ASSESSMENT BY PERFORMANCE ATTRIBUTES OF INDIGENOUS EGG PARASITOIDS (HYMENOPTERA: TRICHOGRAMMATIDAE) FOR BIOCONTROL OF AFRICAN BOLLWORM (*HELICOVERPA ARMIGERA*) IN KENYA.

ANDREW KALYEBI

B.Sc., M.Sc. (Makerere University, Kampala, Uganda)

A THESIS SUBMITTED IN FULFILMENT OF THE REQUIREMENTS FOR THE AWARD OF THE DEGREE OF DOCTOR OF PHILOSOPHY (AGRICULTURAL ENTOMOLOGY) OF KENYATTA UNIVERSITY (NAIROBI, KENYA).

AUGUST 2004

DECLARATION

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

Andrew Kalyebi

Signature.....

Date SEPTEMBER 16, 2004

We as Kenyatta University and ICIPE supervisors confirm that the work reported in this thesis was carried out by the candidate under our supervision. We have read through it and hereby approve the thesis for submission.

Prof. J. M. Mueke (Department of Zoology, Kenyatta University)

Signature.....

Date October 4, 2004

Dr. S. Sithanantham (International Centre of Insect Physiology and Ecology).

Signature.....

Date 17/09/2004

DEDICATION

I dedicate this PhD Thesis to

- My mum, Costanzia Kyakuwaire for her love and constant support.
- My late sister Musawo Florence Nabirye who always wished to see me through but never lived to this day and
- My children, Ernest, Amanda and Arnold to whom it should be a source of inspiration into the future.

ACKNOWLEDGEMENTS

I would like to acknowledge with gratitude my sponsors the Deutscher Akademischer Austauschdienst (DAAD) and the International Centre of Insect Physiology and Ecology (ICIPE) for the financial support they provided during the course of this study.

I wish to thank most sincerely my supervisors, Prof. J.M. Mueke, Dr. S. Sithanantham and Prof. Dr. W. A. Overholt for the deep interest they took in this work and for immense guidance and critical discussions rendered during various stages of the study period.

Dr. S.A. Hassan of the Federal Institute of Biological control, Darmstadt, Germany is greatly acknowledged for advise and full laboratory facilities during my visit to his laboratory and for invaluable discussions on various aspects. My appreciation is expressed to Dr. A. Hertz and O. Zimmermann of BBA, Darmstadt for various discussions on different aspects of *Trichogramma* and for moral support. Dr. F. Schulthess is greatly acknowledged for critical reviews of manuscripts on this work.

The technical assistance given by Mr. Dan. Agengo and Ms. J. Kiluvu in the insect rearing unit, and by Jane Waweru, ICIPE made a significant contribution to the work and are hereby acknowledged.

I thank the colleagues in ARPPIS for a social and friendly environment and for support and comfort during difficult times when everything seemed threatening. Special gratitude goes to Dr. Samira Mohammed who devoted her time to proofread the various chapters and for immense support in various ways.

I remain indebted to my parents and siblings who have been there for me all this time. My deepest gratitude goes to my wife, Esther and our children Ernest, Amanda and Arnold, who endured periods of my prolonged absence from home and for their patience, love and unfailing encouragement. So many people have helped, encouraged and contributed in numerous ways to the commencement and completion of this work. I could not name all of you. My sincere thanks to you. Finally, I am grateful to the Almighty God for giving me the courage, wisdom, strength and motivation to stand the test of time and for enabling me to complete this work successfully.

ABSTRACT

Trichogrammatid egg parasitoids are used extensively for the control of several lepidopteran pests in many areas of the world. They are the most promising biological control agents for inundative releases against the African bollworm *Helicoverpa armigera*, an injurious pest of several agricultural crops in Kenya and elsewhere in Africa. To enhance their potential impact in Kenya as well as eastern Africa, collections of indigenous species/strains (*Trichogramma* sp. nr. mwanzai and *Trichogrammatoidea* sp. nr. *lutea* from low altitude, *Trichogramma* sp. nr. *mwanzai* and *Trichogrammatoidea* sp. nr. *lutea* from medium altitude and *Trichogramma bruni* and *Trichogrammatoidea* sp. nr. *lutea* from high altitude) were made from different altitudes in Kenya and were evaluated following different criteria to select potential candidates for use against *H. armigera*. The major focus was their adaptation to a range of temperature and humidity regimes. The criteria included functional response, lifetime parasitism and development and population growth characteristics at six temperatures (10, 15, 20, 25, 30 and 35°C) and two humidity regimes (40-50 and 70-80%). Preference of parasitoids for target pest and relative suitability for development among five lepidopteran hosts was also investigated, in addition to testing their capacity to attack *H. armigera* eggs occurring on two host plants (tomato and okra) in laboratory and field experiments.

Functional response studies revealed that temperature affected parasitisation rates of the strains significantly, while relative humidity did not. *Trichogrammatoidea* sp.nr. *lutea* from high altitude, *Trichogramma* sp. nr. *mwanzai* from low altitude and *T.* sp. nr. *mwanzai* from medium altitude showed higher parasitism across the widest temperature

range. There was no relationship between source altitude/climate and performance of the strains at the temperatures tested.

Temperature and humidity interactions affected lifetime fecundity and progeny production. The highest parasitism at the two humidity levels was at 30°C for all the strains. The interaction of the two factors also affected adult longevity, which was longer at the lower than higher humidity. Survival followed a type I-survivorship curve at lower temperatures and a type III survivorship curve at the higher temperatures. These studies revealed that *T.* sp. nr. *mwanzai* from both low and medium altitudes and *T.* sp. nr. *lutea* from medium altitude appear as promising candidates for augmentative biocontrol of *H. armigera*.

Temperature and humidity interactions also affected the population growth and development of parasitoids although temperature appeared to be more important. Developmental time was inversely related to temperature. The intrinsic rate of increase was found to increase with increasing temperature up to 30°C. The finite rate of increase also followed the same trend. The net reproduction rate also increased with increasing temperature up to a maximum at 30°C. Both net reproduction and intrinsic rate of increase were higher at the lower humidity. Temperature negatively affected generation time of parasitoids regardless of the humidity level.

Host acceptability studies showed that all the six species/strains did accept all the tested host species, although acceptance levels varied among strains and hosts. Most species/strains showed greater preference for noctuids over pyralids and yponomeutids. Suitability of hosts for progeny development varied from one species/strain to another.

The different species/strains did not show significant differences in parasitising eggs of *H. armigera* when placed on two host plants, namely tomato and okra.

From these studies, *T.* sp. nr. *mwanzai* (M), *T.* sp. nr. *mwanzai* (L) and *T.* sp. nr. *lutea* (M) offer great potential as candidates for augmentative biocontrol of *H. armigera* in Kenya.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER ONE

1.0. GENERAL INTRODUCTION AND LITERATURE REVIEW

1.1 Incidence and distribution of *Helicoverpa armigera*

The African bollworm, *Helicoverpa* (*Heliothis*) *armigera* (Lepidoptera: Noctuidae), is one of the major pests in Africa, attacking a variety of food and cash crops (Annecke and Moran, 1982). It is widely distributed on more than 180 cultivated and uncultivated host plants (Manjunath *et al.*, 1989) and is one of the most important injurious pests of agricultural crops (Van den Berg *et al.*, 1993). In Eastern Africa, it is a sporadic serious pest of cotton, tobacco, maize, sorghum, millet, beans, pigeon pea and other legumes, vegetables (especially tomato), sunflower and citrus (Karel, 1985). In the vegetable crops, the pest generally causes yield reduction due to direct damage on leaves, flowers, buds and fruits and ultimately affects production (Ikin *et al.*, 1993). The species destroys mainly the reproductive parts and also acts as a defoliator. *H. armigera* prefers hairly surfaces for oviposition and this usually coincides with flower production or bud burst. In Kenya, for example, it is one of the most important pests of a wide range of crops including many weeds (Cock *et al.*, 1991; Van den Berg *et al.*, 1993) and export vegetable crops such as peas, beans, capsicum and okra (Oduor *et al.*, 1998; Farrel *et al.*, 1995).

1.1.2 Biology and life cycle of *H. armigera*

Over a reproductive lifetime of 5-10 days, female moths produce many eggs (200-1200) (Reed, 1965b), which are deposited singly on plants (Beeden, 1974). Adults are strongly attracted to crops that provide honeydew or nectar and feeding extends their

lifespan. Under local temperatures in Tanzania for example, larvae develop in 21 days and pupae in 17 days (Reed, 1965b). With a pre-oviposition period of 1-4 days (Singh and Singh, 1975) and an egg development period of 4-5 days (Van den Berg, 1993), the generation time is about 45 days. The larval period lasts about 18 days and fully-grown larvae leave the plant to pupate in soil (3-15 cm deep). A proportion of pupae may enter summer diapause (Reed, 1965a; Hackett and Gatehouse, 1982) or winter diapause (Fitt, 1989), which extends the pupal period. The pupal period depends on temperature. The adult female moths live for about 14 days while the male moths live about 9 days. The longevity of adult moths depends on availability of food and temperature. A high fecundity and a shorter generation time give *H. armigera* great capacity to increase (Fitt, 1989).

1.1.3 Damage and economic impact by *H. armigera*

Helicoverpa armigera is a pest of major importance in most areas where it occurs. Its considerable pest significance is based on the peculiarities of its biology – its mobility, polyphagy, rapid and high reproductive rate and diapause make it particularly well adapted to exploit transient habitats such as man made agroecosystems.

Larvae of *H. armigera* usually live hidden within the fruiting parts of the plant during most of their development. The young larvae cause damage by feeding on soft plant parts rich in protein (especially buds and flowers) and on young pods but the main damage is done by old larvae burrowing into green pods and eating developing seeds (Karel, 1997). The larvae or caterpillars also feed on leaves and buds in some host plants. Normally, they consume only a small portion of the flower or fruit and move to the next, leaving a

trail of damaged flowers or fruits that produce no harvest (Van den Berg *et al.*, 1993). Secondary rots may develop within buds or fruits of vegetables like tomatoes after the insect's attack. The voracious larval feeding behaviour leads to substantial economic loss (Reed and Pawar, 1982). The ability of ovipositing females to locate and utilize a wide range of hosts from a number of families is one of the major factors contributing to the pest status of this moth (Zalucki *et al.*, 1986; Fitt, 1989, Zalucki *et al.*, 1994). *Helicoverpa armigera* is able to cause substantial direct, indirect and cosmetic damage and consequently lead to losses in the quality and quantity within different crops. In Mbita, Kenya, damage by *H. armigera* has been estimated to be about 24% on tomato (ICIPE, 1997).

1.1.4 Control of *H. armigera*

Control of *H. armigera* has mainly relied on the use of insecticides. There is however, a strong ability of *H. armigera* to develop resistance to a variety of insecticides and cases of resistance to organochlorides and pyrethroids in the field have been reported (Wolfenbarger *et al.*, 1981). Besides causing resistance in pests, chemical pesticides are expensive to the farmer, have adverse effects on the environment and cause health hazards (Balk and Koeman, 1984). Moreover, pesticides cause destruction of natural enemy complexes and hence disrupt the natural enemy balance that often exists between pests and their natural enemies (Ehler *et al.*, 1973). This results in secondary pest outbreaks. In Tanzania and the Sudan, the impact of parasitoids on *H. armigera* in cotton declined during the last decade as the use of insecticides increased (Reed, 1965b; Balla, 1981).

Most insecticide applications are targeted at the larval stages, but as these are only really effective when larvae are small, the need to scout for eggs and spray soon afterward is paramount. Young larvae are difficult to find, and older larvae soon burrow into the floral organs where they become less accessible to contact insecticides, require higher doses to kill and cause direct economic loss. Moreover resistant larvae are still susceptible when they are less than 4 days old, so that the targeting of neonates is essential in areas where resistant populations are present (Daly, 1988). The considerable selection pressure, which *H. armigera* has experienced, particularly to the synthetic pyrethroids, which were used predominantly in the early 1980s, has resulted in the development of resistance to the major classes of insecticides in many of the areas where these have been used. Field failures from pyrethroid resistance have been reported from Australia, Thailand, Turkey, India, Indonesia and Pakistan.

In eastern Africa, resistance to pesticides among populations of *H. armigera* (Ikin *et al.*, 1993) makes chemical control less efficient. This scenario has prompted the need for developing Integrated Pest Management (IPM) strategies involving the use of biological and other safer pest control techniques especially for the major pests on vegetable crops. The augmentative release of mass-reared trichogrammatid egg parasitoids is a promising method to reduce egg hatching and subsequent crop damage caused by larval feeding of several lepidopteran pests such as *H. armigera* (Smith, 1996). Although *H. armigera* is recognised as an important pest of vegetables in eastern Africa, no evaluations for the potential impact of its egg parasitoids have been undertaken so far in the region. It is therefore important to assess the potential for utilizing egg parasitoids to control major vegetable pests in Kenya.

1.1.5. Biological control of *H. armigera*

With the deteriorating confidence in the use of insecticides, there is growing emphasis and increased interest in developing an Integrated Pest Management (IPM) approach to avoid resurgences (Gunning *et al.*, 1984; Daly and Murray, 1988; McCaffery *et al.*, 1989). This requires the development of ecologically sound pest control practices that utilize and conserve the natural enemies of insect pests as one of the ways to achieve sustainable crop production. Such practices maximise the contribution of natural enemies to the depression of *H. armigera* populations (Greathead and Waage, 1983). Biological control is especially important to smallerholder farmers with limited capital reserves since it is generally less costly than chemical methods and does not cause degradation of resources. The methods necessary within an IPM framework include among others regulatory control, cultural control, use of sanitary methods and host plant resistance and biological control.

Biological control involves use of naturally occurring enemies such as predators, parasitoids and pathogens in regulating populations of economically important pests including *Heliothis/Helicoverpa* species (Carner and Yearian, 1989). The natural enemies of *H. armigera* have been studied in several countries in Africa (Van den Berg *et al.*, 1988) and most of them are parasitoids, which vary from crop to crop and from country to country within Eastern Africa. One of the most important genera among the egg parasitoids used to control *H. armigera* is *Trichogramma*, tiny wasps of the order Hymenoptera, family Trichogrammatidae and subfamily Chalcidoidea.

1.2 Diversity and distribution of the genus *Trichogramma*

The genus *Trichogramma* is one of the 80 genera in the family Trichogrammatidae. All members of the genus are parasitoids of insect eggs with a few exceptions (Viggiani, 1981). Trichogrammatidae include some of the smallest insects, ranging, from 0.2 to 1.5 mm long. Within the genus *Trichogramma*, there are 145 described species, worldwide. The distribution of the genera in the bio geographical regions of the world was summarised by Yousuf and Shafee (1987). Virtually all larger genera are cosmopolitan with the exception of *Trichogrammatoidea*, which is either not present or poorly represented in the Palaearctic and Nearctic regions. *Trichogramma* is one of the most described species probably because of its importance as a biological control agent.

1.2.1 Taxonomy

Trichogramma are difficult to identify because they are small and have generally uniform morphological characters. Also, certain physical characteristics such as body colour, abundance, body length and hairs were found to vary with season, rearing temperature and host on which the adults were reared (Flanders, 1931, Oldroyd and Ribbands, 1936; Quednau, 1960). Because of these difficulties and the lack of type specimens, species names in the literature in North America before 1980 were largely unreliable (Pinto and Stouthamer, 1994). The taxonomy of *Trichogramma* at species level is being developed and from the 180 species known (Pinto, 1998), about 120 have been described in the last 20 years (Voegele, 1988; Pinto, 1998).

