *et al.*, 1999). This is also closer to the 600-660 bp obtained for the ITS2 regions of *T. minutum*, *T. brassicae* Bezdenko and *T.* near *sibiricum* Sorokina (Sappal *et al.*, 1995). Stouthamer *et al.* (2000) reported that no species-specific differences have been found in the ITS2 DNA sequences of *T. platneri* and *T. minutum* suggesting that both have recently diverged from a common ancestor. The similar PCR product size obtained for ITS2 rDNA of *T. bournieri* and *T.* sp. nr. *mwanzai* could be explained by this similarity.

UPGMA Cluster analysis of RAPD banding patterns revealed that the two Kenyan species, *T. bournieri* and *T.* sp. nr. *mwanzai* had 36 % genetic similarity with respect to the remaining three species. The African species, *T. bournieri* and *T.* sp. nr. *mwanzai* had 40% genetic similarity. The Palaearctic, *T. evanescens* and Nearctic, *T. platneri* had 62% genetic similarity, whereas the Australasian, *T. chilonis* had 42 % genetic similarity with the Palaearctic and Nearctic species.

It is concluded that the Kenyan species are closer to each other genetically than morphologically. However, Nagarkatti and Nagaraja (1977) reported on the presence of so many populations that were phenotypically identical (or nearly so), but genetically different. Pinto *et al.* (1989) also stressed the problems of using simple features for separating species. Therefore, species separation should be based more on genetic characters.

This study has produced new information on the identity of African species of *Trichogramma* and is hoped to foster more research in this area. Further molecular studies on the amplified ITS2 region with restriction enzymes as well as gene sequencing are vital for comprehensive taxonomy of the species.

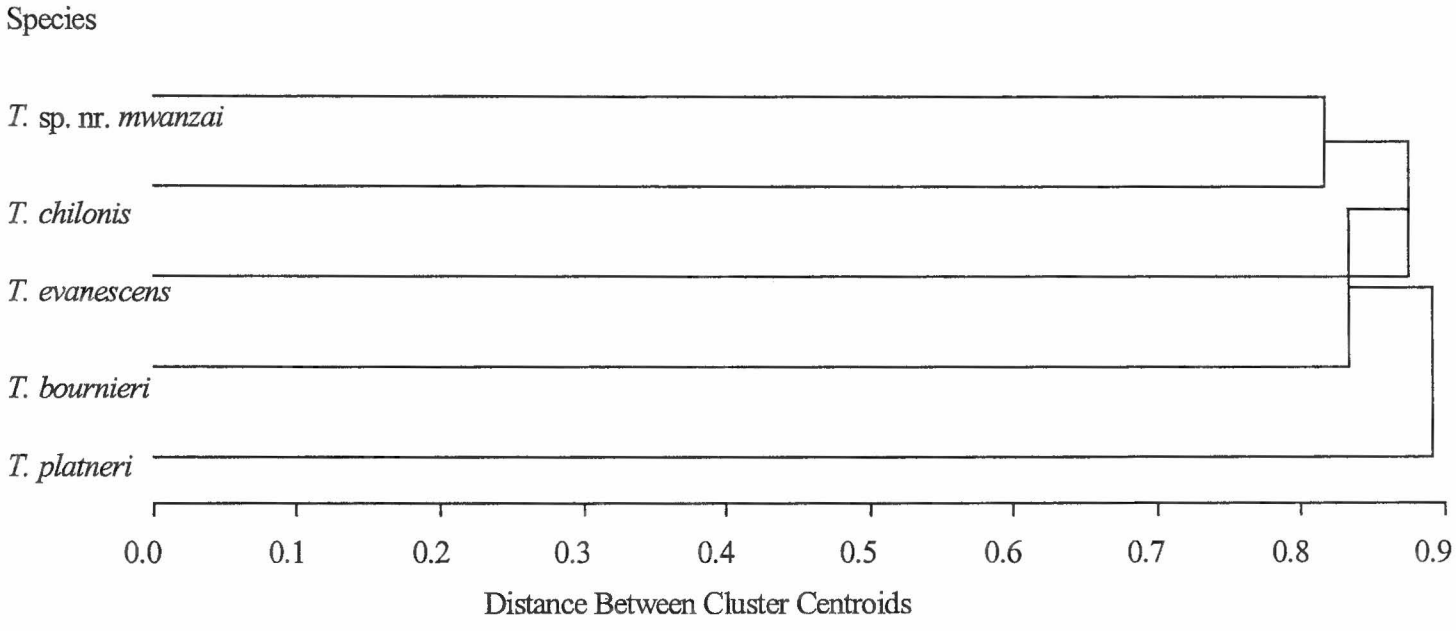


Figure 3.2 Dendrogram showing the morphometric relationships among the five species of *Trichogramma* based on all traits (except BCPS)

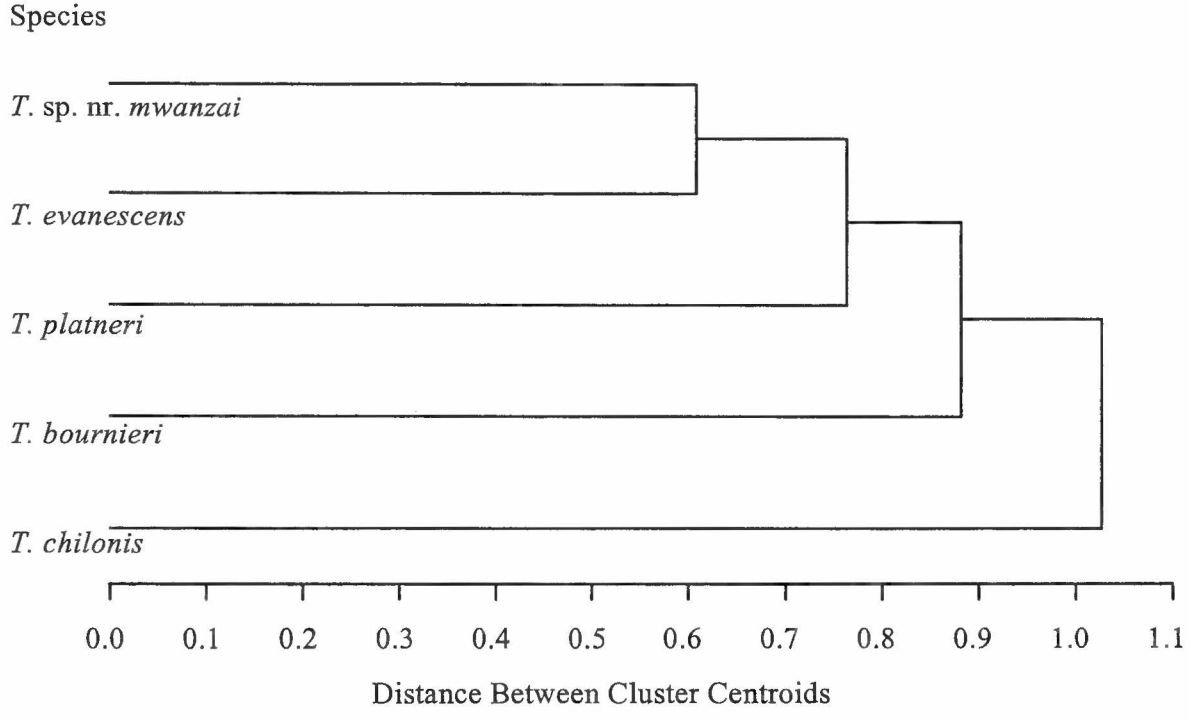


Figure 3.3 Dendrogram showing the morphometric relationships among the five species of *Trichogramma* based on genitalia characters

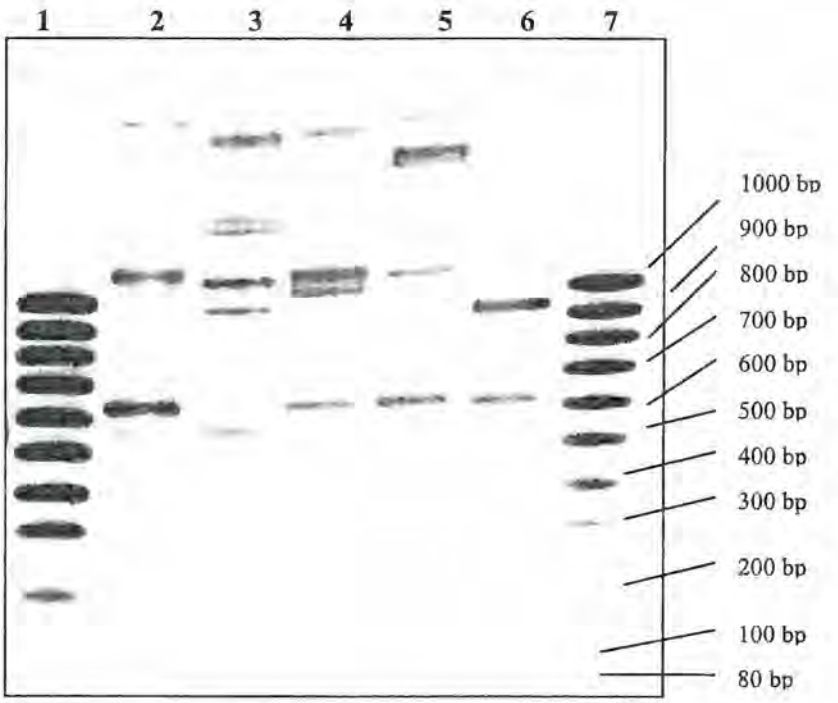


Figure 3.4 Gel showing the PCR-products of RAPD banding patterns for the five *Trichogramma* species. Lane 1: DNA ladder 100 bp, 2: *T. chilonis*, 3: *T.* sp. nr. *mwanzai*, 4: *T. bournieri*, 5: *T. platneri*, 6: *T. evanescens*, 7: DNA ladder 100bp.

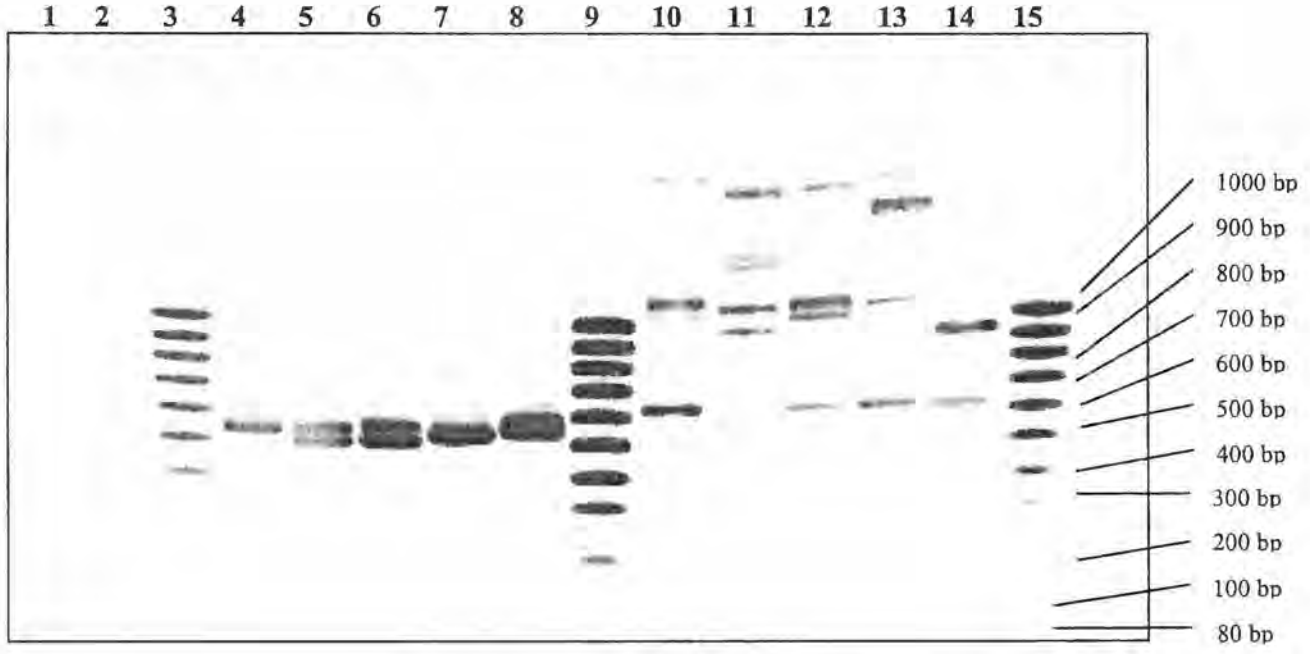


Figure 3.5 Gel showing the PCR-products of the ITS-2 regions and RAPD banding patterns for the five *Trichogramma* species. Lane 1: Control for ITS-2, 2: Control for DO2, 3: DNA ladder 100bp, 4: *T. chilonis*, 5: *T.* sp. nr. *mwanzai*, 6: *T. bournieri*, 7: *T. platneri*, 8: *T. evanescens*, 9: DNA ladder 100bp, 10: *T. chilonis*, 11: *T.* sp. nr. *mwanzai*, 12: *T. bournieri*, 13: *T. platneri*, 14: *T. evanescens*, 15: DNA ladder 100bp.

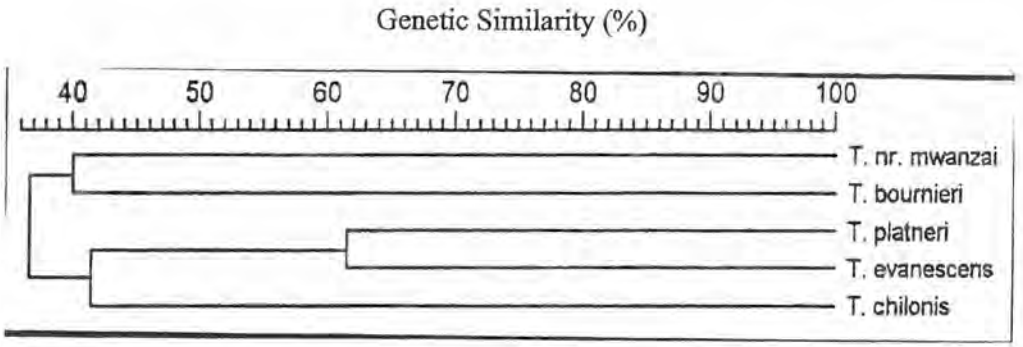


Figure 3.6 Dendrogram of five *Trichogramma* species revealed by UPGMA cluster analysis of genetic similarities based on RAPD-PCR.

Table 3.2 Antennal characters of five *Trichogramma* species

Trichogramma species	Origin	Antennal characters (Mean ± SE) (Number of slides, n = 10)			
		Length of flagellum (FL) (μm)	Width of flagellum (FW) (μm)	Longest seta on flagellum (FSL) (μm)	No. of setae on flagellum (FSNO)
T. bournieri	Kenya (Africa)	161.2 ± 4.57b	30.2 ± 0.53b	77.3 ± 0.54c	19.5 ± 0.86c
T. sp. nr. *mwanzai*	Kenya (Africa)	181.9 ± 3.77a	31.4 ± 0.60ab	83.8 ± 0.94b	25.0 ± 0.45a
T. evanescens	Germany (Europe)	188.5 ± 3.22a	29.9 ± 0.72b	98.6 ± 2.26a	17.5 ± 0.62c
T. platneri	USA (N. America)	164.9 ± 3.43b	31.6 ± 0.60ab	82.3 ± 1.03b	17.8 ± 0.57c
T. chilonis	China (Asia)	184.5 ± 4.35a	32.5 ± 0.60a	77.5 ± 0.60c	22.6 ± 0.85b

- Means followed by the same letter in the same column are not significantly different (P>0.05), Student Newman Keuls (SNK) test.

Table 3.3 Formula of Basiconic Capitate Peg Sensilla (BCPS) of five *Trichogramma* species

Slide	Number of Basiconic Capitate Peg Sensilla (BCPS) on flagellum*				
(Rep)	*T. bournieri*	*T.* sp. nr. *mwanzai*	*T. evanescens*	*T. platneri*	*T. chilonis*
1	1-1-1-1-1	1-2-2-1-1	1-1-1-1-1	1-1-1-1-1	1-2-1-1-1
2	1-1-1-1-1	1-2-2-1-1	1-1-2-1-1	1-1-1-1-1	1-2-2-1-1
3	1-1-1-1-1	1-1-2-1-1	1-1-2-1-1	1-1-1-1-1	1-2-2-1-1
4	1-1-1-1-1	1-2-2-1-1	1-1-2-1-1	1-1-1-1-1	1-2-2-1-1
5	1-1-1-1-1	1-1-2-1-1	1-1-1-1-1	1-1-1-1-1	1-2-2-1-1
6	1-1-1-1-1	1-1-2-1-1	1-1-1-1-1	1-1-1-1-1	1-2-2-1-1
7	1-1-1-1-1	1-1-2-1-1	1-1-2-1-1	1-1-1-1-1	1-2-2-1-1
8	1-1-1-1-1	1-1-2-1-1	1-1-1-1-1	1-1-1-1-1	1-2-2-1-1
9	1-1-1-1-1	1-1-2-1-1	1-1-1-1-1	1-1-1-1-1	1-2-2-1-1
10	1-1-1-1-1	1-1-2-1-1	1-1-2-1-1	1-1-1-1-1	1-2-2-1-1

* The BCPS counting started from the most basal position of the flagellum of antennae.