A major advance in the systematics of *Trichogramma* was the discovery that characteristics of male genitalia could be used to identify species (Nagarkatti and Nagaraja, 1968, 1971, 1977) and this is the primary basis of identification today. Females cannot be identified with the same level of confidence, so collections for identification must include males. In addition to physical characteristics, studies of reproductive compatibility and mode of reproduction also have been especially valuable in identifying species (Pinto and Stouthamer, 1994). Taxonomic support is necessary to identify natural and introduced species to ensure mass cultures are not contaminated by undesired species.

1.2.2 Biology and life cycle

Trichogramma wasps primarily parasitize eggs of moths and butterflies (Lepidoptera). However, certain species of *Trichogramma* also parasitize eggs of beetles (Coleoptera), flies (Diptera), true bugs (Heteroptera), and other wasps (Hymenoptera) and lace wings (Neuroptera). Most indications suggest that trichogrammatids have a greater fidelity to microhabitat than taxon of the host as determining the host range (Wajnberg and Hassan, 1994).

It has been reported that the adult female wasp uses chemical and visual cues to locate a bollworm egg. The chemical cues called kairomones, are on the moth's scales left near the egg by the female moth during oviposition (Nordlund *et al.*, 1981). Some of the kairomones are also bollworm sex pheromones. Egg shape and colour also may be visual clues to the wasp (Ruberson and Kring, 1993). When a suitable host is encountered, the female *Trichogramma* examines the egg by antennal drumming, it drills

a hole into it with her ovipositor and lays one or more eggs within the host egg, depending on its size. 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. A female *Trichogramma* under laboratory conditions parasitises from 1 to 10 bollworm eggs per day or from 10 to 190 during her life span. Large females parasitise more eggs than smaller ones and females feeding on honey and young bollworm eggs live an average of 11 days (Ruberson and Kring, 1993).

When *Trichogramma* finds preferred host eggs, it will usually stay on or near them for a longer time until most of them are parasitised. Less preferable host eggs may be totally rejected or the parasitoid might lay a few eggs before leaving the location to search for more suitable hosts. Therefore, variation in host preference by *Trichogramma* species is an important factor with regard to biological control.

Bollworm eggs in the early stages of development are more suitable for parasitoid development. Older bollworm eggs, especially those in which the head capsule of the larva is visible are not usually parasitised and if they are, parasitoid survival is much lower (Ruberson and Kring, 1993).

The yolk and embryo of the parasitised bollworm eggs are digested before the *Trichogramma* egg hatches. The eggs hatch in about 24 h and the parasitoid larvae develop very quickly. Larvae develop through three instars. During the third instar (3-4 days after host egg was parasitised), dark melanin granules are deposited on the inner surface of the egg chorion, causing the bollworm egg to turn black. Larvae then transform to the inactive pupal stage. After about 4-5 days, the adult wasps emerge from the pupae and escape from the bollworm egg by chewing a circular hole in the eggshell. The black

layers inside the chorion and exit hole are evidence of parasitism by *Trichogramma*. The life cycle from egg to adult requires about 9 days, but varies from 8 days to as many as 17 days at 15.5°C when mid summer temperatures are high (32°C). Adults are most active at a range of 23.8-29°C.

Trichogramma adults emerge from the host eggs in the early morning. Males emerge first and remain at the host egg to mate with emerging females if they are present. Mated females produce male and female offsprings. Unmated females (parthenogenetic) produce only males. The females begin egg laying within a few hours of emergence. *Trichogramma* overwinter as immature forms in host eggs. Some species enter a state of diapause, which allows them to tolerate long periods of subfreezing temperatures. Other species such as *T. pretiosum* slow their rate of development and may be active as adults during warm days. The lack of host eggs in the early springs may be a critical factor in determining the number of *Trichogramma* that are later present to move into field crops in the following season (Lopez and Morrison, 1980).

Trichogramma species are pro-synovigenic insects, that is, the females have 30-80 mature eggs at the time of emergence (Franz and Voegele, 1974) and may continue to mature with time. As in most Hymenoptera, the normal mode of reproduction in *Trichogramma* is arrhenotoky. Haploid males arise from unfertilised eggs and diploid females from fertilised eggs. A less common mode of reproduction is thelytoky, in which unfertilised eggs give rise to diploid female offsprings. Todate, thelytoky has been reported for 14 *Trichogramma* species (Stouthamer, 1997). Thelytokous *Trichogramma* can be converted to arrhenotoky by feeding antibiotics to the infected females or by rearing them at high temperatures (Stouthamer *et al.*, 1990).

1.2.3 Sex ratio in *Trichogramma*

Most *Trichogramma* species, like other Hymenoptera, regulate the sex ratio of their offsprings by arrhenotoky: fertilized zygotes produce females; unfertilised eggs become males (Suzuki *et al.*, 1984). A few *Trichogramma* species are thelytokous and produce only female progeny (Flanders, 1965; Suzuki and Hiehata, 1985). The adjustment of sex ratio to host size has been reported for numerous parasitoid species (Jones, 1982; Waage and Godfray, 1984; Werren, 1984; Waage, 1986). In large hosts, mated *Trichogramma* usually produce highly skewed sex ratios, with female offspring outnumbering male offspring (Suzuki *et al.*, 1984; Waage and Ng, 1984; Waage and Lane, 1984). Sex ratio variation is widespread among biological control agents and can be attributed to a wide range of causes (King, 1987, 1993; Ebbert, 1993; Luck *et al.*, 1993; Sabelis and Nagelkerke, 1993). The variability in sex ratios can either benefit or hinder the practice of biological control. Consistently female biased sex ratios are expected to benefit biological control because of the increased population growth rates and, in the case of parasitoids, because males do not contribute to pest mortality (Waage, 1982; Hassell *et al.*, 1983; Hall, 1993). On the other hand, male biased sex ratios can without a question be a hindrance to successful biological control.

1.3. General approaches in biological control

There are three general approaches to biological control: importation, augmentation and conservation of natural enemies. Each of these techniques can be used either alone or in combination in a biological control program.

Importation of natural enemies, sometimes referred to as classical biological control, is used when a pest of exotic origin is the target of the biocontrol program. Pests are constantly accidentally or intentionally being brought into countries where they are not native. Many of these introductions do not result in establishment or if they do, the organisms may not become pests. However, it is not uncommon for some of these introduced organisms to become pests, due to lack of natural enemies to suppress their populations. In these cases, importation of natural enemies can be highly effective (Caltagirone, 1981). Once the country of origin of the pest is determined, exploration in the native region can be conducted to search for promising natural enemies. If such enemies are identified, they may be evaluated for potential impact on the pest organism in the native country or alternatively imported into the new country for further study. They must first be placed in quarantine for one or more generations to be sure that no desirable species are accidentally imported (diseases, hyperparasitoids etc.).

Augmentation is the direct manipulation of natural enemies to increase their effectiveness and involves the periodic release of a natural enemy that does not occur naturally in sufficient numbers to keep a pest below damaging levels. Augmentation can be carried out by inundative releases or inoculative releases. The inundative approach is achieved by multiple releases of insectary –reared natural enemies. The released insects control pests present at the time, but there is little expectation that later generations will persist at sufficient levels to provide control. This approach requires a large number of natural enemies at the precise time when pest eggs are present and crop and weather are conducive to the release. Inoculative releases involve one or several releases to establish populations of the natural enemy before pest densities have begun to increase. The

natural enemy reproduces on the target pest or an alternate host and its population increases to levels sufficient to control the target pest later in the season.

Conservation is a critical component in any biological control effort and involves identifying the factor(s), which may limit the effectiveness of a particular natural enemy, and modifying them to increase the effectiveness of the beneficial species. In general, conservation of natural enemies involves either, reducing factors, which interfere with natural enemies, or providing resources that natural enemies need in their environment to increase their impact on pests. Examples include preventing the use of broad-spectrum insecticides and planting strip crops in and around the field to provide food and habitat for natural enemies. Insecticides such as those based on *Bacillus thuringiensis* and some insect growth regulators for example have very little or no impact on beneficial arthropods and can be used in IPM programs with *Trichogramma*.

1.3.1 Choice of natural enemies in augmentation

According to Overholt (1997), it is necessary to decide whether to select a native or an exotic natural enemy for an augmentation programme. A primary consideration may be whether the target pest 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. However, if the introduced natural enemies do not provide the level of control desired, then it may be necessary to combine the classical approach with the augmentation approach, whereby exotic natural enemies are mass produced and released to increase population suppression (Overholt, 1997).

Some potential advantages and disadvantages of the use of native and exotic natural enemies for augmentation biocontrol are summarized in Table 1. When collecting natural enemies, it is advisable to collect from the same host species, feeding on the same host plant, as the target of the programme and to collect from areas, which have similar climates to the intended area of introduction.

Table 1: Advantages and disadvantages of native and exotic natural enemies for augmentation biological control.

Origin of natural enemy	Advantages	Disadvantages
Native	Adapted to climate	Effective natural enemies may not be available, particularly for exotic pests
	Little concern about environmental impacts	Need for research to identify natural enemies and rearing procedures
	May stimulate the establishment of a local rearing industry	Need for capital investment to start local rearing industry
	Technical assistance may be locally available	
Exotic	Many natural enemies commercially available	Potential concerns about environmental impacts
	Proven effectiveness in other countries	Cost of importation
	Rearing methods known	Legal (importation restrictions and permits)
		Need for adaptive research
		Natural enemies available may not be adapted to climate
		Potentially poor technical assistance

1.3.2 Modern approaches in augmentation of natural enemies

Because most augmentation involves mass-production and periodic release of natural enemies, this type of biological control has lent itself to commercial development. There are hundreds of biological control products available commercially for dozens of invertebrate and vertebrate pests, weeds, and plant pathogens (Anonymous, 1995).

The practice of augmentation differs from importation and conservation in that making durable changes in an agroecosystem to improve biological control is not the primary goal. Rather, augmentation generally seeks to adapt natural enemies to fit the existing production systems. An excellent example of an augmentative practice that has been successfully adapted to a wide variety of agricultural systems is the inundative release of *Trichogramma* wasps. These minute endoparasitoids of insect eggs are released in crops or forests in large numbers (up to several million/ha) timed to coincide with the presence of pest eggs. *Trichogramma* are the most widely augmented species of natural enemy, having been mass-produced and field released for almost 70 years in biological control efforts. Worldwide, over 32 million hectares of agricultural crops and forests are treated annually with *Trichogramma* spp. in 19 countries, mostly China and republics of the former Soviet Union (Li, 1994).

Trichogramma have been used in all three biological control approaches: Importation, inoculation and inundation. Inundative releases achieve an immediate, nonsustaining reduction in the host population. In inoculative releases, however, it is primarily the progeny of parasitoids released at the beginning of the season that have a latter effect on the host population. Inundative releases have often been used in Europe and the Americas (Bigler, 1986; Hassan, 1982; King *et al.*, 1986, 1985a, b; Smith *et al.*,

1990), whereas countries in Asia and parts of the former USSR have put more emphasis on inoculation and occasional introductions (Voronin, 1982; Wang *et al.*, 1988).

Warmer climates favour multivoltine pests and inoculative releases, because the parasitoids can multiply during the low growing season. Inundative releases, which are timed specifically to the ovipositional period of the pest, are more appropriate in northern climates with uni-or bivoltine host species. Several countries use both strategies through the repeated annual applications of *Trichogramma* (Voronin, 1982; El-Heneidy *et al.*, 1990; Li, 1994). In China, a slight modification of the inoculative approach has been used; *Trichogramma* are released in vegetable gardens adjacent to the target crop in ratios from 1:5 to 1:14 (release garden: target crop) (Wang *et al.*, 1988). Introductions of exotic species have occurred in India (Rawat and Pawar, 1991), North America and Russia. The different approaches to the use of *Trichogramma* have resulted in two different perspectives: the inundative approach, which tends to view the parasitoid as a fast-acting replacement of chemical insecticides, and the inoculative/introduction approach, which sees the parasitoid as one aspect of integrated pest management. According to Hassan (1993), inundative releases for the control of lepidopterous pests are being investigated in more than 50 countries and are commercially used on more than 32 million ha each year. Most augmentative biological control programs release *Trichogramma* as pupae within their host eggs, which are timed to emerge soon after their release in the field (King *et al.*, 1985c). Mixing of *Trichogramma* in different development stages has been used to prolong the effect of the parasitoid in the field (Hassan, 1980, 1985; Hassan and Zhang, 2001). *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).

1.4 Use of *Trichogramma* in biological control

The use of *Trichogramma* for controlling insect pests has recorded attention since the 1920s (Flanders, 1929; Morrison *et al.*, 1978; Olkowski and Zhang, 1990; Newton, 1993; Hassan, 1993). Extensive utilization of this parasitoid has been developed for controlling lepidopterans in corn, rice, sugarcane, vegetables and pines (Hassan, 1993). The efficiency of using *Trichogramma* is dependent on several factors which include selection of suitable species, quality of reared parasitoids, reasonable release rates per hectare, release method (inundative/inoculative), methods of conducting release (mechanical/manual), climate during releases, timing of releases and an integration of other methods of pest control particularly chemical control. Although species of the genus *Trichogramma* attack more than 400 species in 203 genera, 44 families and 7 orders (Bao and Chen, 1989), its world wide commercial use is still limited to a small number. Thus it has a great potential and there is need to develop its applications. For effective commercial use however, it is essential to select suitable parasitoid species (either introduced exotic or indigenous) to control the target pests. Some parasitoid species such as *Trichogramma evanescens, T. pretiosum* and *T. dendrolimi* are quite commonly used in many countries against a number of insect pests because they show plasticity of their response to a diversity of habitats. There are also *Trichogramma* species that are found in only one country, and their utilization is restricted.

It is therefore necessary to understand the importance of identification and distribution of natural populations of *Trichogramma* species, which are well adapted to the given climatic conditions. Information regarding the pest one wishes to control and the crops on which the pest feeds is also vital. Usually, the utilization of an indigenous dominant species is preferred although sometimes introduction of exotic species is also necessary, if they are available.

1.4.1 Functional response of *Trichogramma* species

Functional response is a term that has been used to describe changes in the number of attacks per parasitoid (predator) as host (prey) density changes (Solomon, 1949). The density and spatial patterns of hosts are usually considered to influence parasitoid efficiency (the number of hosts parasitised) (Burnett, 1958; Madden and Pimentel, 1965; Hassell, 1982; Stiling, 1987; Walde and Murdock, 1988). One important aspect in evaluating the efficiency of a natural enemy is to study its performance as an individual by examining the attack rate on the host or prey. Knowledge of the functional response may provide some understanding of the host-natural enemy interactions (Hughes *et al.*, 1992; Kidd and Jervis, 1996). It is also essential to understand 'prey death rates' and 'predator rates of increase' (Lawton *et al.*, 1975) and in the consideration of optimal predator foraging behaviour (Charnov, 1976; Cook and Hubbard, 1977; Comins and Hassell, 1979). However, the ability of a biological control agent to maintain the host population at low densities is the main objective of biological control, therefore it is important to study the parasitoids behaviour at densities characteristic of those encountered in the field.