Table 3.4 Wing characters of five *Trichogramma* species

Trichogramma species	Wing Characters (Mean ± SE) (n=10)					
	Fore wing (μm)			Hind wing (no.)		
	Fore wing length (FWL)	Fore wing width (FWW)	Longest fringe seta (FWFL)	Seta on anterior tract (AT)	Seta on middle tract (MT)	Seta on posterior tract (PT)
T. bournieri	495.2 ± 7.00c	233.7 ± 4.68c	48.6 ± 0.81a	1.3 ± 0.21a	19.8 ± 0.65ab	7.0 ± 0.56a
T. sp. nr. *mwanzai*	502.9 ± 5.52bc	251.9 ± 3.51b	42.6 ± 0.48b	1.3 ± 0.15a	19.9 ± 0.50ab	5.7 ± 0.26b
T. evanescens	524.9 ± 5.39b	267.7 ± 5.54b	46.9 ± 0.82a	2.3 ± 0.47a	18.7 ± 0.58b	7.2 ± 0.36a
T. platneri	547.3 ± 7.01a	289.5 ± 3.47a	48.1 ± 0.43a	2.3 ± 0.33a	16.3 ± 0.56c	5.7 ± 0.21b
T. chilonis	504.4 ± 10.80bc	251.3 ± 6.35b	43.2 ± 0.20b	1.4 ± 0.22a	20.7 ± 0.30a	7.1 ± 0.23a

- Means followed by the same letter in the same column are not significantly different (P>0.05), SNK test.

Table 3.5 Hind tibia of five *Trichogramma* species

Trichogramma species	Hind tibia (Mean ± SE) (n=10) (μm)	
	Hind tibia length (HTL)	Hind tibia width (HTW)
T. bournieri	169.8 ± 2.93a	26.1 ± 0.59ab
T. sp. nr. *mwanzai*	182.1 ± 3.87a	27.0 ± 0.75ab
T. evanescens	169.3 ± 3.20a	24.7 ± 0.75b
T. platneri	174.0 ± 2.19a	28.0 ± 0.82a
T. chilonis	179.9 ± 2.84a	27.3 ± 0.72ab

- Means followed by the same letter in the same column are not significantly different (P>0.05), SNK test.

Table 3.6 Genitalia characters of five *Trichogramma* species

Genitalia Character	Mean ± SE (μm)				
	T. bournieri	*T.* sp. nr. *mwanzai*	*T. evanescens*	*T. platneri*	*T. chilonis*
Genitalia capsule length (GL)	160.1 ± 2.75a	148.4 ± 3.14b	146.3 ± 2.06b	162.3 ± 2.07a	143.8 ± 2.75b
Genitalia capsule width (GW)	49.5 ± 0.90c	49.2 ± 1.00c	50.6 ± 0.62c	53.5 ± 0.76b	58.3 ± 1.11a
Apical distance of genital capsule (AD)	46.1 ± 1.06a	45.6 ± 0.70a	47.7 ± 1.56a	41.5 ± 1.50b	38.9 ± 1.08b
Basal distance of genital capsule (BD)	114.5 ± 2.34a	103.3 ± 2.65b	98.8 ± 2.03b	121.2 ± 1.92a	105.2 ± 2.82b
Intervolsellar process length (IVP)	26.2 ± 0.49a	21.3 ± 0.76b	20.2 ± 0.79b	17.1 ± 0.46c	17.7 ± 0.30c
Volsellar digiti length (VS)	28.0 ± 0.58a	26.6 ± 0.60a	24.8 ± 0.61b	23.2 ± 0.61b	23.0 ± 0.58b
Dorsal lamina length (DLA)	38.8 ± 0.66b	47.1 ± 1.41a	38.9 ± 0.78b	48.6 ± 0.99a	35.7 ± 0.54b
Ventral ridge length (VR)	29.5 ± 1.50d	33.7 ± 1.11c	35.6 ± 0.69c	50.9 ± 0.81a	46.2 ± 1.34b
Aedeagus length (AL)	167.8 ± 2.70a	152.4 ± 2.74b	142.4 ± 2.41b	148.0 ± 2.26b	143.5 ± 2.48b
Constrictions as measured at the base of DLA (CDLA)	38.5 ± 1.21b	35.6 ± 0.70c	33.2 ± 0.96d	38.3 ± 0.75b	45.0 ± 0.75a

- Sample size (n) was 10 individuals except 7 for ventral ridge length (VR) of *T.* sp. nr. *mwanzai* and *T. evanescens*.
- Means followed by the same letter in the same row are not significantly different (P>0.05), SNK test.

Table 3.7 Morphometric comparisons among the five *Trichogramma* species

Trichogramma species	Ratios of morphological characters (Mean ± SE) (n = 10 for all samples)						
	FL/HTL	FL/FW	FSL/FW	GW/GL	AL/HTL	FWFL/FWW	FWFL/HTW
T. bournieri	0.95 ± 0.01c	5.38 ± 0.15bc	2.57 ± 0.06bc	0.31 ± 0.01c	0.99 ± 0.01a	0.21 ± 0.01a	1.87 ± 0.04ab
	(0.88 – 1.03)	(4.54 – 6.42)	(2.31 – 2.91)	(0.29 – 0.33)	(0.96 – 1.01)	(0.17 – 0.24)	(1.67 – 2.00)
T. sp. nr.	1.00 ± 0.01b	5.82 ± 0.12b	2.68 ± 0.05b	0.33 ± 0.01b	0.84 ± 0.01b	0.17 ± 0.002b	1.59 ± 0.05c
mwanzai	(0.94 – 1.07)	(5.17 – 6.33)	(2.36 – 2.83)	(0.30 – 0.35)	(0.80 – 0.88)	(0.16 – 0.18)	(1.33 – 1.89)
T. evanescens	1.12 ± 0.02a	6.35 ± 0.14a	3.32 ± 0.09a	0.35 ± 0.01b	0.84 ± 0.01b	0.18 ± 0.01b	1.92 ± 0.06a
	(1.00 – 1.21)	(5.83 – 7.20)	(2.92 – 3.90)	(0.32 – 0.37)	(0.76 – 0.88)	(0.15 – 0.20)	(1.64 – 2.22)
T. platneri	0.95 ± 0.01c	5.29 ± 0.15c	2.63 ± 0.07b	0.33 ± 0.002b	0.85 ± 0.01b	0.17 ± 0.003b	1.73 ± 0.05bc
	(0.89 – 1.00)	(4.46 – 6.18)	(2.38 – 3.09)	(0.32 – 0.34)	(0.79 – 0.93)	(0.15 – 0.18)	(1.46 – 1.90)
T. chilonis	1.03 ± 0.01b	5.71 ± 0.08bc	2.40 ± 0.04c	0.41 ± 0.01a	0.80 ± 0.01c	0.17 ± 0.01b	1.60 ± 0.05c
	(0.99 – 1.09)	(5.31 – 6.00)	(2.21 – 2.58)	(0.34 – 0.43)	(0.75 – 0.91)	(0.15 – 0.20)	(1.42 – 1.89)
F	24.39	10.28	28.89	47.13	46.68	12.53	8.90
df	4,36	4,36	4,36	4,36	4,36	4,36	4,36
P	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001

- Means followed by the same letter in the same column are not significantly different (P>0.05), SNK test.
- Numbers in parenthesis are the ranges.

CHAPTER 4

4.0 TEMPERATURE-DEPENDENT DEVELOPMENT OF FOUR SPECIES OF *TRICHOGRAMMA*

4.1 INTRODUCTION

Temperature is one of the main environmental factors controlling the development of insects. According to Sharpe and DeMichele (1977), the development of a poikilotherm organism is driven by a rate-determining enzyme or enzyme complex, which has three basic reversible energy states: inactive at low temperature, active at median temperature and inactive at high temperature. The developmental rate, fecundity, longevity, and sex ratio of *Trichogramma* are affected by temperature (Cabello and Vargas, 1988). The size and morphology of *Trichogramma* are also affected by temperature (Gross, 1988; Consoli and Parra, 1995a&b).

The effect of temperature on development of *T. pretiosum* Riley and *T. exiguum* Pinto & Platner (Harrison *et al.*, 1985), *T. galloi* Zucchi (Consoli and Parra, 1995a&b), *T. minutum* Riley (Yu *et al.*, 1984) and *T. maidis* Bezdenko, *T. evanescens* Westwood, *T. dendrolimi* Matsumura, *T. pintoi* Voegele and *T. ostriniae* Pang & Chen (Pavlik, 1990) have been studied. Forsse *et al.* (1992) observed that lower temperatures resulted in lower levels of parasitism under

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laboratory conditions. This has also been verified in field studies (Parker *et al.*, 1971). Chen and Chiu (1986) reported that dispersal of *T. chilonis* Ishii increased with increasing temperature and decreased at relative humidity above 92% and below 33%.

Several researchers have studied the threshold temperatures of different *Trichogramma* species (Harrison *et al.*, 1985; Consoli and Parra, 1995a). Physiological time in degree-days was used to plot the frequency distribution of developmental time (maturation time) (Roux and Baumgärtner, 1995; Jones *et al.*, 1997). Temperature is also critical in mass rearing (Hassan, 1993) and release of *Trichogramma* parasitoids (Smith, 1994).

Trichogramma evanescens was introduced to the Philippines for controlling the Asian corn borer, *Ostrinia furnacalis* Gueneé, with apparently good results (Felkl *et al.*, 1990). Hence, it appears to be a suitable candidate species for warmer climates as well. *Trichogramma chilonis* is a common egg parasitoid of *Helicoverpa armigera* Hübner in Asia (Romeis and Shanower, 1996), but it has been also found on *Plutella xylostella* Linnaeus less frequently (Liu *et al.*, 2000). Furthermore, this species was also recovered in South Africa from eggs of *Eldana saccharina* Walker (Conlong and Hastings, 1984), and from eggs of *Busseola fusca* Fuller and *Chilo partellus* Swinhoe (Kfir, 1991), indicating that it might be indigenous to Africa too.

In Kenya, Ochiel (1989) studied the biology of a native species, *T.* sp. nr. *exiguum* Pinto & Platner on the stem borer *C. partellus* with particular reference to optimum and threshold temperatures for growth and development

as well as the indirect effects of its different lepidopteran hosts. Research towards the understanding of the natural occurrence of trichogrammatid egg parasitoids has been given attention recently in Kenya (Van den Berg, 1993).

The primary objective of this study was to evaluate the response of two native *Trichogramma* species, *T. bournieri* Pintureau & Babault and *T.* sp. nr. *mwanzai* Schulten & Feijen, to different temperature regimes (≤34°C), in order to assess their adaptability to climatic conditions as a selection criterion for inundative biological control in Kenya. A secondary objective was to study the biology of *T. bournieri*, which has not yet been addressed so far. The results are relevant for candidate selection, for mass rearing and future field release programs for biocontrol of *H. armigera* and *P. xylostella* in Kenya.

4.2 MATERIALS AND METHODS

4.2.1 Experimental set-up

Trichogramma bournieri, collected from Mbita at the eastern shores of lake Victoria and *T.* sp. nr. *mwanzai,* collected from Muhaka at the coastal Kenya, from eggs of *Chilo partellus* Swinhoe were used in this study. For comparison, a Palaearctic and an Australasian species, *T. evanescens* and *T. chilonis* originally collected from Germany and China, respectively, were also used. The study was conducted at the Federal Biological Research Centre for Agriculture and Forestry (BBA), Institute for Biological Pest Control, Darmstadt, Germany. All the species used in this study were arrhenotokous (diploid females from fertilized eggs) (Pinto and Stouthamer, 1994). Species identification was re-confirmed following the terminology of Pinto (1999).

Each species of *Trichogramma* was reared in glass vials (75 mm long x 25 mm in diameter) and fed with a mixture of honey (66%), gelatine (1%) and distilled water (33%) and kept in an incubator at 26 ± 1°C and 70 ± 10% relative humidity. Cotton plugs were used as stopper for the vials. Eggs of the Angoumois grain moth, *Sitotroga cerealella* Oliver (Lepidoptera: Gelechidae) were used as hosts for the parasitoids throughout the study. Individual climatic cabinets were maintained at constant temperatures of 13, 18, 25, and 34°C with 70 ± 10% relative humidity and a photoperiod of 16L: 8D. The higher temperature was restricted to 34°C because this was considered to be the maximum temperature under which those parasitoids are being reared and released. To evaluate the mass production capacity 25°C was

used (Hassan, 1993). The lower temperature (13°C) was based on the lowest temperature thresholds for development reported for other species ((Harrison *et al.*, 1985; Consoli and Parra, 1995a). For all temperature levels, the optimum relative humidity (70%) required for mass rearing of *Trichogramma* was used (Hassan, 1993).

In each climatic cabinet sixty 12-24 hours old mated-females of each species were kept. The host eggs were glued with Traganth (Merk, Darmstadt, Germany) on a sheet of paper ($80g/m^2$). Antennal characters were used to distinguish female *Trichogramma* from the male (Pinto *et al.*, 1978). Each adult female was kept in a small glass vial (50 mm long x 12 mm in diameter) with the host eggs (ca. 150-175 eggs per card) and fed for four hours at 26 ± 1°C and 70±10% RH. After four hours of exposure, the females were removed and the parasitised eggs were held simultaneously in individual vials in their respective climatic cabinets.

According to Hassan (1990), pupae of *Trichogramma* (confirmed by the blackening of the egg chorion of the host) were counted within the host eggs beginning from the fourth day after parasitism. Pupae were counted using a binocular microscope (magnification 16x). Since the females used for parasitisation of the test sets came directly out of the rearing chambers at 26°C the term preimaginal development was used instead of fertility.

Developmental time (egg-pupa, pupa-adult and egg-adult) in days, percentage of adult emergence, progeny production (number of parasitoids produced per host egg), and sex ratio were recorded for each species at each

temperature regime. The inverse of the developmental time in days is the developmental rate (Roux and Baumgärtner, 1995). The mean egg to adult developmental rate ((developmental time)$^{-1}$) was plotted against temperature (≤34°C) and a linear regression was fitted.

The lower temperature threshold for development was estimated by the intercept and average number of degree-days required to complete a stage was obtained as the inverse of the slope (Jones *et al.*, 1997). The linear temperature threshold is the zero intercept of the regression line on the x-axis (Wermelinger and Seifert, 1999). Degree-days were used to plot the frequency distribution of developmental time (maturation time) (Roux and Baumgärtner, 1995). Here, the cumulative adult emergence patterns were regressed for comparison purposes.

The preimaginal survivorship was calculated by dividing the number of live individuals until adulthood by the number of eggs laid by the parent females (Maia *et al.*, 2000). Percent emergence of parasitoids was based upon the number of parasitised black host eggs and calculated according to the methods recommended by Van Driesche (1983). Sex ratio was calculated as the proportion of females in the offspring.

4.2.2 Data analysis

Preimaginal development and progeny production data were transformed to $\log_{10}(x+1)$ while all percent data (emergence and proportion of females) were arcsine-transformed before being subjected to analysis of

variance (ANOVA) using the General Linear Models (GLM) procedure (SAS Institute, 1996). The Student Newman Keuls' (SNK) procedure was used to separate the means when necessary. To calculate the lower threshold temperatures for development and duration in degree-days, a linear regression model was evaluated (SAS Institute, 1996).

4.3 RESULTS

4.3.1 Preimaginal development

The highest preimaginal development (number of pupae within the host eggs) per female (Mean ± SE) of 50.37 ± 2.32 was recorded for *T. evanescens* at 25°C (Table 4.1). Preimaginal development decreased as the temperature increased from 25 to 34°C, particularly in *T. evanescens* and *T. bournieri*. Significant differences in preimaginal development were detected between species at the different temperature regimes: 13°C (F=100.25; df=3,236; P=0.0001), 18°C (F=5.14; df=3,236; P=0.0019), 25°C (F=20.49; df=3,236; P=0.0001), and 34°C (F=4.77; df=3,236; P=0.0030). Preimaginal development was not consistent among the species over the different temperatures (F=25.35; df=9,944; P=0.0001).

Trichogramma evanescens showed the greatest preimaginal development at 13°C (18.12 ± 1.34) and 25°C (50.37 ± 2.32), *T. bournieri* at 18°C (29.73 ± 1.31) and *T.* sp. nr. *mwanzai* at 34°C (19.47 ± 1.46). The level of preimaginal development observed was nearly similar in *T.* sp. nr. *mwanzai* at 18, 25 and 34°C. There was no development of *T. bournieri* and, thus, no offspring at the lowest temperature (13°C).