Functional response has been extensively studied by Holling (1959) and reviewed in some detail by the same authour (Holling, 1961, 1966). Holling (1959) demonstrated that a completely general feature of parasitoid or predator attack is 'handling time' and this has a very important effect on their functional response. Handling time has been defined as the interval between a natural enemy first encountering a host or prey and when search (for the next host or prey) is resumed (Varley *et al.*, 1973). Investigation of the effects of environmental factors on the behaviour of *Trichogramma* is essential to understand host-natural enemy interactions. Changes in climatic conditions or in size of the arena searched by the predator may result in a varying predation capacity (Mohaghegh *et al.*, 2001). Holling (1959) suggested a model, the 'disc equation' to illustrate the principal of time budget in behavioural ecology. The model assumes that a parasitoid (predator) spends its time on two kinds of activities: searching for hosts (prey) and prey handling. Holling (1959) and Hassell *et al.* (1977) considered 3 major types of functional responses: Type I (in which predation is proportional to prey density that is, prey mortality due to predation is constant), Type II (search rate is constant and prey mortality declines with prey density) and Type III (in which search rate increases with increasing prey density). Of these, only type III can contribute to stability of predator or parasitoid population interactions (Holling, 1959; Hassell and May, 1973; Murdoch and Oaten, 1975).

1.4.2 Evaluation of natural enemies by use of life tables

Studies evaluating the impact of natural enemies on their hosts fall into two broad categories. The first category involves separate host populations; one population with a

particular natural enemy and the other without. When the resulting densities and mortalities in the population are compared, the difference is attributed to the natural enemy. Such evaluations quantify the impact of the natural enemy largely in terms of mortality caused by an agent to a given generation or a set of generations of the host and the consequent overall reduction in host density (Bellows *et al.*, 1992). However, these evaluations do not provide descriptions of other sources of mortality that act together with the natural enemy. The second category involves the construction and analysis of life tables for the affected generations and this is done for either single or multiple locations or generations. Use of life tables may be geared towards two types of questions about natural enemies (a) they may be used to allow detailed quantification of the mortality caused to host population by the natural enemy; that is, mortality due to natural enemies is compared with other sources of mortality acting on the population and other factors separately quantified. (b) Life tables allow the ecological role of a natural enemy in a particular system (whether it is a source of regulation contributing to stability) to be determined. Southwood (1978) gives a general description of life table construction, organization and terminology.

The impact of parasitoids is generally measured as apparent mortality, that is, the proportion of a host generation in a susceptible stage that is ultimately killed by parasitoids. Mortality is usually equated to percentage parasitism in a generation, but sometimes, high numbers are killed through host feeding or ovipositor piercing of the adult parasitoids (Kidd and Jervis, 1989). Three approaches to study the impact of parasitism are discussed by van Driesch *et al.* (1991) and Bellows *et al.* (1992).

Life tables describe the numbers of separate developmental stages of an organism (L_x) over one (or part of a) generation, and the numbers dying (d_x) from separate stage-specific mortality factors, including natural enemies (Southwood, 1978).

1.4.3 Parasitoid quality

The efficacy of biological control is dependent on the quality of natural enemies produced for release (Hassan and Zhang, 2001). The reliability of biological control would be considerably improved if producers observed product quality standards. Failures of egg parasitoids to control agricultural pests could be due to the use of less suitable strains (Hassan, 1989).

Populations for inundative releases are often selected on the basis that those with high fecundity, emergence, sex ratio (percentage of female to male offspring), longevity, host preference for the target species, host searching ability and tolerance to weather conditions, will be the best. A population with these characteristics is of high quality because these traits are assumed to be ecologically important for these parasitoids when released inundatively. The selection of potential agents and release strategy of natural enemies are critical aspects for the success of these programmes (Ehler, 1990).

Several authors have indicated particular attributes that would increase the biological abilities of the parasitoids and their effectiveness in the field (Pak, 1988; Bigler, 1989; Pak *et al.*, 1991, Pavlik, 1993).

While Parasitoid quality is defined as the performance of a parasitoid in its intended role after release in the field, it is not clear which biological trait(s) are the best to make this prediction. Bigler *et al.* (1991) proposed that a few simple life history traits

such as the number of parasitised eggs or deformed individuals, parasitoid emergence, sex ratio, longevity and activity, be used to estimate quality. A few studies have also attempted to combine several traits into a single quality index to predict field performance (Greenberg, 1991; Cerutti and Bigler, 1995). In the field, *Trichogramma* must also cope with seasonal and daily fluctuations in temperature (Calvin *et al.*, 1984; Pak and van Heiningen, 1985). Tolerance of *Trichogramma* species/strains to environmental conditions characteristic of the field conditions in the release area is important (Hassan, 1994). Hassan (1980) showed that release of a mixture of developmental stages could prolong the effect of the parasitoid in the field and increase efficacy.

1.4.4 Host preference and suitability

Parasitoids of the genus *Trichogramma* are extensively used as biological control agents because they attack eggs of several lepidopteran species that cause serious damage to many kinds of crops. This potential polyphagy is tempered however, by the tendency of most *Trichogramma* to show strong preference to certain host (s), crop (s) and climatic conditions (Hassan and Gou, 1991). Because of these preferences, *Trichogramma* are likely to have a limited host range in any given environment, decreasing the potential for non-target impacts. In designing a biocontrol programme that involves a polyphagous natural enemy or assessing it retrospectively, an essential step entails establishing whether the target pest species will be parasitised relatively frequently by the proposed agent and whether it will serve as a suitable host for the candidate parasitoid. A more

complete interpretation of the polyphagous host relationships requires information on the relative rates of parasitism across host species (host preference) and on host suitability.

One of the primary concepts in selecting for a biological control programme is that a pest should be suitable for the development of the parasite (De Bach, 1964). Suitability of pests needs to be investigated before directing the biological control programme against them. Host suitability encompasses various aspects of the host quality that affects the insect performance (Singer, 1986). The suitability of the pest host for parasitoid development depends on several factors, including environmental factors, the ability to evade the host's internal defensive system, competition with other parasitoids, the presence of toxins detrimental to the parasitoids eggs or larvae and the host's nutritional adequacy (Vinson and Iwantsch, 1980).

Biological control often involves the ability of one organism to locate, interact with, or avoid another organism. Parasitoid wasps of the genus *Trichogramma* employ a vast array of chemical cues in the search and successful attack of host eggs. Moth scales are known to contain contact kairomones that stimulate searching behaviour in *Trichogramma* species (Lewis *et al.*, 1972; Smits, 1982; Noldus and van Lenteren, 1985b; Zaborski *et al.*, 1987). Host accessory gland secretions present on and around host eggs, also cause arrestment and induce host recognition by parasitoids (Noldus and van Lenteren, 1985b; Nordlund *et al.*, 1987; Pak and Je Jong, 1987). Since olfactory cues often control host-parasitoid interactions, an understanding of olfactory response is important in effective use of biological control agents.

1.4.5 Host plant preference

Parasitoids are usually rather specific natural enemies that attack a limited number of insect species. However, their specificity should be considered not only on the level of the insect but also on the level of the plant (Takabayashi *et al.*, 1998). Parasitoids may use hosts that feed on one or a few species of plants only but alternatively, the host of a parasitoid may be a generalist that feeds on a wide range of plant species. Parasitoid behaviour has been studied for several decades and a wealth of knowledge has been obtained on different parasitoid species (Vinson, 1976; Vet and Dicke, 1992; Turlings *et al.*, 1993; Takabayashi and Dicke, 1996). However, to understand differences among parasitoid species, it is important to consider the ecological context in which a parasitoid functions (Vet and Dicke, 1992).

Crop plants may influence the effectiveness of biological control in various ways, one of which is the production of cues that guide natural enemies to infested plants (Dicke, 1996). The impact of host plants on the host range of parasitoids is important as several *Trichogramma* species are found to parasitize a host on certain plants but not on others (Rabb and Bradley, 1968; Abera, 2001). Plant odours have a role to play in host habitat location by *Trichogramma* (Altieri *et al.*, 1981, 1982; Nordlund *et al.*, 1985). Volatile chemicals from the food plant of the host are released in relatively large amounts and provide long range, olfactory information to the parasitoid, whereas chemicals involved in host location, once the parasitoid reaches the microhabitat of its hosts, appear to operate at short range and may originate from the host itself and from its by-products. The parasitoid must first choose a habitat and then search within that habitat for the host (Vinson, 1976). Identification of the parasitoid's habitat preferences is an important

predictor of the ecological host range (Rutledge and Wieldenmann, 1999). Researchers could target more effectively a host species by finding a parasitoid species that has a preference for the habitat of the host.

1.5 OBJECTIVES

1.5.1 General objective

To evaluate the relative adaptation of six indigenous trichogrammatid species/strains to different temperature and humidity regimes as well as their relative preferences to different hosts and host plants.

1.5.2 Specific objectives

(i) To determine and compare the functional responses among six indigenous trichogrammatid species/strains to varying host numbers, under different regimes of temperature and relative humidity

(ii) To evaluate the effect of temperature and relative humidity on parasitism, adult longevity, progeny production and sex ratio of six indigenous trichogrammatid species/strains and select the better adapted strains for augmentative control of *H. armigera*.

(iii) To investigate the effect of temperature and relative humidity on the development and population growth of six indigenous trichogrammatid parasitoid strains so as to assess their climatic adaptability.

(iv) To investigate the relative preference of six trichogrammatid species/strains for the target pest, *Helicoverpa armigera* and its suitability in comparison to other lepidopteran hosts.

(v) To determine the relative preference among trichogrammatid egg parasitoid species/strains for *H. armigera*, when occurring on two host plants, tomato and okra.

1.6 Hypotheses

(i) There is no natural variability between and within the indigenous trichogrammatid species/strains for adaptation to different temperature and humidity regimes.

(ii) The potential of the trichogrammatid species/strains to parasitise *H. armigera* is not dependent upon their host range.

(iii) The performance (percentage egg parasitism) of the trichogrammatid species/strains is not dependent on the host crop.

CHAPTER TWO

2.0. GENERAL MATERIALS AND METHODS

2.1 Rearing of *Trichogramma* in the Laboratory

Trichogramma can be reared in the laboratory 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) that occur commonly as stored product pests in different parts of the world. At the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya, the rice meal moth, *Corcyra cephalonica* was used to rear the egg parasitoids. The parent generation of egg parasitoids recovered from *H. armigera* from the field were offered freshly laid eggs of *C. cephalonica* on a card to parasitise and were fed on a diet comprising of a mixture of 65% honey, 1% gelatine and 33% distilled water prepared on white "Xerox" paper.

Fresh host eggs of *C. cephalonica* were radiated with ultraviolet rays (15 volts UV-lamp) for one hour at a distance of 15 cm prior to their use. Sterilisation of the host eggs is necessary to prevent host larvae from emerging from unparasitised eggs and feeding on the parasitised eggs. After parasitism, eggs were transferred into clean glass vials measuring 2.5 cm diameter by 7.5 cm height to await emergence. Cotton wool was used to cover the glass vials. Upon emergence, the parasitoids were given fresh host eggs and they were fed continuously for maintainance.

The room temperature was maintained at 27±2°C using a portable room heater and 70±10% relative humidity with a photoperiod of 12L: 12D.

2.2. Trichogrammatid species/strains used in the study

The origin of the six species/strains of the native *Trichogramma* and *Trichogrammatoidea* used in the study are presented in Table 2. They were collected from low (L), medium (M) and high (H) altitude locations (< 700, 700-1200 m, > 1200 m, respectively) in Kenya. They were identified by J.C. Monje at the Institute of Phytomedicine, University of Hohenheim (Germany). The trichogrammatids were reared at the International Centre of Insect Physiology and Ecology, Nairobi, Kenya on the rice moth, *C. cephalonica* Stainton (Lepidoptera: Pyralidae). The individuals used were from isofemale lines established from progeny of a single female. Approximately equal numbers of siblings were used to start each colony. All the six species/strains were recoveries from natural parasitisation on *H. armigera* eggs.

At the time of the experiment, *T.* sp. nr. *mwanzai* (M) and *T.* sp. nr. *lutea* (M) had each been in culture for 6 generations; *T.* sp. nr. *lutea* (L) and *T.* sp. nr. *lutea* (H) for 24, while *T. bruni* (H) and *T.* sp. nr. *mwanzai* (L) had been reared for 15 and 106 generations, respectively. The six strains (three of each of the genera *Trichogramma* and *Trichogrammatoidea*) were selected for their high life time fecundity among accessions from the same altitudes. The factitious host *C. cephalonica* was used instead of the target host *H. armigera* because of ease of rearing. Previous work by Muholo (2002) had shown that the two hosts were equally suitable for rearing the six candidate species/strains.

Table 2. Source of the six indigenous Kenyan trichogrammatid species used in the study.

Taxon name	Site of collection	Latitude	Longitude	Elevation (m above sea level)	Min-Max temperature range (°C)	Host plant
Trichogramma sp. nr. *mwanzai* (L)	Muhaka	04° 19' 18.1" S	39° 30' 24.3" E	40	23.2-32.6	Pigeon pea
Trichogrammatoidea sp. nr. *lutea* (L)	Muhaka	04° 19' 18.1" S	39° 30' 24.3" E	40	23.2-32.6	Tomato
Trichogrammatoidea sp. nr. *lutea* (M)	Kwachai	02° 23' 166" S,	038° 00' 319" E	930	16.7-29.3	Tomato
Trichogramma sp. nr. *mwanzai* (M)	Mwea	00° 37' 464" S	037° 21' 801" E	1158	25-31.7	Tomato
Trichogrammatoidea sp. nr. *lutea* (H)	Muguga	01° 14' 590" S	036° 38' 236" E	2227	10.1-23	Tomato
Trichogramma bruni (H)	Muguga	01° 14' 590" S	036° 38' 236" E	2227	10.1-23	Tomato

2.3. Temperature and relative humidity maintenance

The temperatures used in the study were 10, 15, 20, 25, 30 and 35°C at two relative humidity regimes of 40-50 and 70-80%. These temperature regimes covered the minimum and maximum that occur in most of the vegetable growing areas of Kenya while the two humidities cut across altitudes and represent regimes between rainy and non-rainy periods. Humidity was maintained according to procedures described by Hodgman (1948). The recommended salts for regulating humidity levels were placed in a container at the base of cages measuring 30 cm width x 30 cm diameter x 20 cm height. Calcium chloride (0.5 kg) was used to maintain the 40-50% RH at the lower temperatures (10, 15, 20 and 25°C), while ammonium chloride (0.5 kg) was used for 70-80% RH. At higher temperatures (30 and 35°C), ammonium chloride was used to maintain 40-50% RH and cotton wool (soaked in 0.2 liters of water) to maintain the 70-80% RH. The cages were kept closed and sealed with vaseline. A thermo-hygrometer was placed inside the cages to monitor both temperature and humidity levels. Humidities were checked frequently and if required, adjustments were made by addition or removal of water from the salts. The photoperiod in the incubator was set at 12:12 h (L: D).