4.3.2 Developmental time

Trichogramma chilonis and *T. evanescens* completed development at all temperatures tested, but *T. bournieri* and *T.* sp. nr. *mwanzai* failed to complete development at the lowest temperature, 13°C (Table 4.2). The

developmental period for all species decreased as temperature increased. The duration of development from oviposition to adult emergence was about 11-12 weeks shorter at 34°C than at 13°C for *T. chilonis* and *T. evanescens*. There was no significant difference in developmental time during pupa-adult period at 25°C among the four species (F=1.26; df=3,203; P=0.2895). The developmental time of *T.* sp. nr. *mwanzai* was significantly shorter than *T. evanescens* at 18, 25 and 34°C; at 25 and 34°C, it was significantly shorter than the other native species *T. bournieri* and at 25°C (Table 4.2).

The applicability of the linear regression model to describe developmental rates (≤34°C) was tested at P=0.05 (Fig. 4.1). Although the effects of temperature on developmental time over the complete temperature span from lower developmental zero point to upper developmental zero point is represented by non-linear functions, it was accepted as a useful predictive model for egg to adult developmental time of *T. chilonis* and *T. evanescens* within the temperature range tested. However, it was not linear for *T. bournieri* and *T.* sp. nr. *mwanzai* when tested at P=0.05 or P=0.10. The calculated linear temperature thresholds for development and degree-days were 8.8°C and 188 for *T. chilonis* and 9.2°C and 192 for *T. evanescens*, respectively. Adult emergence patterns of the two species were plotted against physiological time units of degree-days (Fig. 4.2).

4.3.3 Survival and progeny production

For all temperatures tested, significant differences were detected in preimaginal survivorship among the species (F=42.55; df=3,682; P=0.0001) but was not consistent among the species (F=57.67; df=8,682; P=0.0001 (Fig.4.3). The percent emergence of the species was significantly different at all temperature regimes: 13°C (F=29.99; df=2,106; P=0.0001), 18°C (F=7.83; df=3,200; P=0.0001), 25°C (F=32.28; df=3,203; P=0.0001), and 34°C (F=99.96; df=3,173; P=0.0001) (Table 4.3).

Adult emergence tended to be lower at 13°C and 34°C, but higher at 18 and 25°C (Table 4.3). Compared to the other three species, *T.* sp. nr. *mwanzai* showed the highest percent emergence of 94 and 95 at 18°C and 25°C, respectively. At the highest temperature (34°C), the percent parasitoid emergence dropped drastically for *T. evanescens* (41) and *T. bournieri* (43). However, *T. chilonis* (92%) and *T.* sp. nr. *mwanzai* (89%) had higher emergence.

No progeny was produced by *T. bournieri* and *T.* sp. nr. *mwanzai* at 13°C. At 34°C, *T.* sp. nr. *mwanzai* produced significantly more progeny (17.48 ± 1.39) than the others (Table 4.4). The highest mean progeny production per female (44.03 ± 2.02) was recorded at 25°C for *T. evanescens*. At 18°C, *T. bournieri* recorded significantly more progeny than the other three species (Table 4.4). There were significant differences in progeny production capacity among the species at all the temperatures tested- 13°C (F=132.18;

df=3,236; P=0.0001), 18°C (F=4.33; df=3,236; P=0.0054), 25°C (F=21.32; df=3,236; P=0.0001), and 34°C (F=11.21; df=3,236; P=0.0001).

4.3.4 Sex ratio

The proportion of females was higher at all temperatures in *T. bournieri* and *T. chilonis*. On the contrary, the proportion of males was higher than females in *T.* sp. nr. *mwanzai* regardless of the temperature (Fig. 4.4). There was no significant difference between the sex ratios of *T. chilonis* and *T. evanescens* at 13°C (F=1.39; df=1,68; P=0.2420). However, significant differences in female proportion existed among the four species at 18°C (F=23.73; df=3,199; P<0.01), 25°C (F=16.07; df=3,203; P<0.01), and 34°C (F=14.09; df=3,160; P<0.01). There was significant difference in the proportion of the females of *T. bournieri* at the different temperature regimes (F=4.51; df=2,150; P<0.01). On the other hand, there was no significant difference in the proportion of females of *T.* sp. nr. *mwanzai* at the different temperature regimes (F= 0.25; df=2,146; P>0.78).

4.4 DISCUSSION

The egg parasitoid *Trichogramma* species tested showed differences in preimaginal development, developmental time, percent emergence, progeny production and sex ratio at the four temperature regimes. There was no development of *T. bournieri* and *T.* sp. nr. *mwanzai* at the lowest temperature of 13°C. Similarly, no development was observed in *T. pretiosum* and *T. exiguum* at 10°C (Harrison *et al.*, 1985).

Trichogramma sp. nr. *mwanzai* appeared to be superior at higher temperature in terms of fertility and progeny production as well as in the overall preimaginal survivorship. The results of the present study indicate that parasitism between 18 and 25°C was higher for all species tested. This observation is also in agreement with previous studies on *T. galloi* (Consoli and Parra, 1995b).

At 25°C, the mean fertility per female of *T. evanescens* was higher while the other three species were lower compared to the level reported for *T. galloi* (Consoli and Parra, 1995b). Pavlik (1990) studied the effect of three different temperatures on five *Trichogramma* species and observed significant differences in parasitisation capacity. This variation was attributed to genetic differences between species (Pavlik, 1990).

For all temperatures tested (≤34°C), linear regression model was appropriate for egg-adult development of *T. chilonis* and *T. evanescens*. However, linear model did not satisfactorily explain egg-pupa and pupa-adult development in all the species tested. Drost *et al.* (1998) has reviewed the

different life-history parameters in relation to temperature. As the temperature increased, the duration of development was found to decrease as in other *Trichogramma* species (Harrison *et al.*, 1985).

The lower threshold temperature for development has been reported to be between 10 and 15°C for *T. pretiosum* and *T. exiguum* (Harrison *et al.*, 1985) and 13.6°C for *T. galloi* (Consoli and Parra, 1995a). The lower threshold temperatures of *T. chilonis* (8.8°C) and *T. evanescens* (9.2°C) are apparently lower than those reported earlier in the literature (Zhang, 1984; Hirashima *et al.*, 1990).

Yu *et al.* (1984) studied the developmental time of *T. minutum* at seven temperatures and reported shorter developmental time than observed in the current study. Consoli and Parra (1995a) on *T. galloi* and Cabello and Vargas (1988) on *T. cordubensis* Vargas & Cabello reported that the sex ratio was unchanged at higher temperatures presumably because the higher temperature tested is below the upper threshold that affects female offspring production. The unchanged sex ratio within the same species (*T.* sp. nr. *mwanzai*) at different temperature regimes could be explained on the same basis. This study is consistent with this report.

Ochiel (1989) observed significant effect of temperature on progeny production of *T.* sp. nr. *exiguum* when tested at 18°C and 30°C. However, this author reported no significant difference in the proportion of the female offspring at all temperatures tested. Both observations agree with the findings of the current studies and the latter particularly confirms the trend observed in

T. sp. nr. *mwanzai*. Temperature was found to affect the female: male ratio, with females being slightly less abundant in *T.* sp. nr. *mwanzai*. This trend has also been reported in *T. pretiosum* and *T. exiguum* when retained at 15°C and 35°C (Harrison *et al.*, 1985). The higher proportion of females observed in *T. bournieri, T. chilonis* and *T. evanescens* is in agreement with the observations reported for *Trichogramma carverae* Oatman and Pinto (Scott *et al.*, 1997).

The present study was restricted to temperature ≤34°C because this was considered to be the maximum temperature under which those parasitoids are being reared and released. If a wider temperature range becomes necessary, additional studies above 34°C are likely to require a non-linear model. The narrow period of adult emergence is likely to facilitate the planning of release (Fig. 4.2). Nevertheless, most *T. chilonis* appear to emerge over a narrow interval than *T. evanescens*. Hence, optimum release strategies may be easier to design for *T. chilonis*. A wider interval would have required detailed calculations on the time of releases. This is because early release would expose the parasitised eggs to predation while late release could cause loss of individuals by early emergence.

Information on responses to different temperature regimes, together with knowledge on host preference and searching capacity as well as mass production potential in general, is useful in the selection of appropriate *Trichogramma* species for biocontrol of the target pests. The results of the present study indicate that developments between 18°C and 25°C are favourable for all species tested. The African species, *T.* sp. nr. *mwanzai*

showed tolerance to high temperature (34°C) and therefore, could be a preferable candidate species for biocontrol programmes in warmer climates.

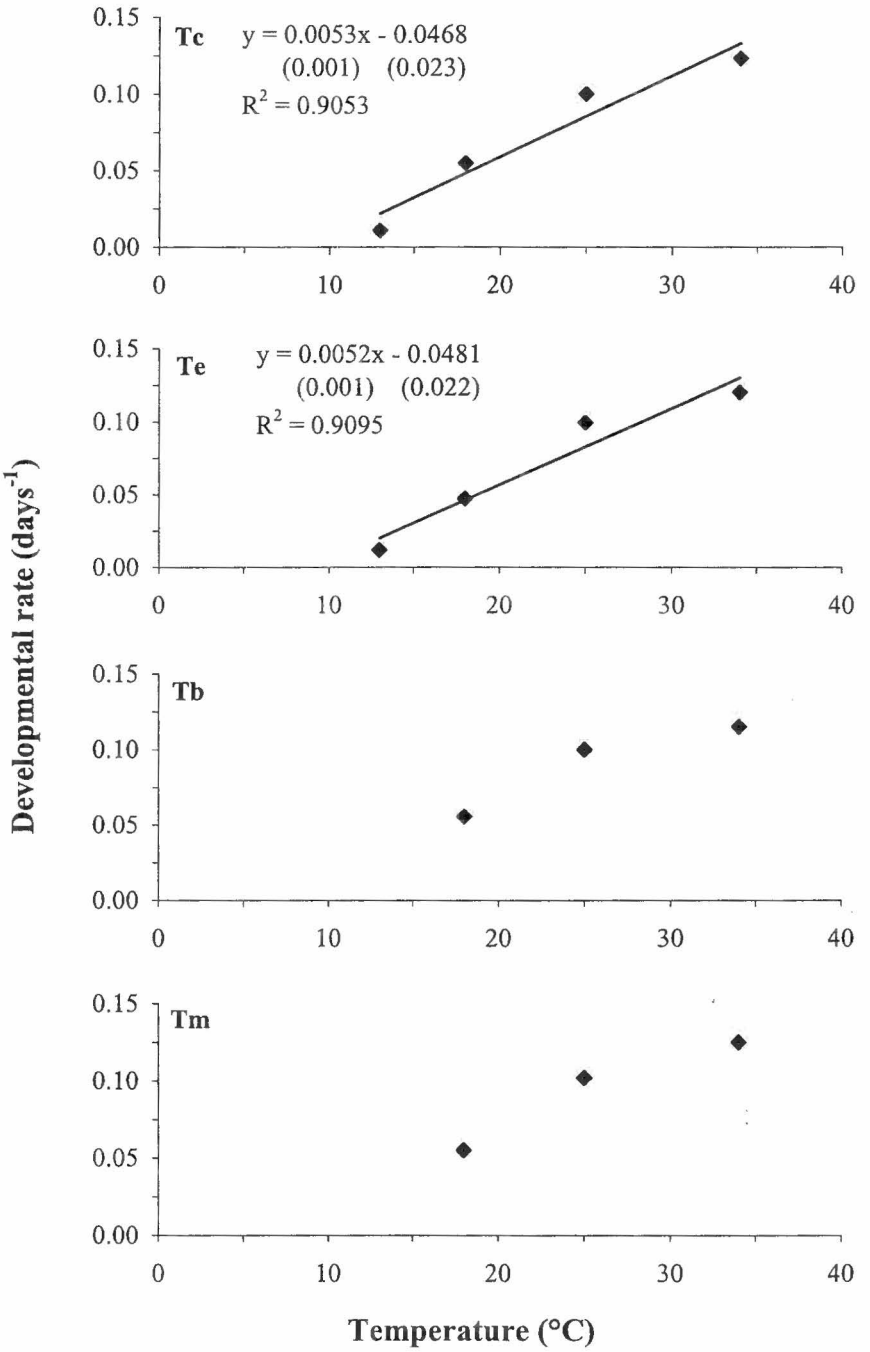

Figure 4.1 Egg to adult developmental rate of *Trichogramma chilonis* (Tc), *T. evanescens* (Te), *T. bournieri* (Tb) and *T.* sp. nr. *mwanzai* (Tm) at four constant temperatures.
Numbers in parenthesis are the standard errors.

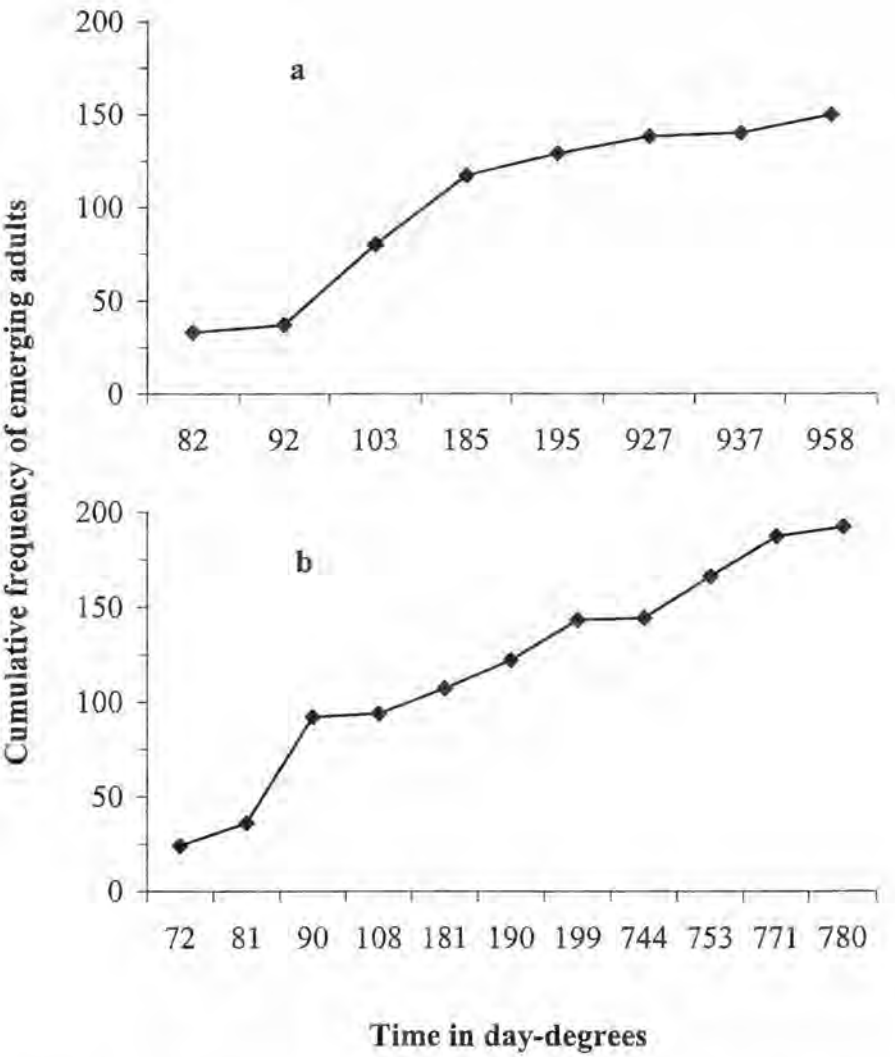

Figure 4.2 Successful adult emergence of *Trichogramma chilonis* (a) and *T. evanescens* (b) displayed on a physiological time basis.

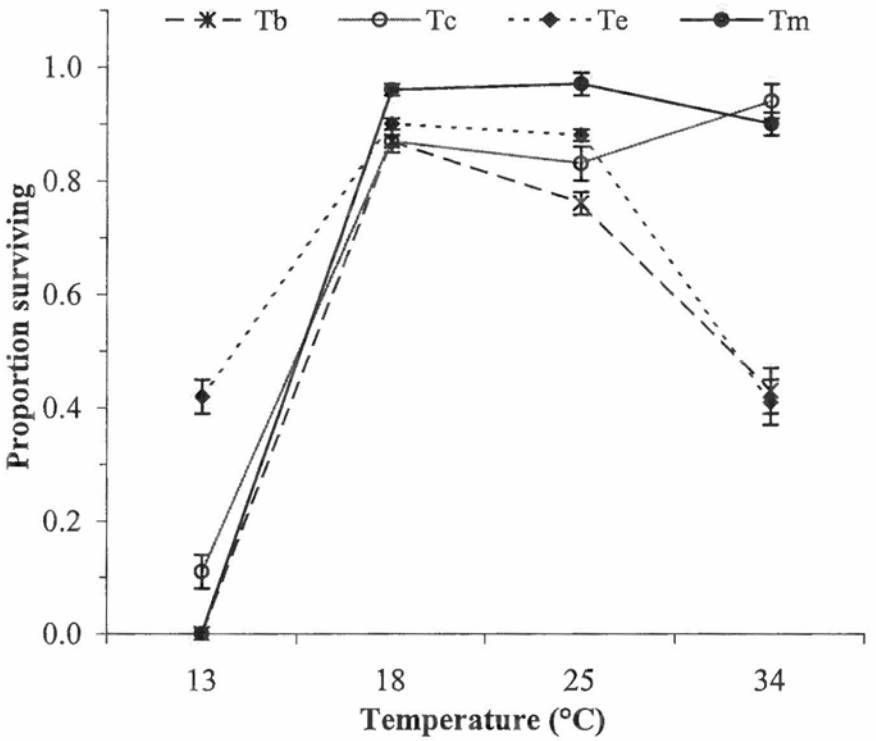

Figure 4.3 Preimaginal survivorship of *T. bournieri* (Tb), *T. chilonis* (Tc), *T. evanescens* (Te) and *T.* sp. nr. *mwanzai* (Tm) at four constant temperature regimes.

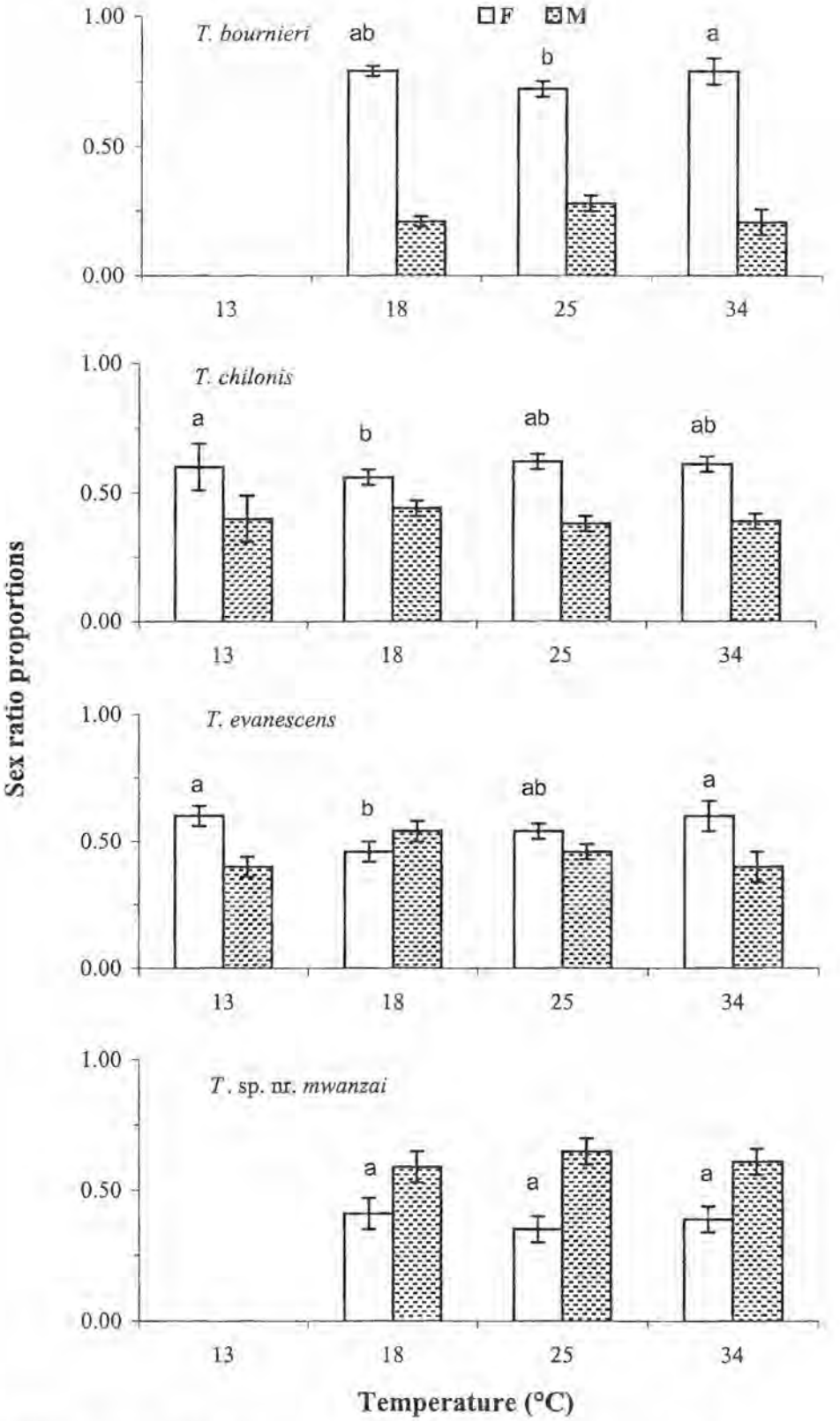

Figure 4.4 Sex ratio of *T. bournieri*, *T. chilonis*, *T. evanescens* and *T.* sp. nr. *mwanzai* at four constant temperature regimes. Means followed by the same letter are not significantly different (P>0.05), SNK.

Table 4.1 Preimaginal development of *Trichogramma bournieri*, *T.* sp. nr. *mwanzai*, *T. chilonis* and *T. evanescens* at four constant temperatures

Trichogramma species	Number of pupae within the host eggs (Mean ± SE) (n = 60)			
	13°C	18°C	25°C	34°C
T. bournieri	ND	29.73 ± 1.31a	25.63 ± 1.75b	5.37 ± 1.83b
T. sp. nr. *mwanzai*	1.13 ± 0.34c	20.08 ± 1.75b	19.82 ± 1.50b	19.47 ± 1.46a
T. chilonis	9.42 ± 1.06b	20.20 ± 1.68b	16.10 ± 1.70c	11.08 ± 1.38b
T. evanescens	18.12 ± 1.34a	21.97 ± 1.86b	50.37 ± 2.32a	12.82 ± 1.53b

- Means followed by the same letter in the same column are not significantly different (P>0.05), Student Newman Keuls (SNK) test.
- Analysis of Variance (ANOVA) performed on $\log_{10}$ transformed preimaginal development. Values presented in Table 4.1 are the original data.
- ND = No development.

Table 4.2 Developmental time of four *Trichogramma* species at four constant temperature regimes

Temperature	*Trichogramma* species	Developmental time in days (Mean ± SE)		
		Egg to Pupa	Pupa to Adult	Egg to Adult
13°C	*T. bournieri*	ND	ND	ND
	T. sp. nr. *mwanzai*	39.17 ± 0.75a (n=52)	ND	ND
	T. chilonis	24.02 ± 0.15b (n=42)	67.62 ± 0.20a (n=21)	91.52 ± 0.32a (n=21)
	T. evanescens	23.00c (n=52)	61.14 ± 0.17b (n=49)	84.14 ± 0.17b (n=49)
18°C	*T. bournieri*	7.00c (n=58)	11.00b (n=58)	18.00c (n=58)
	T. sp. nr. *mwanzai*	7.26 ± 0.10b (n=47)	10.92 ± 0.04b (n=47)	18.17 ± 0.07bc (n=47)
	T. chilonis	7.42 ± 0.10b (n=50)	10.82 ± 0.07b (n=49)	18.24 ± 0.06b (n=49)
	T. evanescens	7.88 ± 0.11a (n=49)	13.29 ± 0.17a (n=49)	21.16 ± 0.12a (n=49)
25°C	*T. bournieri*	5.00a (n=56)	5.00a (n=56)	10.00a (n=56)
	T. sp. nr. *mwanzai*	4.78 ± 0.06b (n=50)	5.04 ± 0.03a (n=50)	9.82 ± 0.06b (n=50)
	T. chilonis	5.00a (n=43)	5.00a (n=43)	10.00a (n=43)
	T. evanescens	5.03 ± 0.02a (n=58)	5.03 ± 0.02a (n=58)	10.07 ± 0.05a (n=58)
34°C	*T. bournieri*	5.00a (n=46)	3.69 ± 0.07c (n=39)	8.69 ± 0.07a (n=39)
	T. sp. nr. *mwanzai*	4.00c (n=52)	4.02 ± 0.02b (n=52)	8.02 ± 0.02c (n=52)
	T. chilonis	4.97 ± 0.03a (n=38)	3.14 ± 0.06d (n=37)	8.11 ± 0.05c (n=37)
	T. evanescens	4.12 ± 0.05b (n=41)	4.28 ± 0.09a (n=36)	8.33 ± 0.08b (n=36)

- Means followed by the same letter in the same column are not significantly different (P>0.05), Student Newman Keuls (SNK) test.
- ND = No development.

Table 4.3 Adult emergences (%) of four *Trichogramma* species at four constant temperature regimes

Trichogramma species	Percent adult emergence at four constant temperature regimes (Mean ± SE)			
	13°C	18°C	25°C	34°C
T. bournieri	ND	86.60 ± 1.45b (n=58)	76.50 ± 1.62c (n=56)	43.15 ± 3.57c (n=46)
T. sp. nr. *mwanzai*	0.00 (n=15)	93.89 ± 1.01a (n=47)	94.72 ± 1.52a (n=50)	88.58 ± 1.69b (n=52)
T. chilonis	10.95 ± 2.78b (n=42)	86.62 ± 2.06b (n=50)	81.23 ± 2.44b (n=43)	91.74 ± 2.86a (n=38)
T. evanescens	41.98 ± 3.03a (n=52)	89.96 ± 1.15b (n=49)	88.00 ± 0.99b (n=58)	40.98 ± 4.03c (n=41)

- Means followed by the same letter in the same column are not significantly different (P>0.05), Student Newman Keuls (SNK) test.
- Analysis of Variance (ANOVA) performed on arcsine transformed percentage emergence. Values presented in Table 4.3 are the original data.
- ND = No development.

Table 4.4 Progeny production of four *Trichogramma* species at four constant temperature regimes

Trichogramma species	Progeny production at four constant temperatures (Mean ± SE) (n = 60)			
	13°C	18°C	25°C	34°C
T. bournieri	ND	25.83 ± 1.23a	20.08 ± 1.49b	7.35 ± 1.04b
T. sp. nr. *mwanzai*	0.0	19.23 ± 1.70b	19.25 ± 1.50b	17.48 ± 1.39a
T. chilonis	0.90 ± 0.20b	17.73 ± 1.47b	13.30 ± 1.48c	10.60 ± 1.34b
T. evanescens	7.48 ± 0.73a	20.15 ± 1.78b	44.03 ± 2.02a	5.78 ± 0.87b

- Means followed by the same letter in the same column are not significantly different (P>0.05), Student Newman Keuls (SNK) test.
- Analysis of Variance (ANOVA) performed on $\log_{10}$ transformed progeny production. Values presented in Table 4.4 are the original data.
- ND = No development.

CHAPTER 5

5.0 LIFE TABLE STUDY OF *TRICHOGRAMMA BOURNIERI* AND *TRICHOGRAMMA* SP. NR. *MWANZAI*

5.1 INTRODUCTION

Trichogramma egg parasitoids (Hymenoptera: Trichogrammatidae) are important biocontrol agents that have successfully been utilized against several lepidopteran pests, especially through inundative releases (Wajnberg and Hassan, 1994). According to Smith (1996), the major principles to be considered in the design of an augmentative biological control programme are selection of the parasitoids, production systems for mass rearing, distribution of the parasitoids and the release as well as impact assessment strategies to be applied in the field.

The choice of the strain should be made on the basis of the attributes that are considered to be adaptable to the ecosystem in which they are released and to the type of release to be made. Life table analysis helps in understanding the dynamics of a population (Southwood, 1978). The basic population parameters like the intrinsic rate of natural increase (r_m), the net reproductive rate (R_o) and the finite population growth rates (λ) are useful in selection of promising species (Southwood, 1978).

To be published as: Abera T. Haile, S. A. Hassan, S. Sithanantham, C.K.P.O. Ogol and J. Baumgärtner. 2001. Comparative life table analysis of *Trichogramma bournieri* Pintureau & Babault and *Trichogramma* sp. nr. *mwanzai* Schulten & Feijen (Hymenoptera: Trichogrammatidae) from Kenya. *Journal of Applied Entomology* (In press).

According to Southwood (1978) and Dent and Walton (1997), there are two types of life tables: the age-specific (cohort, horizontal) and the time-specific (static, vertical). Age-specific life tables are commonly used in entomology than time-specific life tables (Southwood, 1978; Dent and Walton, 1997).

The intrinsic rate of natural increase was used as a criterion for differentiating species of *T. pretiosum* Riley and *T. retorridum* Girault (Orphanides and Gonzalez, 1971). Hassan and Guo (1991) conducted extensive experiments and selected *T. evanescens* Westwood for the control of the European corn borer, *Ostrinia nubilalis* Hübner on the basis of its high fertility. Adult longevity has been also used for comparing *T. chilonis* Ishii and *T. ostriniae* Pang & Chen (Hirashima *et al.*, 1990).

In Kenya, Ochiel (1989) and Lu (1992) have studied the life table parameters of *T.* sp. nr. *exiguum* Pinto & Platner and *T.* sp. nr. *mwanzai* Schulten & Feijen, respectively. Life table studies and evaluation of fitness parameters of species of egg parasitoids that show potential for parasitising the target pests are also useful. However, these parameters have to be defined and should be able to be assessed in the laboratory.

To promote the utilisation of native trichogrammatid species, surveys have recently been undertaken in Kenya (Abera *et al.*, 2000a&b). These include *T. bournieri* Pintureau & Babault and *T.* sp. nr. *mwanzai*, which was earlier reported from Comoros islands and Kenya, respectively (Pintureau and Babault, 1988; Ngi-Song, 1990). This study focussed on inundative releases

and hence, selection of strains with high parasitisation potential. However, the selected strains are expected to perform well in mass rearing programmes (Hassan, 1993). The purpose of this work was to compare the parasitisation potential and the population parameters of the two native *Trichogramma* species, *T. bournieri* and *T.* sp. nr. *mwanzai*.

5.2 MATERIALS AND METHODS

5.2.1 Experimental set-up

Trichogramma sp. nr. *mwanzai* was collected from eggs of *Chilo partellus* Swinhoe on sorghum along the Kenya coast. *Trichogramma bournieri* was collected from eggs of *C. partellus* on maize from Mbita at the eastern shores of lake Victoria. This procedure resulted likely in the selection of strains. Two species used in this study were arrhenotokous (diploid females from fertilized eggs) (Pinto and Stouthamer, 1994). Species identification was re-confirmed following the terminology of Pinto (1999). Antennal characters were used to distinguish female *Trichogramma* from the male (Pinto *et al.*, 1978). The life table studies were conducted at the Federal Biological Research Centre for Agriculture and Forestry, Institute for Biological Pest Control (BBA), Darmstadt in Germany.

Age-specific life tables (Southwood, 1978; Bellows *et al.*, 1992) were constructed using a group of sixty mated 12-24 hours old females each of *T. bournieri* and *T.* sp. nr. *mwanzai*. After mating, each female was kept in a single glass vial (50 mm long x 12 mm in diameter) and fed every day with a mixture of honey (66%), gelatine (1%) and distilled water (33%). Fresh eggs (ca. 150-175) of the Angoumois grain moth, *Sitotroga cerealella* Oliver were offered to each individual female every day until the female died. Parasitised host egg batches were removed daily and incubated at 26 ± 1°C, 70 ± 10% relative humidity and 16L: 8D photoperiod.

Pupae of *Trichogramma* were counted within the host eggs five days after parasitism (Hassan, 1990). The following records were also taken daily at each age interval of the parent: (a) the number of surviving adult females (l_x), (b) the number of females in the reproductive period (l_xr) and (c) fertility rate (m_x) (Dent and Walton, 1997).

5.2.2 Data analysis

The parasitisation potential of the two native species was assessed by comparing the fertility (cumulative and daily), progeny production, oviposition period including the proportion of days spent for oviposition and adult longevity. Due to the absence of superparasitism, the number of parasitised host eggs was used as a measurement of fertility. Cumulative fertility is the total number of eggs produced by females over their lifetime. The difference between the cumulative fertility and the progeny production is used to calculate the intrinsic mortality and survival rate of the immature life stages. Data were subjected to analysis of variance (ANOVA) using the General Linear Models (GLM) procedure (SAS Institute, 1988). Tukey test (P=0.05) was used to separate the means when necessary. Mean fertility and progeny production data was log transformed, and arcsine transformed data for proportion of days spent for egg laying was used for the ANOVA (Sokal and Rohlf, 1981).

The age-specific life table studies involved repeated counting of individuals in a single cohort over time. The span between two consecutive daily periods was represented by its midpoint, that is, the female age plus 0.5, the so-called pivotal age (x). The preimaginal survivorship was calculated by dividing the number of live individuals until adulthood by the number of eggs laid by the cohort. To represent the development of a cohort, data were arranged according to Roux and Baumgärtner (1995) and the following population growth statistics (Birch, 1948; Southwood, 1978; Krebs, 1985) were computed. Population parameter estimates for the two species were compared using the method of Maia *et al.* (2000).