CHAPTER THREE

3.0. FUNCTIONAL RESPONSE OF INDIGENOUS TRICHOGRAMMATID EGG PARASITOIDS AT DIFFERENT TEMPERATURE AND HUMIDITY REGIMES

3.1. Introduction

The African bollworm, *Helicoverpa armigera,* is an injurious pest of several agricultural crops in Africa (Karel, 1985; van den Berg *et al.*, 1993). In tomatoes in Kenya, it can cause substantial direct and indirect damage leading to losses in quality and quantity amounting to 24% (ICIPE, 1997).

Trichogrammatid egg parasitoids are the most promising biological control agents for inundative releases against lepidopterous pests (Alba, 1990; Singh and Jalali, 1994) and for augmentative biocontrol of *H. armigera* in Africa (Sithanantham *et al.*, 2001). The development of an efficient biological control program with egg parasitoids must involve selection of strains with high efficiency against a target pest, in a given environment (Pak, 1988; Hassan, 1994). Climatic adaptation is one important criterion for selecting potential biological control agents (van Lenteren, 1986). Field-testing of natural enemies for selection for climatic adaptation can be very time consuming, especially if several candidates are involved. Hence, laboratory and screenhouse studies are useful for choosing a suitable candidate (Pak and van Lenteren, 1988). According to Hommay *et al.* (2002), *Trichogramma* strains, which are generally well adapted to the local conditions, are the favoured option for biological control of pests.

Among the biological characteristics, searching ability, fecundity, longevity and sex ratio are commonly used for assessing the potential efficacy of a parasitoid.

Trichogramma, are particularly suitable for use as a 'biotic pesticide' in inundative systems (van Lenteren, 1983) and the number of host eggs successfully parasitised by the adult female parasitoid (fecundity) after release in the field, is the key attribute for selecting species/strains. Ballal and Singh (2003) evaluated the effectiveness of three species of *Trichogramma* based on fecundity. Pak and van Lenteren (1988) reported that strains that showed a high potential in the laboratory also had the ability to perform well in the field, whereas Silva *et al.* (2000) and Thomson and Hoffman (2002) found no strong relationship between laboratory and field performance.

An important attribute in evaluating the efficiency of a natural enemy is the attack rate across a range of densities of the host, i.e., its functional response (Berryman, 1999). According to Hassell (1982), Houck and Strauss (1985), and Walde and Murdoch (1988), functional response is key to understanding host-parasitoid dynamics. Holling (1959) described three types of functional response. Type I response describes a linear relationship between the attack rate and host density, where the slope is the parasitoids searching efficiency, which levels off to a plateau when it reaches the maximum attack rate. The type II response is an asymptotic curve that decelerates constantly as host density increases due to the time it takes for the parasitoid to manipulate the host (i.e. the handling time). The asymptote reflects the maximum attack rate. A sigmoid curve is defined as a type III response, where, as host density increases, the response initially accelerates due to the parasitoids becoming increasingly efficient at finding hosts (attack rate increases or handling time decreases) and then levels off under the influence of handling time or satiation (Berryman, 1999; Hassell, 2000).

The generality that weather sets the limits for tritrophic interactions among poikilotherms and influences the level of control that natural enemies exert is well accepted in biological control (Huffaker *et al.*, 1971). Lack of success in biological control programs has often been attributed to high mortality of natural enemies due to climatic extremes. The outstanding success of *Trichogramma evanescens* Westwood to control the Asian corn borer, *Ostrinia furnacalis* Guenee in the Island of Mindanaw, Philippines, was partly attributed to agreeable climatic conditions (high humidity and moderate temperature) (Tran and Hassan, 1986; Tran *et al.*, 1986). Conversely, releases of both wild and mass-reared *Psyttalia concolor* (Szépligeti), a larval parasitoid of tephritids, failed in some parts of Italy (Raspi and Loni, 1994), possibly due to poor temperature adaptation (Fenili and Pegazzano, 1971).

The present study aimed at comparing the functional responses among six indigenous trichogrammatid species/strains to varying host numbers, under different regimes of temperature and relative humidity.

3.2. Materials and Methods

Six trichogrammatid species/strains were used in this study (Table 2 in chapter 2).

3.2.1. Bioassay

Five host egg densities (6, 12, 18, 24 and 30 eggs, per adult parasitoid) were used in the tests. For each host density, eggs were placed on a card, using diluted adhesive gum. Single one-day-old mated, naïve female parasitoids of each of the species/strains were introduced into glass vials (2.5cm diameter x 7.5 cm height) and provided with minute streaks of honey solution (200ml honey: 100ml distilled water: 3g gelatin) as feed. Ten replicates were conducted for each parasitoid species/strain and egg density.

The egg card was inserted into a glass vial, which was placed in a cage maintained at a specific relative humidity, and then in an incubator at a set temperature (±1°C). The temperature and humidity levels used in this study and details of their maintenance are presented in chapter two.

The adult parasitoids and eggs were held at the temperatures/humidity regimes for 8 hours. After 8 hours, the parasitoid wasps were killed and the egg cards kept at 25±2°C and 60-70% RH. Since selection for inundative release was the objective of this study, the primary attribute studied was the number of host eggs parasitised in unit time, across the temperature and humidity regimes. Determination of the number of host eggs parasitised was based on the numbers of host eggs that turned black after 2-3 days as per the procedure of Strand (1986). The parasitisation (attack) rate was calculated as the ratio of the number of hosts parasitised per unit time to the number availed.

3.2.2. Data analysis

The number of eggs parasitised by the species/ strains at different temperature and relative humidity regimes and under different host densities was compared by analysis of variance (ANOVA) using the PROC GLM procedure of SAS (SAS Institute, 2000). Data on numbers of eggs parasitised were log (x+1) transformed before being subjected to the analysis (Sokal and Rohlf, 1981). When ANOVAs were significant, means were separated using the Student Newman Keul's (SNK) test (Zar, 1996).

Polynomial trends were used to determine the optimum temperatures for the performance of each species/strains. In addition to linear regressions, three non-linear mathematical

models (equations 1-3) were fitted to the data on the number of eggs parasitised at each of the host densities (Trexler *et al.*, 1988).

$N_a = N (1-\exp(-a_1))$----------(1)

$N_a = N (1-\exp(a_1/N))$----------(2)

$N_a = a1/(1+a_2\exp(-a_3/N))$----------(3)

N_a is the number of host eggs parasitised; N is the number of host eggs available and a_1, a_2, and a_3 are estimated parameters. Equation 1 generates type I curves (Nicholson and Bailey, 1935), equation 2 produces type II curves (Thompson, 1924) while equation 3 is a logistic equation. The equations were fitted with non-linear regression (Levenberg-Marquardt method). The coefficient of determination of the response curves, which estimates the proportion of variance of the number of hosts parasitised, was used to measure the degree of fit.

3.3. Results

Temperature levels, host egg densities as well as strains significantly affected the number of eggs parasitised ($F = 197.4$; $df = 5,3138$; $P < 0.0001$; $F = 130.1$; $df = 4,3138$; $P < 0.0001$ and $F = 29.9$; $df = 5,3138$; $P < 0.0001$ respectively). The two relative humidity levels tested did not significantly affect the number of eggs parasitised nor the strain performance ($F = 1.83$; $df = 1,3138$; $P = 0.1764$ and $F = 1.63$; $df = 5,3138$; $P = 0.1491$, respectively); their interaction with temperature also did not affect parasitism ($F = 0.6$; $df = 5,3138$; $P = 0.6995$).

Since humidity had no significant effect on the number of eggs parasitised, the data were, pooled for subsequent analyses. For most species/strains, the number of eggs parasitised increased with host density (Figure 3.1).

For all species/strains, a 3rd order polynomial function gave the best fit of parasitisation rates on temperature with a maximum parasitism around 30°C (Table 3.1). Significant differences in parasitisation rates between the strains were observed at the different temperature levels (Figure 3.2). There were no clear trends across temperature in differences in parasitisation rates between species/strains except for *T. bruni* (H), which tended to have the lowest rates under most temperature regimes.

At 10°C, the sigmoid model gave the best fit for three of the strains (Table 3.2). At the other temperatures, except for *T.* sp. nr. *mwanzai* (M) at 15°C, the Nicholson-Bailey model gave the best fit. At 30°C, the linear model also gave a good fit for most strains (Table 3.2).

Table 3.1. Best-fit 3rd degree polynomial functions and optimum temperatures.

Species/strain	Function	R^2	Optimum temperature (°C)
T. sp. nr. *mwanzai* (L)	$y = -0.0001x^3+0.008x^2-0.13x+0.7$	0.95	30
T. sp. nr. *mwanzai* (M)	$y = -0.0001x^3+0.009x^2+0.16x+0.9$	0.99	30
T. sp. nr. *lutea* (M)	$y = -0.0002x^3+0.02x^2-0.32x+1.9$	0.97	30
T. sp. nr. *lutea* (L)	$y = -0.0002x^3+0.01x^2-0.2x+1.3$	0.92	30
T. sp. nr. *lutea* (H)	$y = -0.0002x^3+0.01x^2-0.2x+1.1$	0.95	30
T. bruni (H)	$y = -0.0001x^3+0.01x^2-0.16x+0.9$	0.98	30

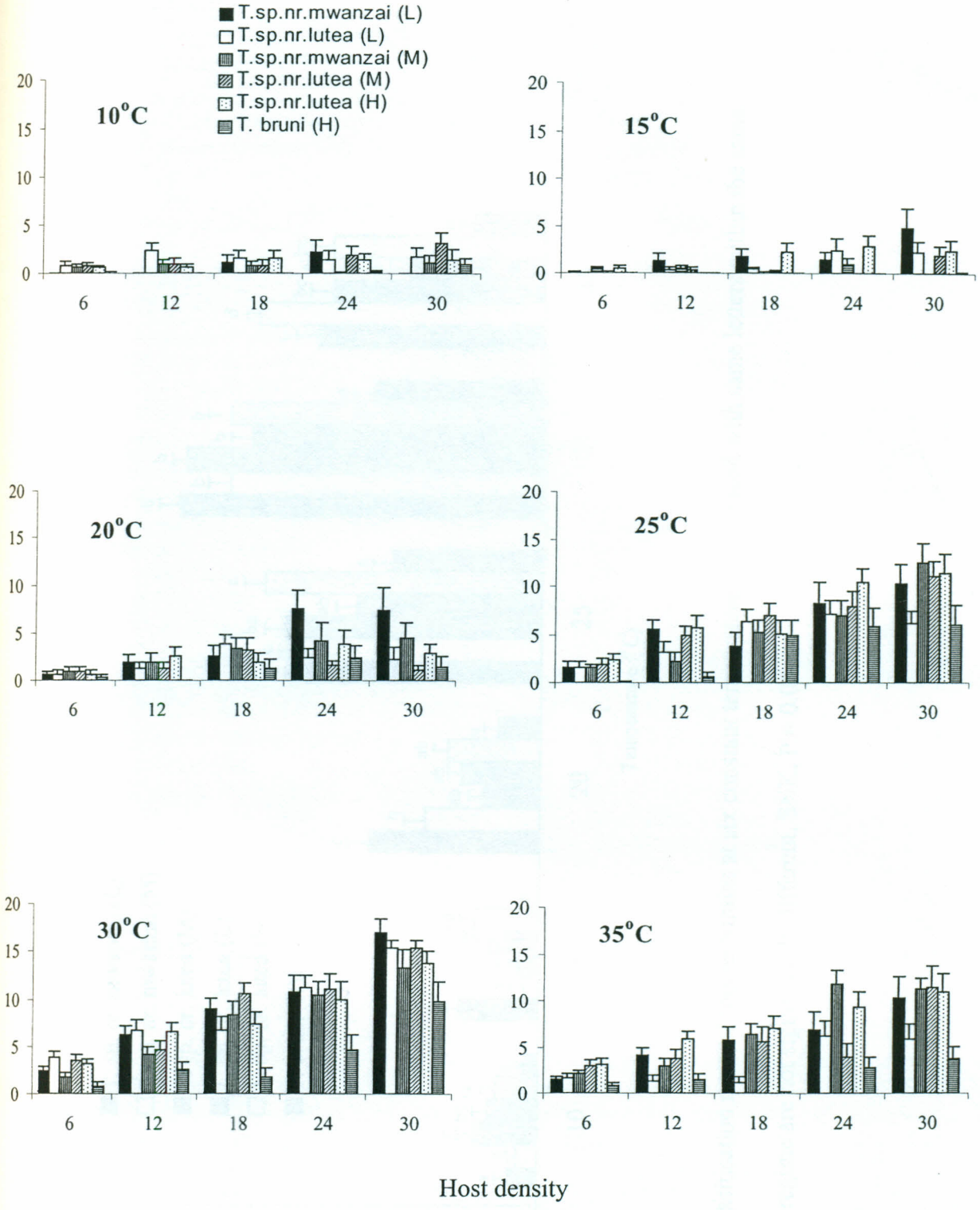

Fig. 3.1. Mean number of eggs parasitised by six species/strains at each host density at six constant temperatures.

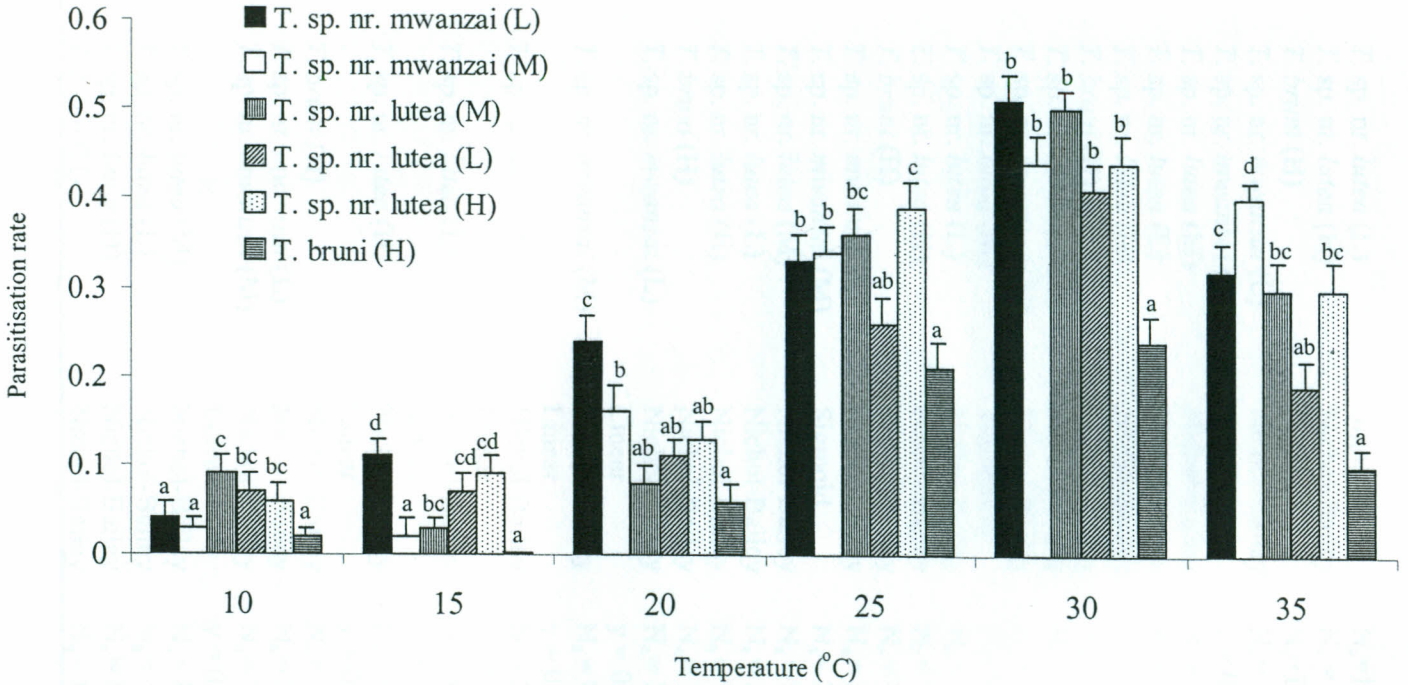

Fig.3. 2. Parasitisation rates of species/strains at six constant temperatures. Bars caped with same letters within the same temperature regime are not significantly different, SNK, P = 0.05.