Accordingly, the dynamics of a closed population subjected to constant age-specific schedules of fertility and mortality for a long period can be represented by

$$N_t = N_0 \; e^{r_m t}$$

Where, N_0 is the number of insects at time zero; N_t is the number of insects at time t; r_m is the intrinsic rate of natural increase, which can be approximated

$$\sum e^{-r_m x} \, l_x m_x = 1$$

In this system the number of times the population will multiply itself per unit time, is the finite rate of increase (λ), which can be obtained from

$$\lambda - e^{r_m} = \text{anti} \;\; \log \;\; e^{r_m} = \frac{N_{t+1}}{N_t}$$

The net reproductive rate (R_o) is the mean net contribution per female to the next generation, expressed in total of offspring females per female, during the entire oviposition period. In other words, it is the probability at birth of being alive at age x is l_x while the mean number of female offspring produced in a unit of time by a female aged x is m_x; the product $l_x m_x$ is obtained for each age group and the sum of these products is the net reproductive rate defined by

$$R_o = \sum l_x m_x$$

Doubling Time (Dt): the time required for a population to double its numbers can be obtained from

$$Dt = \frac{Ln(2)}{r_m}$$

Generation Time (T): the mean length of a generation can be calculated from

$$T = \frac{\log_e R_o}{r_m}$$

To associate variation in the main parameters (r_m and R_o), the Jackknife method (Meyer *et al.*, 1986; Wermelinger *et al.*, 1991) was used. The standard error and confidence interval by using pseudo-values rj of r_m and successively dropping one individual j of the original data set of size n. The pseudo-values rj, which are assumed to be normally distributed were then arranged for calculating the "Jackknife Estimate of r_m". The sex ratio is considered and the standard error and the confidence interval of the mean were calculated (Hulting *et al.*, 1990).

5.3 RESULTS

Table 5.1 shows the parasitisation potential of *T. bournieri* and *T.* sp. nr. *mwanzai*. The difference in female longevity (F=1.66; df=1,118; P=0.2002) and proportion of days spent for egg laying (F=2.61; df=1,117; P=0.1092) was not significant while the oviposition period (F=5.15; df=1,117; P=0.0251) was significant between the two species, *T. bournieri* and *T.* sp. nr. *mwanzai* (Table 5.1).

The difference between the cumulative mean fertility (F=11.00; df=1,118; P=0.0012) and daily mean fertility rate (F=12.51; df=1,117; P=0.0006) as well as the progeny production rate (F=10.86; df=1,118; P=0.0013) was significantly different between the two species (Tables 5.1 and 5.2). *Trichogramma* sp. nr. *mwanzai* recorded 52% of the females in the progeny compared to 72% in *T. bournieri* (Table 5.2). Fig. 5.1 shows that the female adult parasitoids tended to produce greater proportion of males in the progeny as they grew older, and this pattern was conspicuous in *T.* sp. nr. *mwanzai* than in *T. bournieri*.

In the life table analysis, survival of the adult females (l_x), number of females in the reproductive period (l_xr) and mean fertility (m_x) for the two species is summarised in Fig. 5.2. There was no significant difference in the intrinsic rate of natural increase (t=0.034; df=59; P>0.05) and net reproductive rate (t=1.713; df=59; P>0.05) between the two species. Both species had the same finite rate of increase (λ=1.36). They have also similar doubling time 2.27 and 2.24 for *T. bournieri* and *T.* sp. nr. *mwanzai*, respectively (Table 5.2).

5.4 DISCUSSION

Differences in fertility and progeny production were observed between the two native species, *T. bournieri* and *T.* sp. nr. *mwanzai*. Longevity of *T. bournieri* and *T.* sp. nr. *mwanzai* (>6 days) was higher than *T.* sp. nr. *exiguum* (<4 days) recorded for similar temperature levels in Kenya (Ochiel, 1989). Hirashima *et al.* (1990) utilized adult longevity for comparing the potential of two *Trichogramma* species, and found *T. chilonis* to be slightly longer (5.0 days) compared to *T. ostriniae* (4.6 days). Hassan and Guo (1991) conducted extensive experiments for selection of the appropriate *Trichogramma* species for the control of European corn borer, *O. nubilalis*, in which *T. evanescens* and *T. ostriniae* were selected on the basis of their fertility.

Increased longevity should also be matched by greater fecundity, if the species are to be reckoned to be of higher quality, which is favourably reflected in the attributes of *T.* sp. nr. *mwanzai* in the present study. The greater fertility and progeny production ability observed in *Trichogramma* sp. nr. *mwanzai* would be more advantageous than the high proportion of females in *T. bournieri*.

Hirashima *et al.* (1990) also reported that most eggs were deposited on the first day after adult emergence and that the percentage of females in the offspring was highest for both *T. chilonis* and *T. ostriniae*. Another observation they made on the relationships between the adult female age and the ratio of females in the progeny produced compares well with the trend observed in the two trichogrammatid species in the present study. However,

the factor(s) involved in such a shift in the sex ratio of the progeny need further investigation.

The results of Orphanides and Gonzalez (1971) indicated in their study with *T. pretiosum* and *T. retorridum* that the mean fertility of the parental line can differ significantly from its clones and high variation may also occur among the clones. The factor should also be considered in further deployment of the species. Hassan (1993) indicates that the optimum rearing condition for mass production of *Trichogramma* is 27°C temperature and 70% relative humidity. The present study at 26 ± 1°C, 70 ± 10% relative humidity therefore helped to evaluate the suitability for mass production of the two-trichogrammatid species.

In Kenya, Oloo (1987) selected strains of unidentified *Trichogramma* species based on life table parameters. The net reproductive rate, intrinsic rate of natural increase and finite population growth rates were 24.06, 0.35 and 1.41 for the strain Ex-Rusinga, 49.43, 0.43 and 1.54 for Ex-Lambwe and 51.39, 0.44 and 1.55 for Ex-Mombasa, respectively. Based on the relative high biotic potential, Ex-Mombasa strain was selected for field tests on survival, dispersal capacity and parasitisation rate. Although the taxonomic status is unknown, the results showed that there is a potential for finding parasitoid strains with qualities superior to the one reported here.

The present study has also shown that the two native species are comparable in intrinsic and finite rate of increase and doubling time. In similar studies in Kenya by Lu (1992), the intrinsic rate of natural increase and

net reproductive rate at 25°C was 0.334 and 35.9, respectively for *T.* sp. nr. *mwanzai.* This also agrees with the results obtained in this current observation. While the present studies show finite rate of increase of 1.36 for the two species, studies by Ochiel (1989) on another locally occurring species in Kenya, *T.* sp. nr. *exiguum* showed a value of 1.33. These results suggest that the three native species have almost similar finite rates of increase.

The life table study shows that there is no difference between the two strains in their main population parameters (r_m and R_o), however they differ in their parasitisation potential. The result indicates that, at this stage, no preference should be given to one strain when selecting for mass rearing. *Trichogramma* sp. nr. *mwanzai* had the highest parasitisation potential; and therefore this attribute should be considered in future studies.

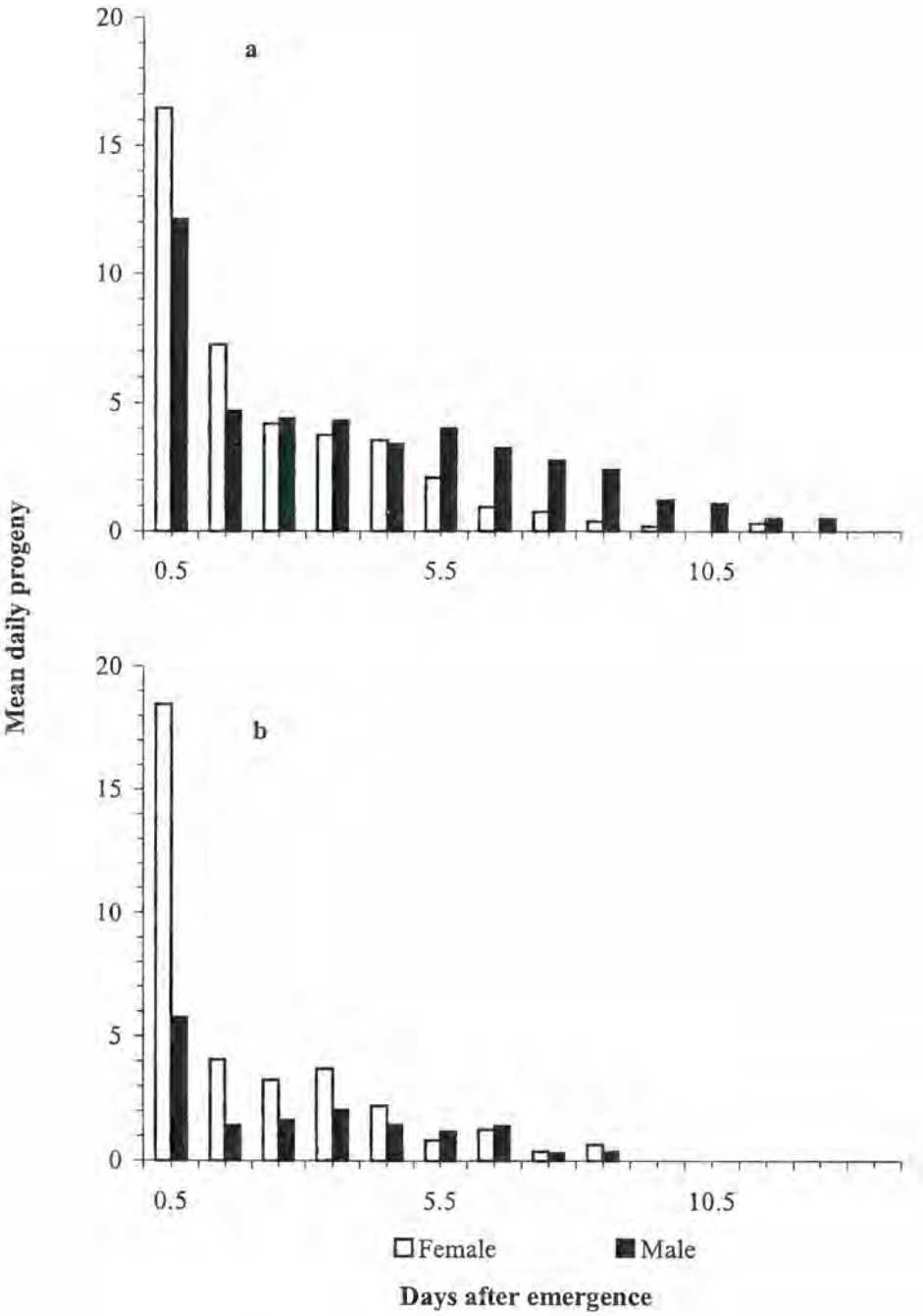

Figure 5.1 Daily progeny production of *Trichogramma* sp. nr. *mwanzai* (a) and *Trichogramma bournieri* (b) at 26±1°C, 70±10% RH and 16L: 8D photoperiod.

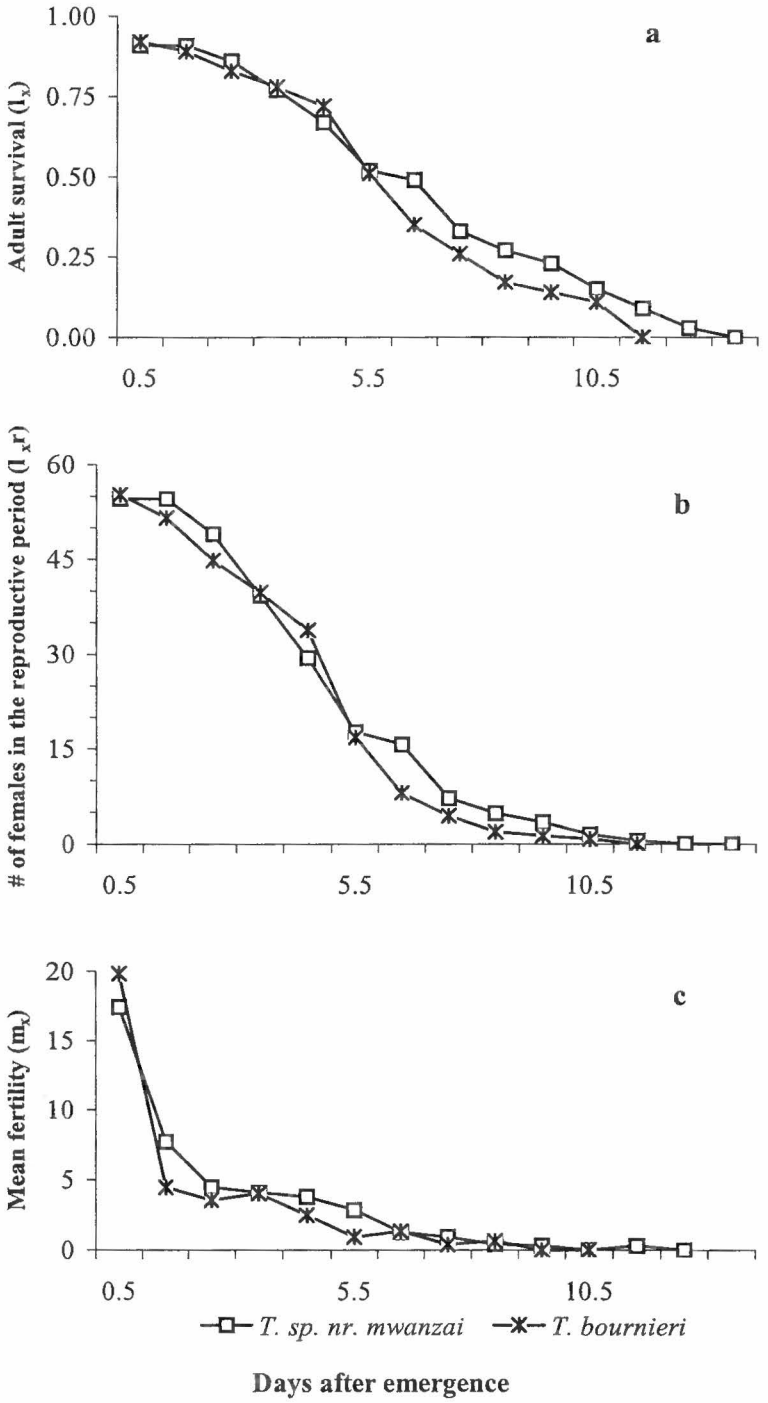

Figure 5.2 Adult survival (a), number of females in the reproductive period (b) and fertility rate (c) of *T.* sp. nr. *mwanzai* and *T. bournieri* at 26±1°C, 70±10% RH and 16L: 8D photoperiod.

Table 5.1 Adult female longevity, oviposition period and fertility of *Trichogramma bournieri* and *Trichogramma* sp. nr. *mwanzai* at 26 ±1°C, 70±10% RH and 16L:8D photoperiod (Mean ± SE)

Trichogramma species (n = 60)	Adult longevity (days)	Oviposition period (days)	Proportion of days spent for egg laying	Fertility (Parasitised host eggs/female)	
				Cumulative	Daily
Trichogramma sp. nr. *mwanzai*	6.85 ± 0.40a	5.37 ± 0.43a	0.75 ± 0.03a	75.97 ± 4.27a	18.11 ± 1.15a
Trichogramma bournieri	6.17 ± 0.35a	4.22 ± 0.28b	0.70 ± 0.03a	47.83 ± 2.23b	13.24 ± 0.76b

- Means followed by the same letter in the same column are not significantly different (P>0.05), Tukey test.
- Analysis of Variance (ANOVA) performed on log transformed cumulative mean fertility and arcsine transformed proportions. Values presented in Table 5.1 are the original data.

Table 5.2 Population growth statistics for *Trichogramma bournieri* and *Trichogramma* sp. nr. *mwanzai* at 26 ±1°C, 70±10% RH and 16L:8D photoperiod

Life Table Growth Statistics	Mean ± SE (n = 60)	
	T. sp. nr. *mwanzai*	*T. bournieri*
Main Population Parameters		
Intrinsic rate of natural increase (r_m)	0.309 ± 0.01a	0.306 ± 0.01a
Net Reproductive Rate (R_o)	35.16 ± 3.49a	31.22 ± 1.80a
Other Population Parameters		
Progeny Production	69.12 ± 3.79a	44.08 ± 2.05b
Mean Generation Time (T)	11.53	11.26
Doubling Time (Dt)	2.24	2.27
Sex Ratio (♀: ♂)	52: 48	72: 28
Preimaginal Survivorship	0.91	0.92
Finite rate of increase (λ)	1.36	1.36

- Means followed by the same letter in the same row are not significantly different (P>0.05), Tukey test.
- Analysis of Variance (ANOVA) performed on log transformed progeny production. Values presented in Table 5.2 are the original data.

CHAPTER 6

6.0 EFFECTS OF HOST INSECT AND HOST PLANT ON PARASITISM AND PROGENY PRODUCTION OF *TRICHOGRAMMA BOURNIERI* AND *T.* SP. NR. *MWANZAI*

6.1 INTRODUCTION

Host habitat location, host location, host acceptance, suitability of the host for the development of the parasitoid, and host regulation are the factors involved in successful parasitism (Vinson and Iwantsch, 1980; Waage and Greathead, 1986; Godfray, 1994; Vinson, 1998). The choice of species suitable for biocontrol should be made on the basis of the attributes that are considered to be advantageous to the ecology in which they are released and to the type of release to be made (Smith, 1996). High fecundity, emergence rate, host preference for the target species, host-searching ability, and tolerance to local conditions would be useful in the selection of suitable *Trichogramma* species for biological control (Hassan, 1990, 1994; Hassan and Guo, 1991; Smith, 1996).