Table 3.2. Results of linear and non-linear functional response curve fits for six strains at different temperatures.

Temperature (°C)	Species/strain	Descriptive model	Equation	R^2
10	*T.* sp. nr. *mwanzai* (L)	Sigmoid	N_a=1.1/(1+15.8exp(0.2N))	0.49
	T. sp. nr. *mwanzai* (M)	Linear	y = 0.03x	0.18
	T. sp. nr. *lutea* (M)	Thompson	N_a=N (1-exp (-1.7/N))	0.29
	T. sp. nr. *lutea* (L)	Sigmoid	N_a=1.75/(1+exp (-1.6N))	0.77
	T. sp. nr. *lutea* (H)	Nichol-Bailey	N_a = N (1-exp (-0.06)	0.72
	T. bruni (H)	Sigmoid	N_a=1.1/(1+27.6exp(-0.1N))	0.77
15	*T.* sp. nr. *mwanzai* (L)	Nichol-Bailey	N_a = N (1-exp (-0.11)	0.82
	T. sp. nr. *mwanzai* (M)	Linear	y = 0.02x	0.03
	T. sp. nr. *lutea* (M)	Nichol-Bailey	N_a = N (1-exp (-0.03)	0.59
	T. sp. nr. *lutea* (L)	Nichol-Bailey	N_a = N (1-exp (-0.07)	0.83
	T. sp. nr. *lutea* (H)	Nichol-Bailey	N_a = N (1-exp (-0.09)	0.82
	T. bruni (H)	Nichol-Bailey	N_a = N (1-exp (-0.002)	0.56
20	*T.* sp. nr. *mwanzai* (L)	Nichol-Bailey	N_a = N (1-exp (-0.24)	0.90
	T. sp. nr. *mwanzai* (M)	Nichol-Bailey	N_a = N (1-exp (-0.16)	0.97
	T. sp. nr. *lutea* (M)	Thompson	N_a=N (1-exp (-1.8/N))	0.22
	T. sp. nr. *lutea* (L)	Nichol-Bailey	N_a = N (1-exp (-0.1)	0.46
	T. sp. nr. *lutea* (H)	Nichol-Bailey	N_a = N (1-exp (-0.12)	0.69
	T. bruni (H)	Nichol-Bailey	N_a = N (1-exp (-0.06)	0.76
25	*T.* sp. nr. *mwanzai* (L)	Nichol-Bailey	N_a = N (1-exp (-0.33)	0.92
	T. sp. nr. *mwanzai* (M)	Sigmoid	N_a=-379/(1-92exp(0.09N))	0.99
	T. sp. nr. *lutea* (M)	Nichol-Bailey	N_a = N (1-exp (-0.36)	0.99
	T. sp. nr. *lutea* (L)	Nichol-Bailey	N_a = N (1-exp (-0.26)	0.85
	T. sp. nr. *lutea* (H)	Nichol-Bailey	N_a = N (1-exp (-0.39)	0.95
	T. bruni (H)	Nichol-Bailey	N_a = N (1-exp (-0.39)	0.95
30	*T.* sp. nr. *mwanzai* (L)	Nichol-Bailey	N_a = N (1-exp (-0.5)	0.98
		Linear	y = 0.51x	0.79
	T. sp. nr. *mwanzai* (M)	Nichol-Bailey	N_a = N (1-exp (-0.44)	0.99
		Linear	y = 0.44x	0.70
	T. sp. nr. *lutea* (M)	Nichol-Bailey	N_a = N (1-exp (-0.5)	0.97
		Linear	y =0.5x	0.82
	T. sp. nr. *lutea* (L)	Nichol-Bailey	N_a = N (1-exp (-0.48)	0.96
		Linear	y =0.41x	0.71
	T. sp. nr. *lutea* (H)	Nichol-Bailey	N_a = N (1-exp (-0.44)	0.98
		Linear	y = 0.44x	0.72
	T. bruni (H)	Nichol-Bailey	N_a = N (1-exp (-0.24)	0.85
35	*T.* sp. nr. *mwanzai* (L)	Nichol-Bailey	N_a = N (1-exp (-0.3)	0.99
	T. sp. nr. *mwanzai* (M)	Nichol-Bailey	N_a = N (1-exp (-0.4)	0.94
		Linear	y = 0.4x	0.74
	T. sp. nr. *lutea* (M)	Nichol-Bailey	N_a = N (1-exp (-0.3)	0.79
	T. sp. nr. *lutea* (L)	Nichol-Bailey	N_a = N (1-exp (-0.19)	0.81
	T. sp. nr. *lutea* (H)	Nichol-Bailey	N_a = N (1-exp (-0.39)	0.96
	T. bruni (H)	Nichol-Bailey	N_a = N (1-exp (-0.1)	0.75

3.4. Discussion

The present study showed that temperature had a significant effect on the functional response of the egg parasitoids tested, while the two relative humidity levels did not. For all species/strains, the highest parasitism was obtained around 30°C. Similar optima were found for developmental rates for other African egg parasitoids reared under constant temperatures (Chabi-Olaye *et al.*, 1997, 2001a, 2004). For most species/strains and at higher temperatures, the functional response was of type I. Kfir (1983) found a type II functional response for *T. pretiosum* Riley, while the parasitism of *Heliothis zea* (Boddie) by *Trichogramma* spp. varied from inverse density-dependent to density-dependent, depending on the distance between host eggs offered to wasps (Morrison *et al.*, 1980); for the Asian corn borer, a linear relationship was found (Shen and Li, 1987).

The differences in response between species/strains in the present study suggest differences in adaptation to different temperature regimes and differences in host searching abilities. Mohaghegh *et al.* (2001), Kfir (1983) and Wang and Ferro (1998) found that the functional response of predators and parasitoids might change from one type to another as environmental conditions (temperatures mainly) change. Such changes may be due to effects on the foraging behaviour of the parasitoids (Guo, 1986; Zhang *et al.*, 1983). In the present study, type I response was most common, followed by type III, while type II was rare. Although a parasitoid with a linear response does not exhibit the theoretical potential for the control of the host, as would be a parasitoid with a sigmoid (type III) functional response (Hassell *et al.*, 1977; Hassell, 1978; Luck, 1985), the presence of a linear response is important for the continued interaction of the host and parasitoid. According to Klomp *et al.* (1980), *Trichogramma* spp. possibly possess a

learning ability to discriminate between parasitised and unparasitised eggs, which may result in the type III response. The type III functional response can lead to density dependence, when host densities are low and thus can potentially stabilize host-parasitoid interactions (Berryman, 1999; Bernstein, 2000). According to Fernandez and Carley (2003), a type III response can also stabilize a Nicholson-Bailey type host-parasitoid system and a parasitoid expressing such response may potentially regulate the host population. In the present study, a relationship between the functional response of the strains and the temperature at the original location could not be clearly established. Some strains showed greater plasticity in their ability to perform at different temperatures regardless of their source climatic conditions. For example, although *T.* sp. nr. *lutea* (H) was collected from the high altitude site (2227m), where temperatures are mostly between 10 and 23°C, it showed higher parasitism at temperatures between 30-35°C than some strains collected from warmer areas. This suggests that this strain has a superior and more plastic tendency for warm temperature adaptation than the others.

At high temperatures of 30 and 35°C, *Trichogrammatoidea* sp. nr. *lutea* (H), *T.* sp. nr. *mwanzai* (L) and *T.* sp. nr. *mwanzai* (M) were superior to the other species/strains. The prevalence of type I responses showed that the parasitoids were able to find and attack hosts even at the lowest host density, which indicates a high searching ability of the parasitoids. Such a candidate may be able to persist in the field and thus, may have the potential to prevent increases of the host population before economic damage occurs (Wiedenmann and Smith, 1993).

Functional response studies in the laboratory have been criticised as being unnatural (Kareiva, 1990), because of differences in the areas where parasitoids have to search to

find hosts (O'Neil, 1989). Such studies are, however, useful in providing the first step for comparing the efficiency of different species/strains (Overholt and Smith, 1990) and also provide a valid means of comparing host finding abilities of the candidate natural enemies (Munyaneza and Obrycki, 1997).

In conclusion, *T.* sp. nr. *lutea* (H), *T.* sp. nr. *mwanzai* (L) and *T.* sp. nr. *mwanzai* (M) were identified as promising candidates for augmentative programs for controlling *H. armigera* in Kenya, based on the functional response studies.

CHAPTER FOUR

4.0. PARASITISM CAPACITY, LONGEVITY AND PROGENY PRODUCTION OF SIX TRICHOGRAMMATID EGG PARASITOID SPECIES/SPECIES AT DIFFERENT TEMPERATURE AND HUMIDITY REGIMES

4.1. Introduction

Egg parasitoids of the genus *Trichogramma* have been used as biological control agents in a variety of agroecosystems (Hassan and Guo, 1991; Pinto and Stouthamer, 1994). They have been utilised successfully as inundative biological control agents against a range of agricultural pests, mainly lepidopterans (Li, 1994; van Lenteren, 2000). Although several species of *Trichogrammatoidea* spp. are considered to be important natural enemies of agricultural pests in various parts of the world (Nagaraja, 1978), information on their biology is limited.

The African bollworm, *H. armigera* is a key pest of tomato in Kenya (Farrell *et al.*, 1995) and an important constraint in the vegetable-based cropping systems in Africa (Ikin *et al.*, 1993), where it is active throughout the year but often becomes more important during warmer seasons. Several native trichogrammatid species occur in Kenya and elsewhere in Africa on this pest (Sithanantham *et al.*, 2001). For efficient use, a choice of better-adapted species/strains based on a thorough understanding of their biology is important.

The efficiency of parasitoids in the field is affected by adverse climatic conditions (Gupta, 1956; Messenger, 1970). The ability of each species (and sometimes each population) to cope with fluctuating environmental conditions in the target area is an

important factor affecting their impact in field situations. According to Hassan (1994) and Voegele (1988), indigenous trichogrammatids tend to be better adapted to local climates, habitat and host conditions than exotic ones.

Performance indices are commonly used to select for desirable traits and utilise such a diversity to improve the impact of beneficial organisms (Young, 1961). *Trichogramma* spp. are particularly suitable for use as a 'biotic pesticide' through inundative releases (van Lenteren, 1983) and the number of host eggs successfully parasitised by the adult female parasitoid within her lifespan (fecundity) after release in the field, is the key attribute for selecting species/strains. The performance of a parasitoid in its intended role after release is an important attribute of its quality (Bigler, 1994; Smith, 1996). Other life history traits (progeny production/sex ratio) are secondary and are important only as efficiency parameters in mass production and hence of limited importance in selecting for an inundative system.

In the present study, the effect of temperature and relative humidity on parasitism, adult longevity, progeny production and sex ratio of six indigenous trichogrammatid species/strains were evaluated to select the better adapted strains for augmentative control of *H. armigera*. The study particularly aimed at finding strains better adapted to higher temperatures (30-35°C), which are generally encountered in pest-prone areas/seasons.

4.2. Materials and Methods

The methodologies adopted were similar to those used by Liu and Smith (2000) in comparing *Trichogramma* adults from accessions (inbred lines) assembled from surveys of eggs of the target pest, *Choristoneura fumiferana* (Clemens) for their fecundity and

longevity and the test candidates were reared for several generations on the factitious host, *Ephestia kuehniella* Zeller. Trichogramma accessions were compared for their parasitisation efficiency in the laboratory as the basis for identifying promising candidates for field release for biocontrol of the target pest. Information about the six species/strains of *Trichogramma* and *Trichogrammatoidea* used in this study is shown in Table 2 (chapter two).

4.2.1. Bioassay

Freshly emerged one-day-old mated females were individually confined in glass vials (2.5 cm x 7.5 cm) and were provided with fine streaks of 10% honey solution. Ten females per strain were used as replicates for each temperature/humidity regime. The glass vials were placed inside a Perspex cage (30 cm width x 30 cm diameter x 20 cm height) maintained at a specific relative humidity and at a set temperature (±1°C) for 24 hours. The temperatures and humidities used in this study and details of their maintenance are presented in chapter two (sub-section 2.3).

Eggs of *C. cephalonica* were provided at *libitum* (30 per day) in a card and were replaced daily with fresh ones. To determine the number of host eggs parasitised, the number of black eggs was counted after 2-3 days (Strand, 1986), under a dissecting compound microscope (magnification x 16). Upon emergence, sex ratio (proportion of females in progeny) was also determined. Longevity of adult parasitoids was determined by checking survival of parental females once a day. Trends in survivorship were determined according to Pearl (1928).

4.2.2. Data analysis

Analysis of Variance (ANOVA) using the procedure mixed (Proc Mixed, SAS Institute, 2000) was used to examine main effects (temperature, relative humidity and strain) and their interactions on parasitism, progeny production, sex ratio as well as adult longevity. To stabilise the variance, the data on numbers of eggs parasitised and adults emerged were log (x+1) transformed before being subjected to analysis (Sokal and Rohlf, 1981). When ANOVAs were significant, means were separated using the Student Newman Keul's (SNK) test at P=0.05 (Zar, 1996). A correlation analysis was used to examine the relationship between adult longevity and parasitism for the different strains.

4.3. Results

4.3.1. Parasitism

The interactions of temperature, relative humidity and strain affected the number of eggs parasitised (Table 4.1). At 70-80% RH, differences in the number of eggs parasitised were observed between strains at all temperatures, except at 30°C (Table 4.2). At 10°C, *T.* sp. nr. *mwanzai* (M) parasitised more eggs than *T. bruni* (H). Whereas *T.* sp. nr. *mwanzai* (M), *T.* sp. nr. *lutea* (M) and *T.* sp. nr. *mwanzai* (L) parasitised more eggs than *T.* sp. nr. *lutea* (H) at 15°C, *T.* sp. nr. *mwanzai* (L) parasitised more eggs than *T.* sp. nr. *lutea* (L). *Trichogrammatoidea* sp. nr. *lutea* (L) also parasitised more eggs than *T.* sp. nr. *lutea* (H). At 20°C, *T.* sp. nr. *lutea* (H) parasitised more eggs than *T.* sp. nr. *lutea* (L), *T.* sp. nr. *mwanzai* (L) and *T. bruni* (H), while at 35°C, *T.* sp. nr. *mwanzai* (L) parasitised the highest number of eggs. Additionally, *T.* sp. nr. *mwanzai* (M) parasitised more eggs

than *T.* sp. nr. *lutea* (L), *T.* sp. nr. *lutea* (H) and *T. bruni* (H). *Trichogrammatoidea* sp. nr. *lutea* (M) also parasitised more eggs than *T.* sp. nr. *lutea* (H) and *T. bruni* (H).