According to Nordlund (1994), host-habitat location, host location and host acceptance in *Trichogramma* is mediated by a number of stimuli, particularly chemical stimuli. Host-habitat location is the important first step in the process and these minute insects exhibit preferences for certain habitat types. A variety of plants, including *Amaranthus*, maize and tomato produce chemical stimuli that mediate host-habitat location of *Trichogramma* (Nordlund, 1994).

Kaiser *et al.* (1989) reported that *T. evanescens* Westwood, *T. pretiosum* Riley and *T. maidis* (=*brassicae*) Bezdenko respond to components of the sex pheromone released by most female moths. Recognition of specific kairomones was also acquired by learning, through the association of the host and its kairomones during oviposition (Van Alphen and Vet, 1986). Bjorksten and Hoffmann (1998) also reported that host acceptance by *Trichogramma* has been influenced by adult learning through oviposition experiences and pre-adult learning through development inside a host. Younger eggs were preferred by trichogrammatid egg parasitoids where survival and reproductive success is highest (Strand, 1986).

The number of *Trichogramma* pupae developing within the host eggs showed preference for the parasitoid for laying eggs, whereas the progenies produced showed the suitability of the host (Hassan and Guo, 1991; Wührer and Hassan, 1993; Hassan, 1994). Several researchers have studied the role of host insects and host plants on parasitism and progeny production by different *Trichogramma* species in the laboratory under choice and no choice situations and field tests (Taylor and Stern, 1971; Curl and Burbutis, 1978; Torreno and Cadapan, 1984; Pak, 1988; Corrigan and Laing, 1994; Romeis *et al.,* 1997, 1998, 1999; Liu *et al.*, 1998; Monje *et al.*, 1999). However, few studies (Ochiel, 1989; Guang and Oloo, 1990) were conducted on native species of *Trichogramma* in Kenya.

The objective of this study was to determine the influence of three host insects and two host plants on the performance of the native *T. bournieri* Pintureau & Babault and *T.* sp. nr. *mwanzai* Schulten & Feijen as part of the criterion in the selection of suitable species for biocontrol of *H. armigera* on tomato in Kenya.

6.2 MATERIALS AND METHODS

6.2.1 Rearing of egg parasitoids

Trichogramma bournieri and *T.* sp. nr. *mwanzai* were collected from eggs of *Chilo partellus* Swinhoe on maize and sorghum plants at Mbita and Muhaka in Kenya, respectively (Abera *et al.*, 2000a). Eggs of *Corcyra cephalonica* Stainton and *C. partellus* were used as standard hosts for laboratory and field cage experiments, respectively. *Trichogramma bournieri* and *T.* sp. nr. *mwanzai* were first reared for 21 and 24 generations on *C. partellus*, respectively. Eggs of *C. partellus* were obtained from the Animal Rearing and Quarantine Unit of the International Centre of Insect Physiology and Ecology (ICIPE). The parasitoids were also later reared for 36 generations on *C. cephalonica* eggs in the laboratory using the methods of Singh and Jalali (1994). The rearing was conducted at ambient temperatures of 24-29°C, 50-80% relative humidity and a photoperiod of 12L: 12D.

6.2.2 Effect of host insect

In a laboratory host preference experiment, *T. bournieri* and *T.* sp. nr. *mwanzai* were separately offered with the choice between eggs of the target pest, *C. partellus* and eggs of the standard laboratory host, *C. cephalonica*. Choice tests were performed using the method of Pak (1988) and Hassan (1990, 1994). Both species used in this study were arrhenotokous (diploid females from fertilized eggs) (Pinto and Stouthamer, 1994). A single mated *Trichogramma* female (12 to 24 hours old) was released in a glass tube (75

mm long x 25 mm in diameter) together with one egg card of *C. cephalonica* (ca. 80 eggs/card) and two batches of *C. partellus* (ca. 40 eggs/batch). Freshly laid host eggs were used for all experiments.

The egg cards/batches were glued at the corner of a piece of paper measuring 2 cm x 2 cm (Hassan, 1994). Individual eggs of *C. cephalonica* were glued with Traganth (Merk, Darmstadt, Germany) on a sheet of paper (80 g/m^2), where as *C. partellus* eggs were laid on butter paper (Ochieng *et al.*, 1985). Feeding of the parasitoids were done using a mixture of honey (66%), gelatine (1%) and distilled water (33%) and placed at the centre of the paper daily until the fifth day. Cotton plugs were used as stopper for the vials.

A completely randomised design (CRD) was used for each species and the test was replicated thirty times (n = 30 females) with ca. 80 x 2 eggs per replicate. On the fifth day, parasitised eggs of each host insect were transferred into another glass tube and larvae from non-parasitised eggs were removed. Fertility, progeny production and sex ratio of the emerging parasitoids were recorded. Fertility refers to the number of parasitised host eggs in which the *Trichogramma* is at the prepupal stage (Hassan, 1990). The experiment was conducted at ambient temperatures of 24-29°C, 50-80% relative humidity and a photoperiod of 12L: 12D.

In the no choice laboratory test, completely randomised design was used for each species and the test was replicated thirty times (n = 30 females) with ca. 80 x 2 eggs per replicate. Eggs of *Helicoverpa armigera* Hübner, *C. cephalonica* and *C. partellus* were offered individually to *T. bournieri* and *T.*

sp. nr. *mwanzai* parasitoids under no choice condition. Eggs of *H. armigera* were laid on cloths (Thorpe, 1984). Due to shortage of eggs, *H. armigera* was not included in the choice experiment above. In addition to fertility, progeny production and sex ratio, the egg to adult developmental time was recorded.

6.2.3 Effect of host plant

The choice experiment was carried out using 4-6 weeks old potted plants of maize (*Zea mays* L.) and tomato (*Lycopersicon esculentum* Mill.) kept in cages measuring 50 cm long x 65 cm wide x 100 cm high. Two maize and two tomato plants were kept in each cage. Inside the cage, every maize plant was placed next to tomato. Freshly laid egg batches of *C. partellus* (4 batches/plant) were placed on the upper and lower leaves of the plant. Mated and 12 to 24 hours old female *Trichogramma* (ca. 50) of each species were released separately at the centre of the cage (Hassan and Guo, 1991; Hassan, 1994). The release was conducted during the morning hours. At the centre of the cage, diet that consists of honey (66%), gelatine (1%) and distilled water (33%) was placed for the parasitoids to feed after the release. A completely randomised design was used for each species and the test was replicated in three cages with four plants per cage.

The no choice experiment was conducted using *C. partellus* egg batches of exposed to the target *T. bournieri* and *T.* sp. nr. *mwanzai* individually using maize or tomato plants. Four maize or tomato plants were kept in each cage. Freshly laid egg batches of *C. partellus* (4 batches/plant)

were placed on the upper and lower leaves of the plant. Mated female *Trichogramma* (ca. 50) 12 to 24 hours old of each species were released separately at the centre of the cage. Diet that consists of honey (66%), gelatine (1%) and distilled water (33%) was placed at the centre of the cage for the parasitoids to feed after the release. A completely randomised design was used for each species and the test was replicated in three cages with four plants per cage.

The egg batches were collected on the fifth day and kept in glass tubes and monitored in the laboratory at ambient temperatures of 24-29°C, 50-80% relative humidity and a photoperiod of 12L: 12D. Data on parasitism, emergence rate, progeny production and sex ratio were recorded.

6.2.4 Data analysis

All frequency data were analysed using the Chi-square (χ^2) tests. Percent parasitism and emergence was calculated based on the methods outlined by Van Driesche (1983). Fertility and progeny production data were transformed to $\log_{10}$ (x+1) while all percentage data (emergence of adults and proportion of females) were arcsine-transformed before being subjected to analysis of variance (ANOVA) using the General Linear Models (GLM) procedure (SAS Institute, 2000). When F-values showed significance at P=0.05, means were separated using Student-Newman-Keuls (SNK) test.

6.3 RESULTS

6.3.1 Effect of host insect

In the laboratory choice experiments, a significant preference for *C. cephalonica* compared to *C. partellus* was shown by *T. bournieri* (χ^2=12.77; df=1; p=0.001) and *T.* sp. nr. *mwanzai* (χ^2=94.16; df=1; p=0.001). *Trichogramma* sp. nr. *mwanzai* produced significantly higher progenies in *C. cephalonica* compared to *C. partellus* (χ^2=25.33; df=1; p=0.001). The two hosts did not differ in progeny production by *T. bournieri* (χ^2=0.005; df=1; p=0.942) (Fig. 6.1).

The proportion of females was significantly higher in *C. cephalonica* compared to *C. partellus* (χ^2=13.83; df=1; p=0.001) for *T.* sp. nr. *mwanzai*. There was no significant difference in the proportion of females between the two hosts for *T. bournieri* (χ^2=0.23; df=1; p=0.632) (Fig. 6.1). Under choice laboratory experiments, there was no significant difference (P>0.05) between *T. bournieri* and *T.* sp. nr. *mwanzai* in fertility and progeny production on both *C. partellus* and *C. cephalonica*.

In the no choice laboratory experiments, *T. bournieri* and *T.* sp. nr. *mwanzai* showed greater acceptance to *C. partellus* and *C. cephalonica* compared to *H. armigera* (Tables 6.1 and 6.2). Progeny production by *T. bournieri* and *T.* sp. nr. *mwanzai* was also significantly different among the host insects. Both parasitoids produced large number of progenies in *C. partellus* and *C. cephalonica* compared to *H. armigera*. Egg to adult developmental time in *T.* sp. nr. *mwanzai* was significantly different among

the hosts (Table 6.2).

Under the no choice laboratory experiments, there was no significant difference between *T. bournieri* and *T.* sp. nr. *mwanzai* in fertility (F=1.30; df=1,29; p=0.2642) and progeny production (F=0.76; df=1,29; p=0.3901) on *C. cephalonica*. In the case of *C. partellus*, there was no significant difference in fertility (F=2.14; df=1,29; p=0.1540) but progeny production was significantly different (F=5.16; df=1,29; p=0.0308) between the two parasitoids.

6.3.2 Effect of host plant

In the field cage choice experiment using *C. partellus* eggs, both species showed a stronger preference for maize plant than for tomatoes. In the choice experiments, *T. bournieri* attained significantly higher percent parasitism (χ^2=41.81; df=1; p=0.001), progeny production (χ^2=28.45; df=1; p=0.001) and proportion of females (χ^2=24.89; df=1; p=0.001) on maize plant compared to tomatoes (Fig. 6.2). There was significant difference between *T. bournieri* and *T.* sp. nr. *mwanzai* in percent parasitism (F=7.47; df=1,20; p=0.0128) and progeny production (F=5.88; df=1,20; p=0.0249) on maize. However, there was no significant difference (P>0.05) between the two species on tomatoes.

In the no choice experiments in the field cage, percent parasitism was significantly higher in maize than in tomatoes in both species. However, there was no significant difference in percent emergence and proportion of females

between the host plants for both species (Tables 6.3 and 6.4). There was no significant difference (P>0.05) between *T. bournieri* and *T.* sp. nr. *mwanzai* in percent parasitism and progeny production on both maize and tomatoes.

6.4 DISCUSSION

6.4.1 Effect of host insect

There was a strong host insect and host plant influence on both parasitism and progeny production of *T. bournieri* and *T.* sp. nr. *mwanzai*. Based on the two biological parameters, parasitism and progeny production, *H. armigera* eggs appeared to be less suitable for both *T. bournieri* and *T.* sp. nr. *mwanzai*. When *C. cephalonica* and *C. partellus* eggs were compared for the two biological parameters, there were no significant differences between the parasitoids under the choice laboratory studies.

In Kenya, Guang and Oloo (1990) had found that *Trichogramma* sp. nr. *mwanzai* showed no significant difference in progeny production on *C. partellus*, *Busseola fusca* Fuller and *Sitotroga cerealella* Oliver while on *Eldana saccharina* Walker, it produced significantly fewer progeny. Further, the parasitoids did not accept the eggs of the silk worm, *Bombyx mori* Linnaeus even under a no choice situation.

In a separate study in Kenya, Ochiel (1989) studied the host preference of *Trichogramma* sp. nr. *exiguum* Pinto & Platner on *C. partellus*, *B. fusca*, *Galleria mellonella* Linnaeus and *H. armigera*. The parasitoid showed a marked preference for *H. armigera* despite being reared on *C. partellus* for 14 generations (Ochiel, 1989). The strong preference shown in the present study by *T. bournieri* and *T.* sp. nr. *mwanzai* for *C. cephalonica* and *C. partellus* could be attributed to learning as a result of rearing for over 50 generations on

both hosts. However, the parasitoids still maintained their preference to the original host, *C. partellus*.

Similarly, a strong host preference was observed in *T. semifumatum* Perkins for *S. cerealella* than the cabbage looper, *Trichoplusia ni* Hübner after the parasitoids was reared on *S. cerealella* for over 100 generations (Taylor and Stern, 1971). Torreno and Cadapan (1984) also reported that *T. australicum* Girault and *T. chilotreae* Nagarkatti were highly influenced by their previous rearing hosts. Strong effect of an oviposition experience (adult learning) has been reported in an Australian egg parasitoid, *T.* sp. nr. *ivelae* Pang & Chen when tested on three hosts (Bjorksten and Hoffmann, 1998).

Corrigan and Laing (1994) observed no significant effect of the rearing host species, *Ephestia kuehniella* Zeller and *S. cerealella* on percent emergence and sex ratio of *T. minutum* Riley. The effect of rearing host has been observed in *T. dendrolimi* Matsumura where significantly higher parasitism was recorded on the Chinese oak silk moth, *Antheraea pernyi* Guerin-Meneville compared to *C. cephalonica* (Liu *et al.*, 1998). Hence, this study is in agreement with the findings by Liu *et al.* (1998). The high fertility and progeny production observed in *C. cephalonica* probably indicates the suitability of the host for mass rearing (Singh and Jalali, 1994).

In the laboratory tests, *T. nubilale* Ertle & Davis had successfully parasitised eggs of 17 out of 21 species of Lepidoptera (Curl and Burbutis, 1978). The host specificity of candidate species should be considered among

other factors for successful biological control programs using *Trichogramma* (Pak, 1988).

6.4.2 Effect of host insect

In the field cage experiment using *C. partellus* eggs, both species showed a stronger preference for maize plant than for tomatoes. A strong association between both parasitoids and maize plant has been observed in the field cage choice and the no choice experiments, as percent parasitism and progeny production were significantly higher in maize compared to tomatoes. Furthermore, *T.* sp. nr. *mwanzai* failed to parasitise *C. partellus* eggs on tomatoes.

Hassan and Guo (1991) tested 20 *Trichogramma* strains on the European corn borer, *Ostrinia nubilalis* Hübner on maize plants in the cage as well as with the standard host *S. cerealella* in the laboratory. *Trichogramma ostriniae* Pang & Chen and *T. evanescens* were selected based on their preference to the parasitise *O. nubilalis* on maize. Kaiser *et al.* (1989) reported that *T. brassicae* Bezdenko could associate the odour of maize with the presence of *O. nubilalis* egg masses. *Helicoverpa armigera* on sorghum was parasitised to high levels by *T. chilonis* compared to pigeon pea (Romeis *et al.*, 1997). The phenomena observed in *T.* sp. nr. *mwanzai* is consistent with the reports of Kaiser *et al.* (1989) and Romeis *et al.* (1997).

The influence of host plants observed in this study agrees with earlier reports by Vinson and Iwantsch (1980) and Vinson (1998). However, a thorough understanding of the role of host plants in the foraging behaviour should be assessed before field releases.

The study confirms the influence of host insects and host plants on parasitism and progeny production of the two native parasitoid species. Both species showed strong preference to *C. partellus* in the laboratory. The strong preference to the maize plant is also vital in locating the host habitat. Therefore, both species could be candidate for biological control of *C. partellus* on maize in Kenya.