At 40-50%RH, there were no differences between strains at 10 and 20°C, whereas at 15°C, *T.* sp. nr. *mwanzai* (M) and *T.* sp. nr. *lutea* (M) parasitised more eggs than *T.* sp. nr. *lutea* (H) and *T. bruni* (H) (Table 4.3). At 25°C, *T.* sp. nr. *lutea* (M) parasitised more eggs than *T. bruni* (H) and at 30°C, *T.* sp. nr. *mwanzai* (M) parasitised more eggs than *T. bruni* (H). At 35°C, *T.* sp. nr. *mwanzai* (M) parasitised more eggs than *T.* sp. nr. *lutea* (H) and *T. bruni* (H).

Generally, the number of eggs parasitised by most strains at the two humidity levels increased with increasing temperature to a maximum at 30°C.

Parasitism by most strains did not differ between the two relative humidity levels at most temperature regimes. Whereas parasitism was higher at the lower humidity for *T.* sp. nr. *lutea* (L) and *T.* sp. nr. *mwanzai* (L) at 20°C, *T.* sp. nr. *lutea* (H) at 35°C and *T.* sp. nr. *mwanzai* (M) at 30°C, it was higher at the higher humidity for *T.* sp. nr. *mwanzai* (L) at 25 and 35°C, *T.* sp. nr. *lutea* (H) at 10°C and *T.* sp. nr. *mwanzai* (M) at 10 and 25°C.

4.3.2. Progeny production

Progeny production was similarly significantly affected by the interaction of temperature, humidity and strain (Table 4.4). In general, progeny production at the two humidity levels increased with increasing temperature, following the same pattern as the number of eggs parasitised with respect to temperature (Figure 4.1).

4.3.3. Sex ratio

Sex ratio was affected by the interactions of temperature and strain ($F = 1.6$; $df = 25,472$; $P = 0.03$). Since humidity had no affect on sex ratio ($F = 0.4$; $df = 5,472$; $P = 0.5$), the data were, pooled for subsequent analyses.

Differences in sex ratio were only observed between strains at 20, 25 and 30°C ($P < 0,05$). Across all temperatures, there was no clear relationship between temperature and progeny sex ratio for the majority of the trichogrammatid strains studied (Figure 4.2).

4.3.4. Adult longevity

The longevity of adult parasitoids was significantly influenced by temperature, relative humidity and strain ($F = 138.69$; $df = 5,648$; $P < 0.0001$, $F = 28.6$; $df = 1,648$; $P < 0.0001$ and $F = 6.09$; $df = 5,648$; $P < 0.0001$, respectively). The interactions of temperature and relative humidity ($F = 10.32$; $df = 5, 648$; $P < 0.0001$), temperature and strain ($F = 1.87$; $df = 25,648$; $P = 0.0066$) as well as relative humidity and strain ($F = 4.68$; $d f= 5,648$; $P = 0.0003$) also affected adult longevity.

At 70-80%RH, significant differences in longevity were observed between strains at 10 and 15°C (Table 4.5). Whereas *Trichogramma bruni* (H) lived longer than *T.* sp. nr. *lutea* (M) at 10°C, *T.* sp. nr. *mwanzai* (M) lived longer than *T.* sp. nr. *lutea* (M) at 15°C. There were no significant differences in longevity between strains at 20, 25, 30 and 35°C. Adult longevity also varied between temperatures for the different strains.

At 40-50%RH, adult longevity did not differ significantly between the strains at 10, 15 and 20°C while differences were observed at 25, 30 and 35°C (Table 4.6). Whereas *T.* sp. nr. *mwanzai* (L), *T.* sp. nr. *mwanzai* (M) and *T.* sp. nr. *lutea* (M) lived longer than *T.*

bruni (H) at 25°C, *T.* sp .nr. *lutea* (L) and *T.* sp. nr. *mwanzai* (M) lived longer than *T.* sp. nr. *mwanzai* (L) and *T. bruni* (H) at 30°C. *Trichogramma* sp. nr. *mwanzai* (L) lived longer than *T. bruni* (H) at 35°C. Adult longevity at 40-50%RH also varied between temperatures for the different strains. Generally, there was an inverse relationship between adult longevity and temperature.

For *T.* sp. nr. *lutea* (L), adult longevity did not differ significantly between the two relative humidity regimes at most temperature regimes, but was longer at the lower humidity than higher humidity at 20°C. *Trichogramma* sp. nr. *mwanzai* (L) lived longer at 40-50%RH at 20, 25 and 35°C. Whereas *Trichogrammatoidea* sp. nr. *lutea* (H) lived longer at 20 and 30°C at 40-50%RH, *T. bruni* (H) lived longer at 10 and 15°C at 70-80%RH. *Trichogramma* sp. nr. *mwanzai* (M) lived longer at 40-50%RH than at 70-80%RH at 20, 25 and 30°C. *Trichogrammatoidea* sp. nr. *lutea* (M) lived longer at 40-50%RH at 20 and 25°C.

Longevity of adults was positively correlated with the total number of host eggs parasitised by the various strains ($r = 0.26$; $P = 0.004$ for *T.* sp. nr. *lutea* (L), $r = 0.48$; $P < 0.0001$ for *T.* sp. nr. *mwanzai* (L), $r = 0.35$; $P < 0.0001$ for *T.* sp. nr. *lutea* (H), $r = 0.34$; $P = 0.0002$ for *T. bruni* (H), $r = 0.33$; $P = 0.004$ for *T.* sp. nr. *mwanzai* (M), $r = 0.54$; $P < 0.004$ for *T.* sp. nr. *lutea* (M), respectively).

The survival of the strains followed a type III survivorship curve at the higher temperatures (30 and 35°C) and a type I survivorship curve at the lower temperatures (Figure 4.3).

Table 4.1: Effects of temperature, relative humidity and strain on parasitism.

Source	*F*	*df*	*P*
Temperature	221.7	5, 648	< 0.0001
RH	1.2	1,648	0.28
Strain	36.9	5,648	< 0.0001
Temperature*RH	5.2	5,648	< 0.0001
Temperature*Strain	7.2	25,648	< 0.0001
RH*Strain	3.1	5,648	0.005
Temperature*RH*Strain	3.2	25,648	< 0.0001

Table 4.2: Parasitism capacity of six trichogrammatid strains at six constant temperatures at 70-80%RH.

Temperature (°C)	*T.* sp. nr. *lutea* (L)	*T.* sp. nr. *mwanzai* (L)	*T.* sp. nr. *lutea* (H)	*T. bruni* (H)	*T.* sp. nr. *mwanzai* (M)	*T.* sp. nr. *lutea* (M)	*F*	*df*	*P*
10	0.2± 0.1ab C	0.3± 0.2ab C	0.4± 0.2ab B	0.2± 0.1b D	0.8± 0.2a D	0.2± 0.2ab C	2.51	5,54	0.03
15	0.8± 0.3b C	2.2± 0.4a C	0.7± 0.4c B	0.9± 0.3abc CD	2.2± 0.3ab C	2.0± 0.4ab C	5.06	5,54	0.0007
20	2.4± 0.6b BC	2.0± 0.7b C	5.5± 0.6a A	1.2 ±0.6b BC	4.6± 0.7ab B	3.0± 0.7ab BC	5.13	5,54	0.0006
25	9.3± 1.1a A	9.4± 1.0a B	7.2± 1.0a A	2.7 ±1.0b AB	9.4± 1.0a A	10.6± 1.0a A	8.19	5,54	< 0.0001
30	12.5± 1.8a A	7.5± 2.0a B	8.8± 1.9a A	5.0± 2.0a A	8.6± 2.0a A	12.1± 1.9a A	2.23	5,54	0.06
35	4.3± 1.1abc B	19.8± 1.5e A	0.6± 1.2ab B	0a BCD	10.7± 1.6d A	8.3 ±1.6cd AB	17.5	5,54	< 0.0001
F	41.9	41.5	25.1	10	44.4	17			
df	5,54	5,54	5,54	5,54	5,54	5,54			
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001			

Means followed by the same lower case letter in each row and those followed by the same upper case letter in each column are not significantly different at P=0.05 (SNK).

Table 4.3: Parasitism capacity of six trichogrammatid strains at six constant temperatures at 40-50%RH.

Temperature (°C)	*T.* sp. nr. *lutea* (L)	*T.* sp. nr. *mwanzai* (L)	*T.* sp. nr. *lutea* (H)	*T. bruni* (H)	*T.* sp. nr. *mwanzai* (M)	*T.* sp. nr. *lutea* (M)	*F*	*df*	*P*
10	0.2± 0.1a D	0.2± 0.1a B	0.1± 0.1a D	0.0± 0.1a C	0.2± 0.1a D	0.2± 0.1a C	0.4	5,54	0.86
15	1.4± 0.4ab CD	1.8± 0.4ab B	1.1± 0.4b CD	1.1± 0.4b BC	2.7± 0.4a C	3.1± 0.5a B	3.9	5,54	0.005
20	4.1± 0.6a BC	4.3± 0.6a A	3.9± 0.6a B	2.7± 0.7a AB	3.9± 0.6a C	4.5 ±0.6a B	1.1	5,54	0.4
25	7.3± 1.0ab AB	6.4± 0.9ab A	6.3± 1.1ab AB	2.9± 1.2b AB	6.7± 0.9ab B	8.3± 1.0a A	2.7	5,54	0.03
30	9.8± 1.3ab A	7.1 ±1.7ab A	8.5± 1.4ab A	5.8 ±1.7b A	13.4± 1.3a A	10.3 ±1.4 ab	3.3	5,54	0.01
35	6.7± 1.6abc AB	6.0 ±1.0abc A	4.0± 1.2ab BC	0a BC	13.3± 0.7c A	6.2 ±1.4abc AB	4.8	5,54	0.0007
F	10.7	19.5	15.8	7.8	66	24.6			
df	5,54	5,54	5,54	5,54	5,54	5,54			
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001			

Means followed by the same lower case letter in each row and those followed by the same upper case letter in each column are not significantly different at P=0.05 (SNK test).

Table 4.4: Effects of temperature, relative humidity and strain on progeny production.

Source	*F*	*df*	*P*
Temperature	213.2	5,648	< 0.0001
RH	3.6	1,648	0.06
Strain	27.0	5,648	< 0.0001
Temperature*RH	6.8	5,648	< 0.0001
Temperature*Strain	7.1	25,648	< 0.0001
RH*Strain	4.1	5,648	0.001
Temperature*RH*Strain	3.4	25,648	< 0.0001

Table 4.5. Adult longevity (days) of six trichogrammatid strains at different temperatures at 70-80% RH.

Temperature (°C)	*T.* sp. nr. *lutea* (L)	*T.* sp. nr. *mwanzai* (L)	*T.* sp. nr. *lutea* (H)	*T. bruni* (H)	*T.* sp. nr. *mwanzai* (M)	*T.* sp. nr. *lutea* (M)	*F*	*df*	*P*
10	16.5±3.1ab A	10.6±3ab A	15.1±2.5ab A	17.4±1.3a A	15.5±2.8ab B	8.1±3.0b AB	2.9	5,54	0.02
15	18.4±4.2ab A	14±2.4ab A	12.8±2.7ab AB	16.5±1.9ab A	25.6±2.4a A	12.7±3.4b A	2.4	5,54	0.045
20	10±1.9a AB	6.7±2.1a AB	9.6±1.7a AB	9.1±1.4a B	7.8±2.2a C	6.1±1.2a ABC	1.1	5,54	0.4
25	5.7±1.5a BC	6.7±1.3a ABC	6.5±1.3a BC	6±0.9a B	7.2±1a C	5.8±1.2a ABC	0.3	5,54	0.9
30	4.2±0.9a BC	2.4±0.3a BC	2.7±0.4a C	2.6±0.4a C	2.4±0.3a CD	3.1±0.5a BC	1.5	5,54	0.2
35	2.1±0.4a C	1.1±0.1a C	1.8±0.3a C	1.2±0.1a C	1.5±0.3a D	1.4±0.3a C	2	5,54	0.1
F	9.6	8.9	11.7	45.1	27.2	6.2			
df	5,54	5,54	5,54	5,54	5,54	5,54			
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	0.0001			

Means followed by the same lower case letter in each row and those followed by the same upper case letter in each column are not significantly different P=0.05 (SNK test).

Table 4.6. Adult longevity (days) of six trichogrammatid strains at different temperatures at 40-50% RH.

Temperature (°C)	*T.* sp. nr. *lutea* (L)	*T.* sp. nr. *mwanzai* (L)	*T.* sp. nr. *lutea* (H)	*T. bruni* (H)	*T.* sp. nr. *mwanzai* (M)	*T.* sp. nr. *lutea* (M)	*F*	*df*	*P*
10	13.4±2.9a AB	16.3±2.7a A	16.5±3.4a AB	8.6±1.6a AB	19.3±3.1a A	10.2±2.8a AB	2	5,54	0.09
15	16.7±3.4a A	15.7±3.7a A	19.2±2.7a A	10.9±1.7a A	19.3±3.7a A	10.9±2.7a AB	1.6	5,54	0.2
20	18.1±2a A	15.2±2.7a A	22.2±3.1a A	11.3±1.6a A	18.2±2.7a A	17.2±2.7a A	1.5	5,54	0.2
25	9.2±1.7ab AB	15.1±1.9a A	8.1±2.2ab B	5.4±0.8b BC	13.2±1.7a AB	12.4±2.1ab A	3.9	5,54	0.004
30	5.9±0.7a BC	2.4±0.3b B	4.4±0.5ab C	2.9±0.5b CD	5.8±0.6a BC	4.2±0.4ab BC	7.4	5,54	< 0.0001
35	1.5±0.3ab C	2.6±0.3a B	1.8±0.4ab C	1.0b D	1.8±0.3ab C	1.5±0.3ab C	3.8	5,54	0.005
F	11	12.7	15.1	18.3	15.5	8.8			
df	5,54	5,54	5,54	5,54	5,54	5,54			
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001			

Means followed by the same lower case letter in each row and those followed by the same upper case letter in each column are not significantly different P=0.05 (SNK test).