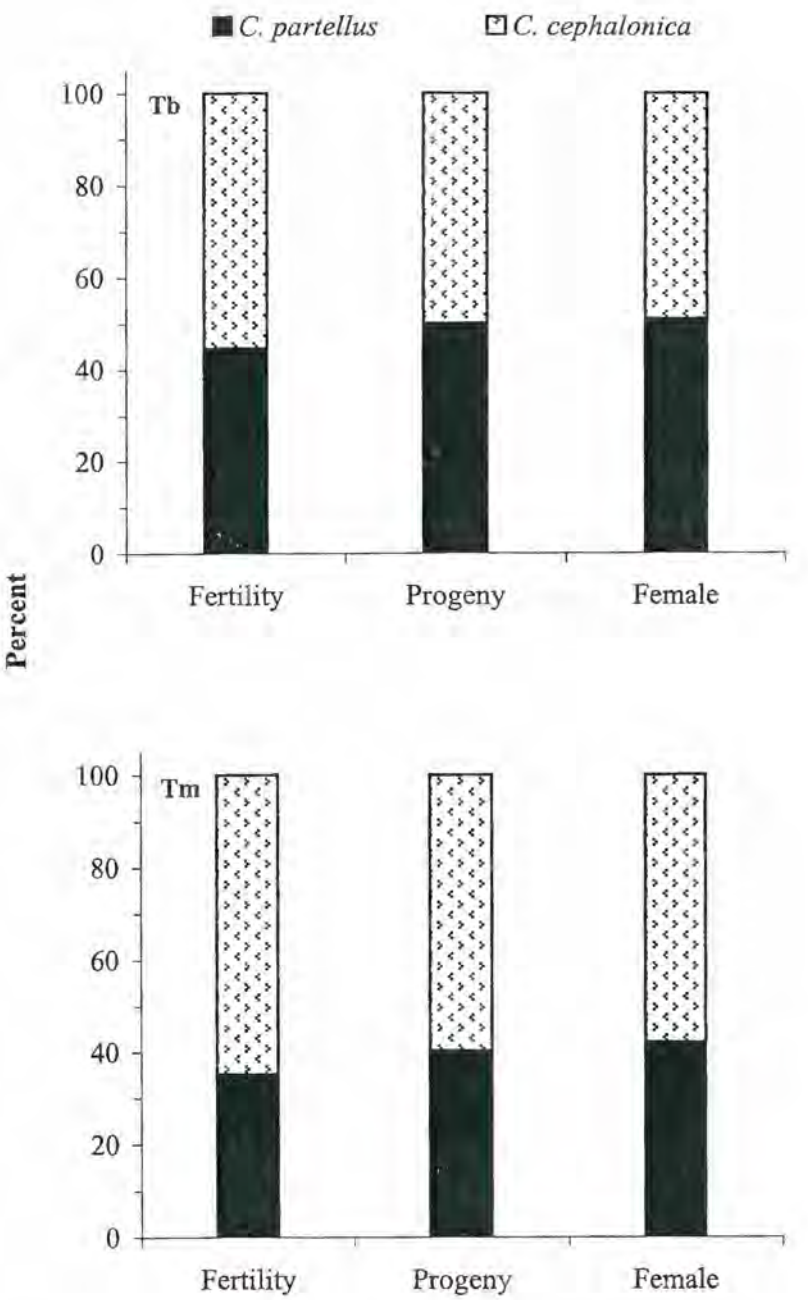

Figure 6.1 Effect of host insect on fertility, progeny production and sex ratio of *Trichogramma bournieri* (Tb) and *Trichogramma* sp. nr. *mwanzai* (Tm) in the laboratory choice experiment.

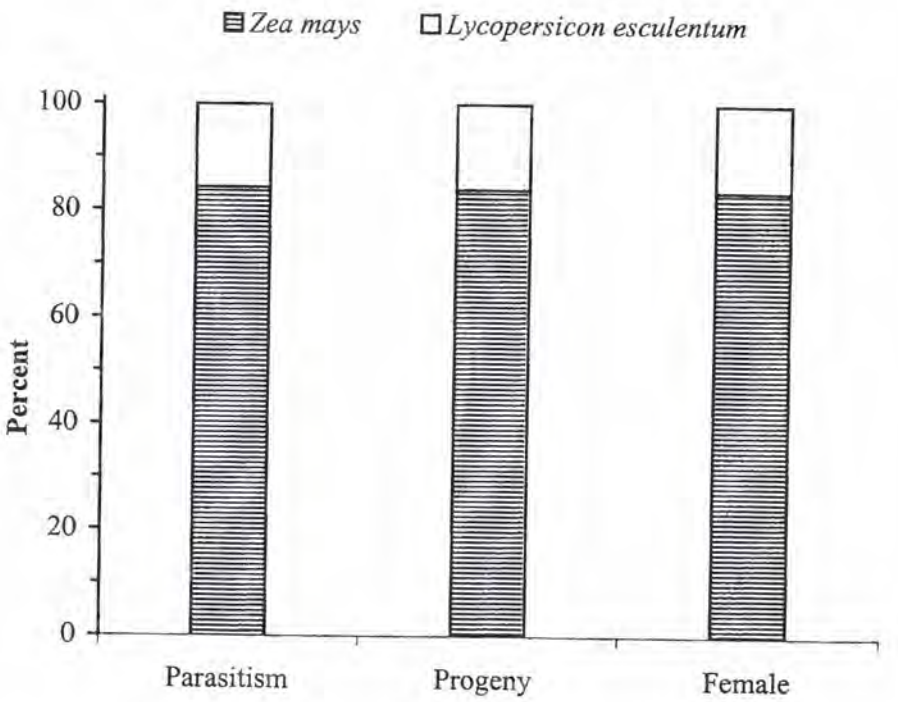

Figure 6.2 Effect of host plant on parasitism, progeny production and sex ratio of *T. bournieri* in the field cage choice experiment.

Table 6.1 Effect of host insect on parasitism, progeny production, sex ratio and developmental time of *Trichogramma bournieri* in the laboratory no choice experiment (Mean ± SE)

Host Insects		Parasitised eggs (per female)	Progeny Production (per female)	Proportion of Females	Developmental Time (days)
Chilo partellus		26.2 ± 2.3a	17.3 ± 2.1a	0.81 ± 0.04a	10.3 ± 0.1a
Corcyra cephalonica		40.2 ± 3.3a	23.7 ± 2.2a	0.84 ± 0.02a	10.0a
Helicoverpa armigera		2.5 ± 0.7b	4.2 ± 1.3b	0.73 ± 0.09a	10.3 ± 0.2a
	F	64.34	30.52	0.52	3.47
	df	2,58	2,58	2,37	2,37
	P	0.0001	0.0001	0.6003	0.0416

- Means followed by the same letter in the same column are not significantly different (P>0.05), SNK test.
- Analysis of Variance (ANOVA) performed on $\log_{10}$ transformed parasitised eggs and progeny production and arcsine transformed proportion of females. Values presented in Table 6.1 are the original data.
- n = 30 (number of *Trichogramma* females).

Table 6.2 Effect of host insect on parasitism, progeny production, sex ratio and developmental time of *Trichogramma* sp. nr. *mwanzai* in the laboratory no choice experiment (Mean ± SE)

Host Insects		Parasitised eggs (per female)	Progeny Production (per female)	Proportion of Females	Developmental Time (days)
Chilo partellus		27.5 ± 1.6a	21.6 ± 1.7a	0.83 ± 0.02a	10.5 ± 0.1b
Corcyra cephalonica		35.2 ± 3.8a	22.50 ± 2.6a	0.83 ± 0.02a	10.1 ± 0.1b
Helicoverpa armigera		1.0 ± 0.2b	1.7 ± 0.5b	0.75 ± 0.09a	11.5 ± 0.4a
	F	94.03	71.67	0.04	11.12
	df	2,58	2,58	2,37	2,39
	P	0.0001	0.0001	0.9644	0.0002

- Means followed by the same letter in the same column are not significantly different (P>0.05), SNK test.
- Analysis of Variance (ANOVA) performed on $\log_{10}$ transformed parasitised eggs and progeny production and arcsine transformed proportion of females. Values presented in Table 6.2 are the original data.
- n = 30 (number of *Trichogramma* females).

Table 6.3 Effect of host plant on parasitism, emergence, progeny production and sex ratio of *Trichogramma bournieri* in the field cage no-choice experiment using *Chilo partellus* eggs (Mean ± SE)

Host Plants		Parasitism (%)	Emergence (%)	Progeny Production (no.)	Proportion of Females
Zea mays		20.2 ± 5.1a	98.5 ± 1.5a	16.0 ± 4.1a	0.85a
Lycopersicon esculentum		1.8 ± 1.8b	100.0a	1.5 ± 1.5b	0.94a
	F	12.41	1.13	17.60	0.86
	df	1,44	1,9	1,44	1,9
	P	0.0010	0.3165	0.0001	0.3780

- Means followed by the same letter in the same column are not significantly different (P>0.05), SNK test.
- Analysis of Variance (ANOVA) performed on $\log_{10}$ transformed progeny production and arcsine transformed percentages and proportions. Values presented in Table 6.3 are the original data.
- n = 48 (number of *Chilo partellus* egg batches, ca. 40 eggs per batch).

Table 6.4 Effect of host plant on parasitism, emergence, progeny production and sex ratio of *Trichogramma* sp. nr. *mwanzai* in the field cage no-choice experiment using *Chilo partellus* eggs (Mean ± SE)

Host Plants		Parasitism (%)	Emergence (%)	Progeny Production (no.)	Proportion of Females
Zea mays		14.8 ± 4.3a	84.3 ± 8.8a	10.8 ± 3.4a	0.72 ± 0.1a
Lycopersicon esculentum		3.7 ± 1.8b	100.0a	2.9 ± 1.4b	0.87 ± 0.1a
	F	5.49	2.13	4.64	1.65
	df	1,44	1,13	1,44	1,12
	P	0.0237	0.1678	0.0368	0.2226

- Means followed by the same letter in the same column are not significantly different (P>0.05), SNK test.
- Analysis of Variance (ANOVA) performed on $\log_{10}$ transformed progeny production and arcsine transformed percentages and proportions. Values presented in Table 6.4 are the original data.
- n = 48 (number of *Chilo partellus* egg batches, ca. 40 eggs per batch).

CHAPTER 7

7.0 GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

7.1 GENERAL DISCUSSION AND CONCLUSIONS

Trichogramma egg parasitoids (Hymenoptera: Trichogrammatidae) are extensively used in augmentative biological control of lepidopteran pests worldwide. *Trichogramma* are particularly good natural enemies of lepidopteran pests, because they parasitise and kill the pest in the egg stage, before the crop is damaged. However, research on the natural occurrence and use of this group of biocontrol agents has been limited in Africa. In Kenya, the few earlier studies have been focussed on host range, adaptation and quantitative response of *Trichogramma* species. The objective of the present study was to assemble and identify the native *Trichogramma* species occurring in different locations and assesses the potential of candidate species in the integrated management of the African bollworm, *Helicoverpa armigera* Hübner and the Diamondback Moth, *Plutella xylostella* Linnaeus in Kenya.

Using surveys and field trials in Kenya, five native egg parasitoids species, namely *Trichogramma bournieri* Pintureau & Babault, *Trichogramma* sp. nr. *mwanzai* Schulten & Feijen, *Trichogramma* sp. nr. *bruni* Nagaraja, *Trichogrammatoidea* sp. nr. *lutea* Girault and *Trichogrammatoidea* sp. were recovered from eggs of *H. armigera*, *P. xylostella* and *Chilo partellus* Swinhoe (Chapter 2).

Trichogramma bournieri and *T.* sp. nr. *bruni* are the first records for Kenya and probably the latter for Africa. The natural occurrence of *Trichogrammatoidea* sp. nr. *lutea* on *P. xylostella* eggs was also the first record for Kenya as well as for eastern Africa. Natural egg parasitism on *P. xylostella* and *C. partellus* in the fields reached up to 18% and 39%, respectively whereas on *H. armigera* it was very low (below 5%). *Trichogramma* sp. nr. *mwanzai* parasitised *C. partellus* eggs on kale but failed to parasitise *P. xylostella* eggs under natural conditions.

A strong association was observed between *Trichogramma* sp. nr. *mwanzai* and sorghum or maize. On the other hand, all the *P. xylostella* eggs sampled were found to be parasitised only by *Trichogrammatoidea* sp. nr. *lutea*. *Trichogramma* sp. nr. *mwanzai* occurred in different altitudes, host insects and plants and so could be a preferred candidate for being further utilised in a broad-based biological control programme in Kenya.

The most problematic area in *Trichogramma* research is its taxonomy due to their small size and morphological homogeneity. About 150 specimens of native egg parasitoids were identified based on the male genitalia and other morphological traits (Nagarkatti and Nagaraja, 1971, 1977). Morphometric studies conducted on the taxonomy of the native *Trichogramma* species (Chapter 3) revealed that the species could be distinguished using characteristics such as genital capsule, intervolsellar process, dorsal lamina and ventral ridge.

Trichogramma sp. nr. *mwanzai* was morphologically more similar to the Australasian *T. chilonis* Ishii than to *T. bournieri*. The genitalia of *T.* sp. nr. *mwanzai* was more similar to the Palaearctic species, *T. evanescens* Westwood, than to the genitalia of *T. bournieri*. Further studies based on genetic characters using molecular techniques (RAPD-PCR) showed *T. bournieri* and *T.* sp. nr. *mwanzai* had 40% genetic similarity. It is concluded that the two native Kenyan species are more similar genetically than morphologically.

In order to assess the relative suitability of the two native species of *Trichogramma* for biocontrol, their temperature tolerance, life table parameters and responses to host insect and host plant were determined. Chapter 4 deals with studies on the effect of temperature on *T. bournieri* and *T.* sp. nr. *mwanzai* compared also with *T. evanescens* and *T. chilonis*. There was no development of *T. bournieri* and *T.* sp. nr. *mwanzai* at 13°C. *Trichogramma* sp. nr. *mwanzai* appeared to be superior at higher temperatures in terms of fertility and progeny production.

At all the four temperatures tested, *T.* sp. nr. *mwanzai* had the highest preimaginal survivorship. The developmental period for all the species decreased as the temperature increased. Sex ratio was female-biased at all temperatures for *T. bournieri* and *T. chilonis*. In contrast, sex ratio was male-biased in *T.* sp. nr. *mwanzai*. Since *T.* sp. nr. *mwanzai* showed tolerance to the higher temperature (34°C), it could well be regarded as a preferable candidate species for biocontrol programmes in warmer climates.

As part of the selection criteria (Smith, 1996), a life table study was conducted on *T. bournieri* and *T.* sp. nr. *mwanzai* at 26 ± 1°C, 70 ± 10% relative humidity and 16L: 8D photoperiod (Chapter 5). There was no significant difference between the two species in the intrinsic rate of natural increase and the net reproductive rate. The life table study, however, showed that *T.* sp. nr. *mwanzai* had a significantly higher fertility than *T. bournieri.*

The effects of host insect and host plant on the performance of *T. bournieri* and *T.* sp. nr. *mwanzai* were investigated both in the laboratory and in field cage under choice and no choice situations (Chapter 6). There was a strong host insect and host plant influence on the parasitism and progeny production of *T. bournieri* and *T.* sp. nr. *mwanzai.*

In the field cage experiment using *C. partellus* eggs, both the native species showed better performance on maize compared to tomatoes. Parasitism and progeny production were significantly higher on maize compared to tomatoes. The study confirmed the influence of host insects and host plants on the performance of the two native species. The superior performance of *T.* sp. nr. *mwanzai* in the laboratory studies as well as in the field cage tests suggested that it could be a preferred candidate species for biological control programmes.

Based on its natural occurrence in different ecological zones, tolerance to different temperature regimes, high fertility and progeny production as well as host specificity the native *T.* sp. nr. *mwanzai* could be a candidate species for future mass rearing and field release programmes for biocontrol of different lepidopteran pests in Kenya.

7.2 RECOMMENDATIONS

Information on the natural egg parasitism of target lepidopteran pests in Kenya and other eastern African countries is required for designing a successful biological control programme. Further research in the following areas is of paramount importance for sustainable use of *Trichogramma* egg parasitoids in the framework of IPM.

i. Surveys and further field trials to determine the natural occurrence of *Trichogramma* egg parasitoids at different ecological zones in Kenya and eastern Africa.

ii. Basic studies on the biology and ecology of native *Trichogramma* egg parasitoids.

iii. Continuing the molecular identification of *Trichogramma* species by amplifying the ITS-2 region with restriction enzymes and determining the gene sequence.

iv. Behavioural studies investigating the role of host plants and host insects in the foraging of *Trichogramma*.

v. Development of field release and impact assessment strategies.