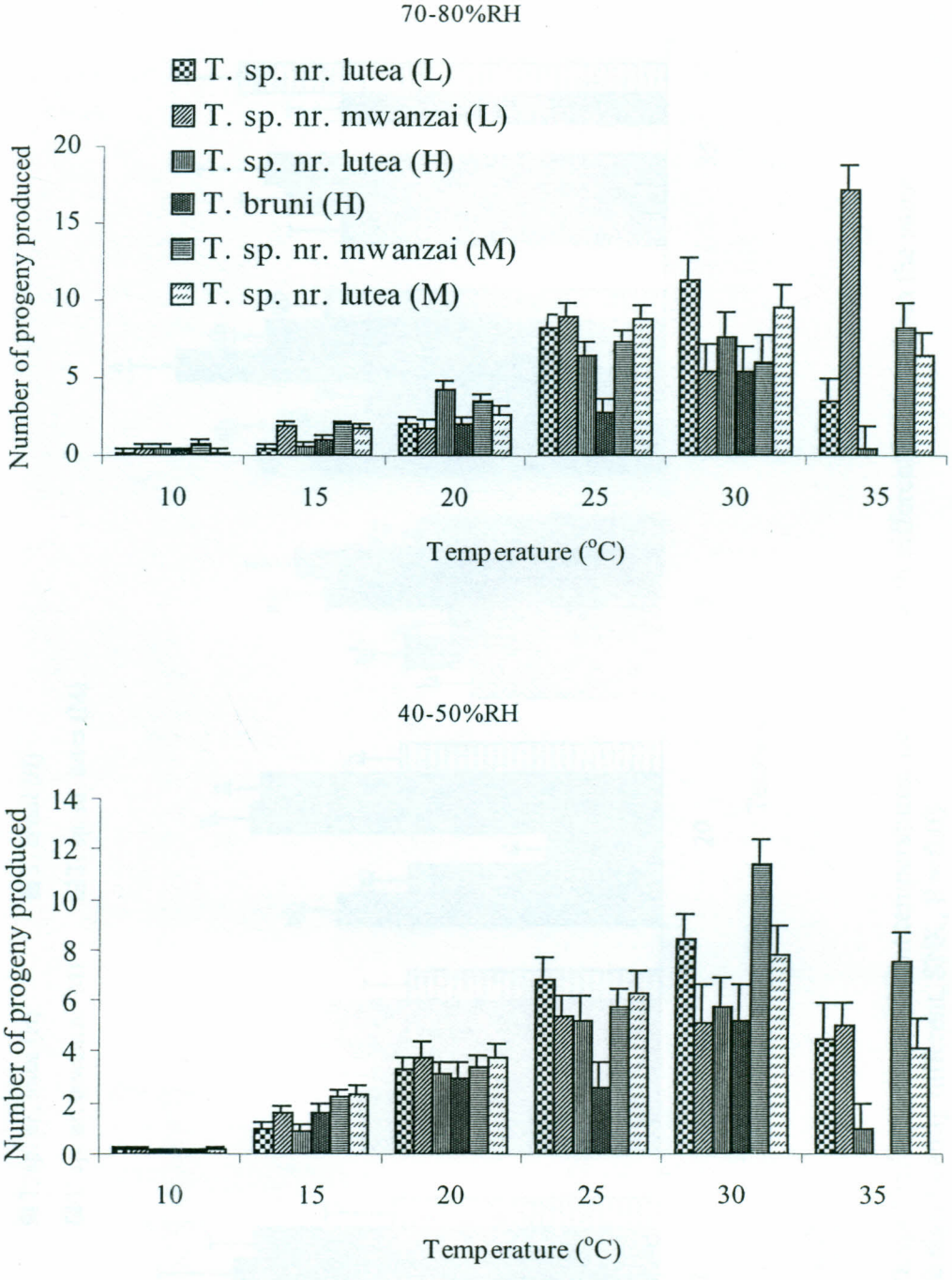

Fig.4.1. Progeny production by six trichogrammatid species/strains at six temperatures at two humidity regimes

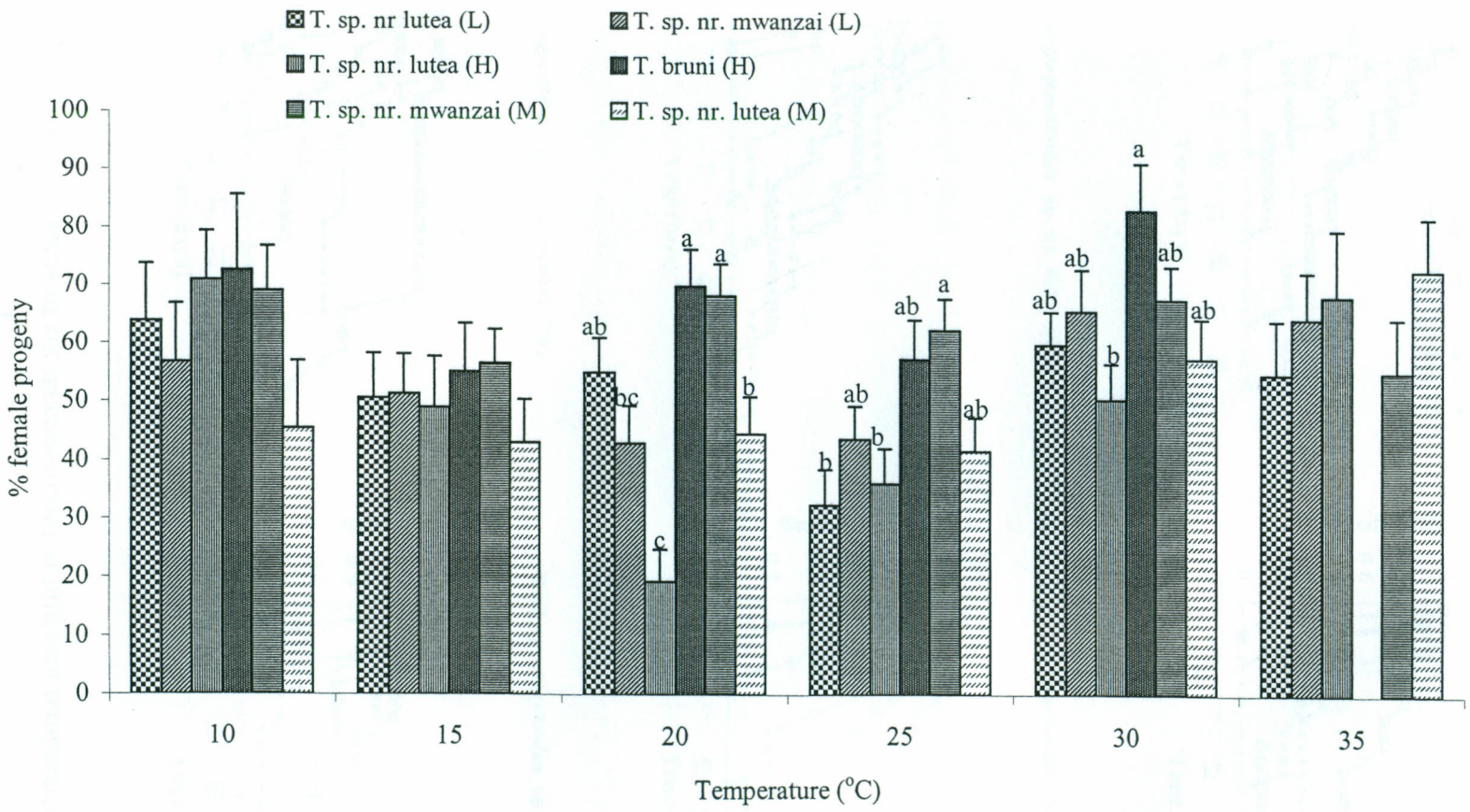

Fig.4.2. Sex ratio of six species/strains at different temperatures. Bars caped with different letters within the same temperature regime are significantly different, SNK, P = 0.05

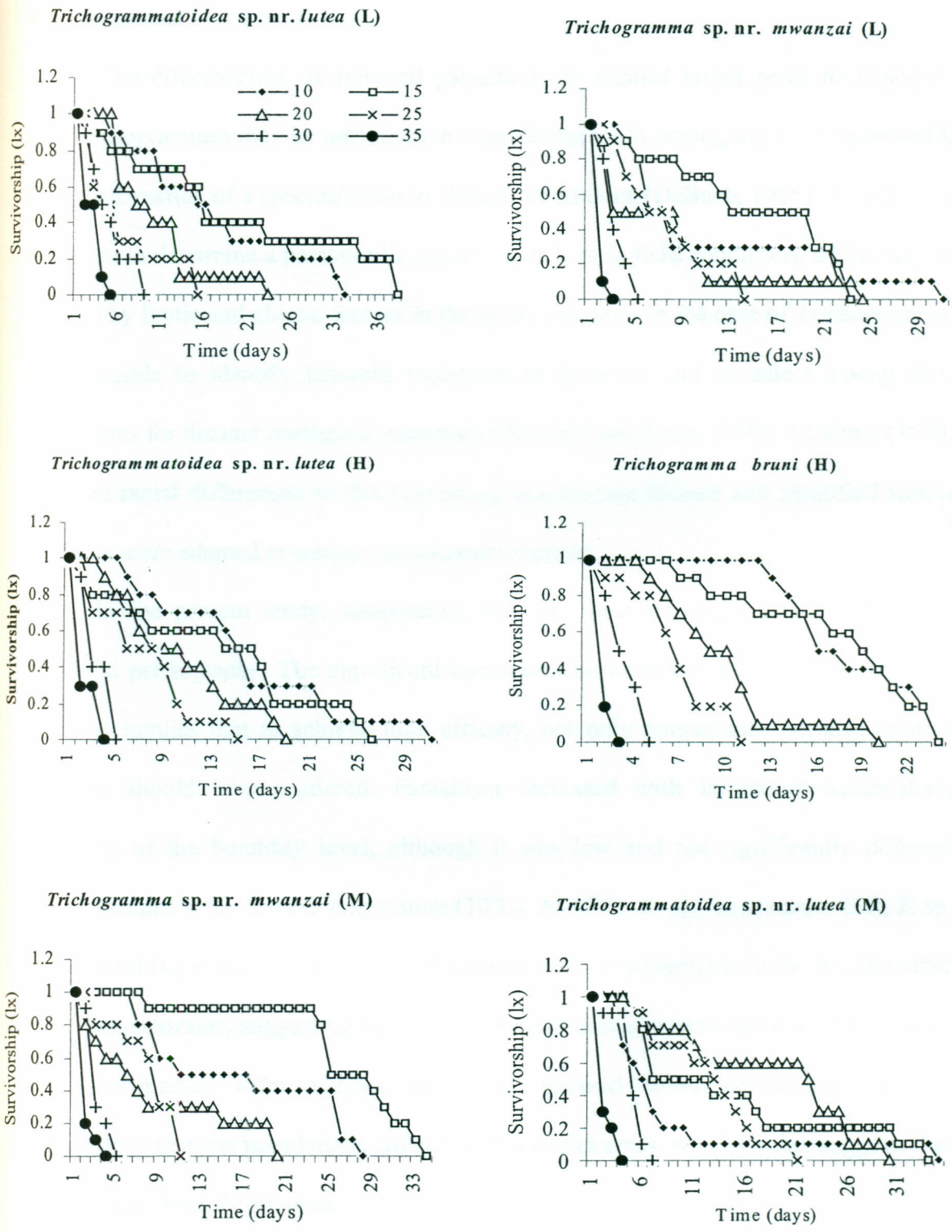

Fig.4.3. Survivorship curves of six species/strains at different temperatures

4.5. Discussion

The effectiveness of released parasitoids to control target pests in biological control programmes may be attributed to many factors, but among the most important is lack of adaptation of a species/strain to climatic conditions (DeBach, 1965). Besides, it is important to determine a parasitoid's impact on its host in field conditions, which may be affected by biotic and abiotic factors in the agroecosystem. In the case of *Trichogramma*, it is possible to identify inherent variations in diversity and so select among their populations for distinct biological responses (Bleicher and Parra, 1990). Abraham (1970) observed racial differences in *Trichogramma australicum* Girault and identified strains that were more adapted to warmer temperature regimes.

In the present study, temperature was the most important factor influencing parasitoid performance. The significant interaction between temperature and humidity, however, implies that to achieve high efficacy, not only temperature but also relative humidity should be considered. Parasitism increased with increasing temperature, regardless of the humidity level, although it was low and not significantly different between strains at the lowest temperature (10°C). At 15°C, *T.* sp. nr. *mwanzai* (M), *T.* sp. nr. *mwanzai* (L) and *T.* sp. nr. *lutea* (M) showed a higher parasitic activity than the other three species/strains, suggesting better performance in cooler environments. These three strains, originated from warmer areas, but showed better performance at low temperatures than the populations collected from cooler areas, which further suggests that they are more broadly adapted.

Trichogramma sp. nr. *mwanzai* (M), *T.* sp. nr. *lutea* (M) and *T.* sp. nr. *mwanzai* (L) were better suited to performance at high temperatures, hence could be expected to perform

well in warmer areas. Conversely, *Trichogramma bruni* (H) was unable to parasitise at 35°C, suggesting poor performance at such warmer temperatures. This strain originated from high altitude, where mean monthly temperatures are low (10-23°C), and was apparently not well suited to the higher temperature regimes. *Trichogramma* sp. nr. *mwanzai* (L) performed better than other species/strains, which may have been related to its history of laboratory rearing. This taxon was reared much longer than the other species/strains, and thus may have been inadvertently selected to perform well under laboratory conditions. However, since all the species/strains were stringently reared continuously as isofemale lines, there was little chance of adaptation to the laboratory environment suggesting therefore that *T.* sp. nr. *mwanzai* (L) may be intrinsically superior.

Progeny production followed a similar trend as parasitism across the different temperature and humidity regimes. Ochiel (1989) observed significant effect of temperature on progeny production on *T.* sp. nr. *exiguum* when tested at 18 and 30°C. In the present study, *T.* sp. nr. *mwanzai* (L), *T.* sp. nr. *mwanzai* (M) and *T.* sp. nr. *lutea* (M), produced higher progeny at the higher temperatures and thus may be good candidates for deploying in the warmer areas of Kenya. Park *et al.* (2000) found that emergence of *Trichogramma dendrolimi* was lower at higher (30-32°C) as well as at lower temperatures (26°C) but was highest at 28°C. In the present study, apparently because of the higher parasitism and progeny production, the optimum temperature for the candidate species/strains was found to be around 30°C.

There was no clear relationship between sex ratio and temperature for most species/strains at the two humidity levels. Similarly, Lund (1934) found no consistent

relationship between temperature and sex ratio for *Trichogramma minutum*. Ochiel (1989) also reported no significant difference in sex ratio for *T.* sp. nr. *exiguum* with temperature. Harrison *et al.* (1985), however, reported that temperature affected the sex ratio of *Trichogramma exiguum* and *Trichogramma pretiosum*, with females slightly less abundant at lower and upper developmental temperatures. A similar trend has been reported by Haile *et al.* (2002a) for *Trichogramma* sp. nr. *mwanzai* in Kenya. The present results, however, showed that temperature affected the sex ratio of some strains, but not all.

Generally, there was an inverse relationship between longevity and temperature. Similarly, Harrison *et al.* (1985) found that the longevity for *T. exiguum* Pinto and Platner and *T. pretiosum* Riley significantly decreased with increase in temperature (15-30°C). The same trend has also been reported by Yu *et al.* (1984) for *T. minutum* Riley. The differences observed in longevity between strains within the different temperatures and humidity levels are suggestive of differences in adaptation to environmental conditions. The ability of a parasitoid to perform in a particular agro-ecosystem is a critical factor in evaluating the potential of a parasitoid as a natural enemy (Tillman and Powell, 1991). It is therefore important that the choice of the strain should be made on the basis of the climatic conditions of the area of release.