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Appendix 1 Field trial layout for studying the natural occurrence and parasitism of egg parasitoids on *Helicoverpa armigera*, *Plutella xylostella* and *Chilo partellus* in Muhaka, coastal Kenya (1999/2000)

Design: Latin square (6 treatments x 6 replications = 36 plots)

Crops: 1 = Tomato 2 = Okra 3 = Kale 4 = Pigeon pea 5 = Sunflower 6 = Sorghum

Plot size: 5.6m x 5.6m = 31.36 m^2 **Total area:** 41m x 41m = 1681 m^2

Appendix 2. List of native egg parasitoids samples collected during the survey and field trials (1998-2000) and maintained as live cultures at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya

Egg Parasitoid Sample	Date of Collection	Place of Collection	Host Insect	Host Plant	Field Trial Code	Identified Scientific Name (Dr. J.C. Monje, University of Hohenheim, Germany)
Kenya-01	15. 10. 1998	Muhaka	*Chilo partellus*	Sorghum	Preliminary Survey	*Trichogramma* sp. nr. *mwanzai* Schulten & Feijen
Kenya-02	22. 11. 1998	Mbita	*Chilo partellus*	Maize	Preliminary Survey	*Trichogramma bournieri* Pintureau & Babault
Kenya-03	06. 09. 1999	Muhaka	*Helicoverpa armigera*	Pigeon pea	Y1 W5 S9 P27 Sp12	*Trichogramma* sp. nr. *mwanzai*
Kenya-04	16. 09. 1999	Muhaka	*Helicoverpa armigera*	Pigeon pea	Y1 W6 S12 P24 Sp6	*Trichogrammatoidea* sp.
Kenya-05	23. 09. 1999	Muhaka	*Helicoverpa armigera*	Pigeon pea	Y1 W7 S14 P5 Sp5	*Trichogrammatoidea* sp. nr. *lutea* Girault
Kenya-06	23. 09. 1999	Muhaka	*Helicoverpa armigera*	Pigeon pea	Y1 W7 S14 P31 Sp9	*Trichogrammatoidea* sp.
Kenya-07	29. 11. 1999	kibwezi	*Corcyra cephalonica*	Maize	Transect Survey	*Trichogramma* sp. nr. *mwanzai*
Kenya-08	17. 10. 1999	Mtitoandei	*Corcyra cephalonica*	Maize	Transect Survey	*Trichogramma* sp. nr. *mwanzai*
Kenya-09	29. 11. 1999	Mtitoandei	*Corcyra cephalonica*	Maize	Transect Survey	*Trichogramma* sp. nr. *mwanzai*
Kenya-10	29. 11. 1999	Mtitoandei	*Corcyra cephalonica*	Maize	Transect Survey	*Trichogramma* sp. nr. *mwanzai*
Kenya-11	29. 11. 1999	Mtitoandei	*Corcyra cephalonica*	Maize	Transect Survey	*Trichogramma* sp. nr. *mwanzai*
Kenya-12	29. 11. 1999	Mtitoandei	*Corcyra cephalonica*	Maize	Transect Survey	*Trichogramma* sp. nr. *mwanzai*
Kenya-13	29. 11. 1999	Mtitoandei	*Corcyra cephalonica*	kale	Transect Survey	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-14	29. 11. 1999	Shimba Hills	*Corcyra cephalonica*	Maize	Transect Survey	*Trichogramma* sp. nr. *mwanzai*
Kenya-15	29. 11. 1999	Shimba Hills	*Corcyra cephalonica*	Maize	Transect Survey	*Trichogramma* sp. nr. *mwanzai*
Kenya-16	30. 11. 1999	Muhaka	*Corcyra cephalonica*	Maize	Preliminary Survey	*Trichogramma* sp. nr. *mwanzai*
Kenya-17	30. 08. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W4 S7 P1 Sp12	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-18	30. 08. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W4 S7 P1 Sp15	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-19	30. 08. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W4 S7 P12 Sp10	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-20	30. 08. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W4 S7 P26 Sp4	*Trichogrammatoidea* sp. nr. *lutea*

Cont. Appendix 2

Egg Parasitoid Sample	Date of Collection	Place of Collection	Host Insect	Host Plant	Field Trial Code	Identified Scientific Name (Dr. J.C. Monje, University of Hohenheim, Germany)
Kenya-21	30. 08. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W4 S7 P33 Sp13	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-22	02. 09. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W4 S8 P12 Sp7	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-23	02. 09. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W4 S8 P17 Sp2	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-24	16. 09. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W6 S12 P26 Sp10	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-25	20. 09. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W7 S13 P12 Sp7	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-26	20. 09. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W7 S13 P22 Sp11	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-27	20. 09. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W7 S13 P26 Sp4	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-28	23. 09. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W7 S14 P26 Sp5	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-29	23. 09. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W7 S14 P26 Sp9	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-30	27. 09. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W8 S15 P26 Sp2	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-31	30. 09. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W8 S16 P1 Sp3	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-32	30. 09. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W8 S16 P1 Sp16	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-33	30. 09. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W8 S16 P12 Sp6	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-34	30. 09. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W8 S16 P12 Sp14	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-35	30. 09. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W8 S16 P17 Sp2	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-36	30. 09. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W8 S16 P22 Sp13	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-37	30. 09. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W8 S16 P33 Sp1	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-38	04. 10. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W9 S17 P17 Sp1	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-39	07. 10. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W9 S18 P22 Sp6	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-40	18. 10. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W11 S21 P1 Sp7	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-41	18. 10. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W11 S21 P22 Sp6	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-42	28. 10. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W12 S24 P22 Sp13	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-43	28. 10. 1999	Muhaka	*Plutella xylostella*	kale	Y1 W12 S24 P26 Sp15	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-44	09. 08. 1999	Muhaka	*Chilo partellus*	kale	Y1 W1 S1 P26 Sp2	*Trichogramma* sp. nr. *mwanzai*
Kenya-45	23. 08. 1999	Muhaka	*Chilo partellus*	Sorghum	Y1 W3 S5 P34 Sp11	*Trichogramma* sp. nr. *bruni* Nagaraja
Kenya-46	23. 08. 1999	Muhaka	*Chilo partellus*	Sorghum	Y1 W3 S5 P34 Sp14	*Trichogramma* sp. nr. *mwanzai*
Kenya-47	26. 08. 1999	Muhaka	*Chilo partellus*	Sorghum	Y1 W3 S6 P6 Sp7	*Trichogramma* sp. nr. *mwanzai*
Kenya-48	30. 08. 1999	Muhaka	*Chilo partellus*	kale	Y1 W4 S7 P12 Sp14	*Trichogramma* sp. nr. *mwanzai*
Kenya-49	30. 08. 1999	Muhaka	*Chilo partellus*	kale	Y1 W4 S7 P17 Sp4	*Trichogramma* sp. nr. *mwanzai*
Kenya-50	02. 09. 1999	Muhaka	*Chilo partellus*	Sorghum	Y1 W4 S8 P6 Sp10	*Trichogrammatoidea* sp. nr. *lutea*

Cont. Appendix 2

Egg Parasitoid Sample	Date of Collection	Place of Collection	Host Insect	Host Plant	Field Trial Code	Identified Scientific Name (Dr. J.C. Monje, University of Hohenheim, Germany)
Kenya-51	06. 09. 1999	Muhaka	*Chilo partellus*	Sorghum	Y1 W5 S9 P7 Sp15	*Trichogramma* sp. nr. *mwanzai*
Kenya-52	06. 09. 1999	Muhaka	*Chilo partellus*	Sorghum	Y1 W5 S9 P34 Sp6	*Trichogramma* sp. nr. *mwanzai*
Kenya-53	09. 09. 1999	Muhaka	*Chilo partellus*	Sorghum	Y1 W5 S10 P29 Sp4	*Trichogramma* sp. nr. *mwanzai*
Kenya-54	16. 09. 1999	Muhaka	*Chilo partellus*	Sorghum	Y1 W6 S12 P6 Sp2	*Trichogramma* sp. nr. *mwanzai*
Kenya-55	16. 09. 1999	Muhaka	*Chilo partellus*	Sorghum	Y1 W6 S12 P6 Sp4	*Trichogramma* sp. nr. *mwanzai*
Kenya-56	16. 09. 1999	Muhaka	*Chilo partellus*	Sorghum	Y1 W6 S12 P6 Sp7	*Trichogramma* sp. nr. *mwanzai*
Kenya-57	16. 09. 1999	Muhaka	*Chilo partellus*	Sorghum	Y1 W6 S12 P34 Sp8	*Trichogramma* sp. nr. *mwanzai*
Kenya-58	23. 09. 1999	Muhaka	*Chilo partellus*	Sorghum	Y1 W7 S14 P7 Sp4	*Trichogramma* sp. nr. *mwanzai*
Kenya-59	23. 09. 1999	Muhaka	*Chilo partellus*	Sorghum	Y1 W7 S14 P14 Sp8	*Trichogramma* sp. nr. *mwanzai*
Kenya-60	23. 09. 1999	Muhaka	*Chilo partellus*	Sorghum	Y1 W7 S14 P14 Sp16	Not identified
Kenya-61	23. 09. 1999	Muhaka	*Chilo partellus*	Sorghum	Y1 W7 S14 P34 Sp16	*Trichogramma* sp. nr. *mwanzai*
Kenya-62	27. 09. 1999	Muhaka	*Chilo partellus*	Sorghum	Y1 W8 S15 P14 Sp4	*Trichogramma* sp. nr. *mwanzai*
Kenya-63	04. 10. 1999	Muhaka	*Chilo partellus*	kale	Y1 W9 S17 P22 Sp10	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-64	29. 11. 1999	Mtitoandei	*Corcyra cephalonica*	Maize	Transect Survey	*Trichogramma* sp. nr. *mwanzai*
Kenya-65	06. 12. 1999	kasarani	*Corcyra cephalonica*	Tomato	Transect Survey	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-66	29. 11. 1999	Mtito Andei	*Corcyra cephalonica*	Maize	Transect Survey	Not identified
Kenya-67	29. 11. 1999	Mtito Andei	*Corcyra cephalonica*	Maize	Transect Survey	Thelytokous species
Kenya-68	13. 01. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W2 S4 P12 Sp1	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-69	13. 01. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W2 S4 P33 Sp1	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-70	13. 01. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W2 S4 P33 Sp5	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-71	17. 01. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W3 S5 P12 Sp11	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-72	17. 01. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W3 S5 P12 Sp14	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-73	17. 01. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W3 S5 P22 Sp13	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-74	17. 01. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W3 S5 P12 Sp13	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-75	17. 01. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W3 S5 P12 Sp9	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-76	17. 01. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W3 S5 P1 Sp10	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-77	17. 01. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W3 S5 P17 Sp2	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-78	17. 01. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W3 S5 P33 Sp2	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-79	17. 01. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W3 S5 P22 Sp4	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-80	13. 01. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W3 S5 P22 Sp6	*Trichogrammatoidea* sp. nr. *lutea*

Cont. Appendix 2

Egg Parasitoid Sample	Date of Collection	Place of Collection	Host Insect	Host Plant	Field Trial Code	Identified Scientific Name (Dr. J.C. Monje, University of Hohenheim, Germany)
Kenya-81	17. 01. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W3 S5 P33 Sp8	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-82	17. 01. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W3 S5 P12 Sp4	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-83	17. 01. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W3 S5 P33 Sp13	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-84	20. 01. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W3 S6 P12 Sp7	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-85	03. 02. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W5 S10 P22 Sp4	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-86	03. 02. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W5 S10 P22 Sp16	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-87	07. 02. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W6 S11 P12 Sp11	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-88	07. 02. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W6 S11 P22 Sp6	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-89	07. 02. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W6 S11 P22 Sp10	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-90	07. 02. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W6 S11 P1 Sp5	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-91	07. 02. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W6 S11 P1 Sp8	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-92	07. 02. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W6 S11 P12 Sp4	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-93	03. 02. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W5 S10 P12 Sp8	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-94	10. 02. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W6 S12 P26 Sp9	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-95	14. 02. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W7 S13 P22 Sp4	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-96	17. 01. 2000	Muhaka	*Helicoverpa armigera*	Okra	Y2 W3 S5 P18 Sp15	*Trichogrammatoidea* sp.
Kenya-97	06. 01. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W1 S2 P29 Sp9	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-98	10. 01. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W2 S3 P21 Sp15	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-99	20. 01. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W3 S6 P7 Sp11	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-100	20. 01. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W3 S6 P21 Sp4	*Trichogramma* sp. nr. *mwanzai*
Kenya-101	14. 02. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W7 S13 P21 Sp13	Thelytokous species
Kenya-102	17. 01. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W3 S5 P7 Sp12	*Trichogramma* sp. nr. *mwanzai*
Kenya-103	20. 01. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W3 S6 P29 Sp1	*Trichogramma* sp. nr. *mwanzai*
Kenya-104	24. 01. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W4 S7 P14 Sp6	*Trichogramma* sp. nr. *mwanzai*
Kenya-105	20. 01. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W3 S6 P6 Sp1	*Trichogramma* sp. nr. *mwanzai*
Kenya-106	24. 01. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W4 S7 P21 Sp5	*Trichogramma* sp. nr. *mwanzai*
Kenya-107	24. 01. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W4 S7 P7 Sp14	*Trichogramma* sp. nr. *mwanzai*
Kenya-108	24. 01. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W4 S7 P21 Sp6	*Trichogramma* sp. nr. *mwanzai*
Kenya-109	31. 01. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W5 S9 P6 Sp5	Not identified
Kenya-110	31. 01. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W5 S9 P29 Sp3	*Trichogrammatoidea* sp. nr. *lutea*

Cont. Appendix 2

Egg Parasitoid Sample	Date of Collection	Place of Collection	Host Insect	Host Plant	Field Trial Code	Identified Scientific Name (Dr. J.C. Monje, University of Hohenheim, Germany)
Kenya-111	27. 01. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W4 S8 P6 Sp3	*Trichogramma* sp. nr. *mwanzai*
Kenya-112	20. 01. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W3 S6 P12 Sp9	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-113	03. 02. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W5 S10 P22 Sp8	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-114	07. 02. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W6 S11 P17 Sp13	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-115	14. 02. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W7 S13 P17 Sp1	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-116	03. 02. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W5 S10 P22 Sp15	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-117	03. 02. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W5 S10 P22 Sp10	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-118	10. 02. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W6 S12 P33 Sp12	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-119	07. 02. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W6 S11 P17 Sp7	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-120	14. 02. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W7 S13 P26 Sp9	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-121	17. 02. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W7 S14 P22 Sp5	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-122	02. 03. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W9 S18 P17 Sp6	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-123	28. 02. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W9 S17 P26 Sp2	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-124	14. 02. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W7 S13 P14 Sp1	Thelytokous species
Kenya-125	06. 03. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W10 S19 P34 Sp7	*Trichogramma* sp. nr. *mwanzai*
Kenya-126	14. 02. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W7 S13 P29 Sp16	*Trichogramma* sp. nr. *mwanzai*
Kenya-127	14. 02. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W7 S13 P34 Sp8	*Trichogramma* sp. nr. *mwanzai*
Kenya-128	21. 02. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W8 S15 P14 Sp3	*Trichogramma* sp. nr. *mwanzai*
Kenya-129	09. 03. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W10 S20 P14 Sp6	*Trichogramma* sp. nr. *mwanzai*
Kenya-130	09. 03. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W10 S20 P29 Sp6	*Trichogramma* sp. nr. *mwanzai*
Kenya-131	13. 03. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W11 S21 P6 Sp7	*Trichogramma* sp. nr. *mwanzai*
Kenya-132	13. 01. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W2 S4 P7 Sp9	*Trichogramma* sp. nr. *mwanzai*
Kenya-133	24. 01. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W4 S7 P21 Sp4	*Trichogramma* sp. nr. *mwanzai*
Kenya-134	23. 03. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W12 S24 P6 Sp1	Not identified
Kenya-135	28. 02. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W9 S17 P29 Sp4	*Trichogramma* sp. nr. *mwanzai*
Kenya-136	17. 01. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W3 S5 P17 Sp12	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-137	07. 02. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W6 S11 P22 Sp1	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-138	10. 02. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W6 S12 P26 Sp2	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-139	10. 02. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W6 S12 P33 Sp6	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-140	14. 02. 2000	Muhaka	*Plutella xylostella*	Kale	Y2 W7 S13 P33 Sp4	*Trichogrammatoidea* sp. nr. *lutea*

Cont. Appendix 2

Egg Parasitoid Sample	Date of Collection	Place of Collection	Host Insect	Host Plant	Field Trial Code	Identified Scientific Name (Dr. J.C. Monje, University of Hohenheim, Germany)
Kenya-141	16. 03. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W11 S22 P6 Sp6	*Trichogramma* sp. nr. *mwanzai*
Kenya-142	31. 01. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W5 S9 P7 Sp3	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-143	17. 02. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W7 S14 P21 Sp16	*Trichogramma* sp. nr. *mwanzai*
Kenya-144	14. 02. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W11 S22 P7 Sp3	*Trichogramma* sp. nr. *mwanzai*
Kenya-145	13. 03. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W11 S21 P7 Sp2	*Trichogramma* sp. nr. *mwanzai*
Kenya-146	28. 02. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W11 S21 P29 Sp8	*Trichogramma* sp. nr. *mwanzai*
Kenya-147	13. 03. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W11 S21 P34 Sp4	*Trichogramma* sp. nr. *mwanzai*
Kenya-148	13. 03. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W11S21 P34 Sp6	*Trichogramma* sp. nr. *mwanzai*
Kenya-149	02. 03. 2000	Kasarani	*Corcyra cephalonica*	Pigeon pea	Transect Survey	*Trichogrammatoidea* sp. nr. *lutea*
Kenya-150	26. 02. 2000	Kibwezi	*Corcyra cephalonica*	Amaranthus	Transect Survey	*Trichogramma* sp. nr. *mwanzai*
Kenya-151	16. 03. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W11 S22 P14 Sp8	*Trichogramma* sp. nr. *mwanzai*
Kenya-152	20. 03. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W12 S23 P14 Sp1	*Trichogramma* sp. nr. *mwanzai*
Kenya-153	20. 03. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W12 S23 P14 Sp1	*Trichogramma* sp. nr. *mwanzai*
Kenya-154	20. 03. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W12 S23 P14 Sp1	*Trichogramma* sp. nr. *mwanzai*
Kenya-155	20. 03. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W12 S23 P14 Sp1	*Trichogramma* sp. nr. *mwanzai*
Kenya-156	16. 03. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W11 S22 P6 Sp1	*Trichogramma* sp. nr. *mwanzai*
Kenya-157	16. 03. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W11 S22 P6 Sp1	Not identified
Kenya-158	16. 03. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W11 S22 P6 Sp1	Not identified
Kenya-159	16. 03. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W11 S22 P6 Sp1	Not identified
Kenya-160	16. 03. 2000	Muhaka	*Chilo partellus*	Sorghum	Y2 W11 S22 P29 Sp3	Not identified