Different researchers have used different biological attributes in evaluating *Trichogramma* performance. Hirashima *et al.* (1990) used adult longevity to compare the potential of two *Trichogramma* species in which *Trichogramma chilonis* Ishii was superior (5 days) compared to *Trichogramma ostriniae* Pang and Chen (4.6 days). Hassan and Guo (1991) selected appropriate *Trichogramma* species to control the European corn

borer, *Ostrinia nubilalis* Hubner, on the basis of their fertility. It is, however, essential that longevity be matched by greater fecundity, if a generational response is desired. The two attributes were found in *T.* sp. nr. *mwanzai* (L), *T.* sp. nr. *mwanzai* (M) and *T.* sp. nr. *lutea* (M) in the present study. The existence of a positive correlation between the daily fecundity and adult longevity by the species/strains suggests good performance. Because *H. armigera* and *C. cephalonica* are equally suitable hosts (Muholo, 2002), the three strains are potentially useful for the control of *H. armigera*. Bourchier and Smith (1996) and Dutton *et al* (1996) also found fecundity on a factitious host to predict field parasitism.

Laboratory studies at different temperatures provide useful information on the performance of potential insects (Wang *et al.*, 1997). They also indicate the potential effectiveness of parasitoids in the field (Force and Messenger, 1964). However, according to Omer *et al.* (1996), it is important that verifications are done in the field where conditions are variable.

In conclusion, the three strains; *T.* sp. nr. *mwanzai* (L), *T.* sp. nr. *mwanzai* (M) and *T.* sp. nr. *lutea* (M) appear to be the most promising candidates for use in augmentative biocontrol programmes against *H. armigera* in warmer areas where the pest is a serious problem.

CHAPTER FIVE

5.0. POPULATION GROWTH AND DEVELOPMENT OF TRICHOGRAMMATID EGG PARASITOIDS AT DIFFERENT TEMPERATURE AND HUMIDITY REGIMES.

5.1. Introduction

Egg parasitoids (Hymenoptera: Trichogrammatidae) are used extensively for the control of several lepidopteran pests in many areas of the world (Li, 1994). Commercial use of *Trichogramma* spp. egg parasitoids for augmentative biological control of key lepidopteran pests has been reported in over 30 countries (Wajnberg and Hassan, 1994). The effectiveness of *Trichogramma* spp. in the field depends on several factors, the major ones being its searching behaviour (habitat, host location), host preference (recognition, acceptance, suitability) and tolerance to environmental conditions (Hassan, 1994).

Climatic tolerance of natural enemies is a key factor that determines species establishment and effectiveness in augmentative biological control programs (DeBach, 1965; Messenger *et al.*, 1976a,b; Hokkanen, 1985), and thus has been incorporated into the process of evaluating candidate natural enemies (Kot, 1979). For inundative biological control, adaptation of natural enemies to climatic (weather) conditions during the crop growing season is critical. Tolerance of candidate *Trichogramma* species/strains to extreme weather conditions in the relevant area is therefore important. Studies have shown that temperature influences several biological characteristics of insects, including sex ratio (Godfray, 1994), adult life span, survival, fecundity and fertility (Singh and

Ashby, 1985;Yang *et al.*, 1994; Dreyer and Baumgartner, 1996) and developmental rates (Taylor, 1981; Pedigo, 1989).

The construction of life tables is useful to gain insight into the population dynamics of a species (Southwood, 1978) and has been widely employed in entomological and ecological studies to develop an understanding of age-specific mortality and reproductive rates (Carey, 1993). Life tables provide an excellent method for comparative inter- and intraspecifc studies (Bleicher and Parra, 1990).

In biological control, the intrinsic rate of natural increase has been used as a bioclimatic index to compare natural enemies (Messenger, 1970). It is useful in comparing different species and/or populations of the same species and also in evaluations of natural enemies on different hosts (Orphanides and Gonzalez, 1971; Nechols *et al.*, 1989). Environmental factors tend to operate in an interactive manner in natural ecosystems. Temperature and moisture are considered to be important climatic factors affecting life history processes of insects (Odum, 1983).

In Kenya, Ochiel (1989) studied the development and life table attributes of *Trichogramma* sp. nr. *exiguum* Pinto and Platner at different temperatures, while Haile *et al.* (2002a) compared the development of three exotic and one native species at different temperatures. Haile *et al.* (2002b) also compared life table attributes of *T. bournieri* and *T.* sp. nr. *mwanzai* at 26±1°C and 70±10%RH. The present study was conducted to investigate the effect of temperature and relative humidity on the development and population growth of six indigenous trichogrammatid parasitoid strains from Kenya to assess their climatic adaptability.

5.2. Materials and Methods

Six parasitoid species/strains (Table 2) were used in this study. The species/strains were compared on the basis of developmental time in *C. cephalonica* and on life table population growth parameters at six temperatures and two relative humidities.

5.2.1. Bioassay

To determine the developmental time, one day- old mated naïve female parasitoids were confined individually in glass vials (2.5 cm diameter by 7.5 cm height). They were provided diet in the form of minute streaks of 10% honey solution on the inside of the vials. Each female was then provided with 30 eggs (one day-old) of *C. cephalonica* fixed on a card with dilute gum. The glass vials containing the adult female and the host eggs were placed inside rectangular Perspex cages (30 cm width x 30 cm diameter x 20 cm height) with an opening on one side, which was sealed with a lid cover. They were placed in incubators adjusted to the six constant temperatures (chapter two). The relative humidity in the cages was maintained at either 40-50% or 70-80%. Ten females (replicates) were used per strain for each treatment. Each day, the eggs were replaced with fresh ones (one day old) until the female parasitoid died. Temperature and humidity were maintained as in chapter two.

Parasitoid emergence was monitored every 24 hours. The number of eggs parasitised, adults emerged and sex ratio (the proportion of female progeny) were recorded. Daily records of age specific fecundity (m_x) and age specific longevity (survival) (l_x) of each female were also kept and used in constructing life tables (Dent and Walton, 1997).

5.2.2. Data Analysis

Analysis of variance (ANOVA), Proc GLM (SAS Institute, 2000) was used to examine the effect of temperature and relative humidity on the developmental time of the parasitoid strains. When ANOVAs were significant, mean separation was done using Student-Newman-Keul's (SNK) test at P=0.05 (Zar, 1996). Age-specific life tables (Southwood, 1978; Bellows *et al.*, 1992) were constructed using a group of 10 females for each treatment. L_x (age specific survival rate) and mx (age specific fecundity) tables were constructed using age increments of 0.5 days (Andrewartha and Birch, 1954). A mid point, the pivotal age (x), represented the span between two consecutive daily periods.

For any particular age group, x was the pivotal age in days, l_x was the survival rate (proportion of individuals alive) at the beginning of the age interval and m_x was the mean number of eggs successfully parasitised at the pivotal age of the female.

Life table statistics were calculated using the Jacknife procedure described by Hulting *et al.* (1990). Differences among intrinsic rates of increase and net reproductive rates between strains at the different temperature and humidity levels were evaluated using the student-Newman-Keuls test based on Jacknife associated standard errors (Hulting *et al.*, 1990; Zar, 1996). Data on the following biological parameters; net reproductive rate (R_o), innate capacity of natural increase (r_m), finite rate of increase (λ) and mean generation time (GT) at the different temperature and humidity regimes were used for evaluating the parasitoid strains.

5.3. Results

5.3.1. Developmental time

Temperature and relative humidity levels significantly affected the developmental time of the parasitoids species/strains ($F = 419.2$; $df = 5,2256$; $P < 0.0001$; $F = 6.9$;$df = 1,2256$; $P = 0.0086$, respectively). Variations in developmental time were also observed between strains ($F = 160.9$;$df = 5,2256$; $P < 0.0001$). The interaction of the three factors also significantly affected developmental time ($F = 3.84$; $df = 24, 2256$; $P < 0.0001$). Most of the strains developed at each of the temperature-humidity levels evaluated except *T. bruni* (H), which did not develop at 35°C at both the humidity levels. Significant differences in developmental times were observed between strains at all the temperature-humidity levels evaluated, except at 10°C at 40-50%RH ($F = 2.61$;$df = 5,24$;$P = 0.0506$).

Significant differences in developmental time were observed between the strains at the different temperatures at the two relative humidities (Table 5.1 and 5.2). Generally, parasitoid developmental time was inversely related to temperature. Parasitoid development did not differ significantly between the two humidity levels tested at 10 and 15°C. Whereas, *T.* sp. nr. *lutea* (L) took longer to develop at 70-80%RH at 20°C, *T.* sp. nr. *lutea* (H) and *T. bruni* (H) had longer developmental times at 40-50%RH. At 25°C, *T.* sp. nr. *mwanzai* (L), *T.* sp. nr. *lutea* (H), *T.* sp. nr. *mwanzai* (M) and *T.* sp. nr. *lutea* (M) took longer to develop at 40-50% RH. Whereas *T.* sp. nr. *mwanzai* (L), *T. bruni* (H) and *T.* sp. nr. *mwanzai* (M) took longer to develop at 30°C, at the lower humidity, *T.* sp. nr. *mwanzai* (M) took longer to develop at 35°C at the higher humidity.

5.3.2. Life table parameters

Data on population growth parameters; Ro (net reproductive rate), r_m (intrinsic rate of natural increase), λ (finite rate of increase), and GT (generation time) of the six-trichogrammatid parasitoid species/strains at the different temperatures at the two humidity levels (70-80% and 40-50%) are presented in Tables 5.3 and 5.4, respectively.

5.3.2.1. Net reproductive rate (R_o)

The highest net reproductive rate (R_o) for most of the strains at 70-80%RH was reached at 30°C except for *T.* sp. nr. *mwanzai* (L) and *T.* sp. nr. *mwanzai* (M), which had their highest rates at 25°C (Table 5.3). At the lower relative humidity level (40-50%), most strains except *T.* sp. nr. *mwanzai* (L) and *T.* sp. nr. *lutea* (M) also had their highest net reproductive rates at 30°C (Table 5.4). At each temperature, significant differences in the net reproductive rate were observed between parasitoid strains at each of the two relative humidity levels tested.

At 70-80%RH, at 10°C, *T.* sp. nr. *mwanzai* (M) had a higher net reproductive rate than *T.* sp. nr. *lutea* (L), *T.* sp. nr. *mwanzai* (L) and *T.* sp. nr. *lutea* (M). Similarly, *T.* sp. nr. *lutea* (H) had a higher net reproductive rate than *T.* sp. nr. *lutea* (M). At 15°C, *T.* sp. nr. *mwanzai* (M) had the highest net reproductive rate among all the strains. *Trichogramma* sp. nr. *mwanzai* (L) had a higher net reproductive rate than *T.* sp. nr. *lutea* (H) and *T.* sp. nr. *lutea* (M). At 20°C, net reproductive rate for *T.* sp. nr. *mwanzai* (M), *T.* sp. nr. *lutea* (H) and *T.* sp. nr. *lutea* (L) were higher than for *T.* sp. nr. *lutea* (L). At 25°C, *T.* sp. nr. *mwanzai* (M) had a higher net reproductive rate than *T.* sp. nr. *lutea* (L), *T.* sp. nr. *lutea* (H) and *T. bruni* (H) while the net reproductive rate for *T.* sp. nr. *lutea*

(M) was higher than *T.* sp. nr. *lutea* (H) and *T. bruni* (H) and that of *T.* sp. nr. *mwanzai* (L) was also higher than for *T.* sp. nr. *lutea* (H). At 30°C, *T.* sp. nr. *lutea* (M) had a higher net reproductive rate than *T.* sp. nr. *mwanzai* (L), *T.* sp. nr. *lutea* (H), *T. bruni* (H) and *T.* sp. nr. *mwanzai* (M) while at 35°C, *T. bruni* (H) did not replace itself and the net reproductive rate for *T.* sp. nr. *lutea* (M) was higher than for *T.* sp. nr. *lutea* (L).

At the lower relative humidity (40-50%), at 10°C, *T.* sp. nr. *mwanzai* (M) and *T.* sp. nr. *mwanzai* (L) had higher net reproductive rates than *T. bruni* (H) and *T.* sp. nr. *lutea* (M) while at 15°C; *T.* sp. nr. *mwanzai* (M) had a higher net reproductive rate than *T.* sp. nr. *lutea* (L), *T.* sp. nr. *lutea* (H) and *T. bruni* (H). At 20°C, *T.* sp. nr. *mwanzai* (M) had a higher net reproductive rate than *T.* sp. nr. *mwanzai* (L), *T.* sp. nr. *lutea* (H) and *T. bruni* (H) while at 25°C, the net reproductive rate for *T.* sp. nr. *mwanzai* (M) was higher than for *T.* sp. nr. *lutea* (L), *T.* sp. nr. *lutea* (H), *T. bruni* (H) and *T.* sp. nr. *lutea* (M). Additionally, the net reproductive rate for *T.* sp. nr. *mwanzai* (L) was higher than for *T.* sp. nr. *lutea* (L), *T.* sp. nr. *lutea* (H) and *T. bruni* (H). *Trichogrammatoidea* sp. nr. *lutea* (M) also had a higher net reproductive rate than *T.* sp. nr. *lutea* (L), *T.* sp. nr. *lutea* (H) and *T. bruni* (H). At 30°C, *T.* sp. nr. *mwanzai* (M) had the highest net reproductive rate. However, *T.* sp. nr. *lutea* (L) had a higher net reproductive rate than *T.* sp. nr. *mwanzai* (L), *T.* sp. nr. *lutea* (H) and *T. bruni* (H) while *T.* sp. nr. *lutea* (M) had a higher net reproductive rate than *T.* sp. nr. *mwanzai* (L) and *T. bruni* (H). The net reproductive rate for *T.* sp. nr. *lutea* (H) was also higher than that of *T.* sp. nr. *mwanzai* (L) and at 35°C; *T. bruni* (H) did not replace itself while *T.* sp. nr. *mwanzai* (L) had the highest net reproductive rate. In general, the net reproductive rates for most parasitoid strains were higher between 25°C and 30°C.

5.3.2.2. Intrinsic rate of natural increase (r_m)

The highest intrinsic rate of natural increase for most of the strains at 70-80%RH was reached at 30°C except for *T.* sp. nr. *mwanzai* (M), which was reached at 25°C. At the lower relative humidity level (40-50%), the intrinsic rate of increase for *T.* sp. nr. *lutea* (L), *T.* sp. nr. *lutea* (H), *T. bruni* (H) and *T.* sp. nr. *lutea* (M) was highest at 30°C while that of *T.* sp. nr. *mwanzai* (L) and *T. bruni* (H) was reached at 25°C and 20°C, respectively. Significant differences in the intrinsic rate of natural increase were also observed between the parasitoid strains at the different temperatures at the two relative humidity levels tested.

At 70-80%RH, at 10°C, *T. bruni* (H) had the lowest intrinsic rate of increase. The intrinsic rate of increase for *T.* sp. nr. *lutea* (M) was lower than for *T.* sp. nr. *mwanzai* (M), *T. bruni* (H), *T.* sp. nr. *lutea* (H) and *T.* sp. nr. *mwanzai* (L). At 15°C, *T.* sp. nr. *mwanzai* (M) had the highest intrinsic rate of increase. The intrinsic rate of increase for *T.* sp. nr. *mwanzai* (L) was significantly higher than for *T.* sp. nr. *lutea* (L) and *T.* sp. nr. *lutea* (H) while at 20°C, *T.* sp. nr. *mwanzai* (M) had a higher intrinsic rate of increase than *T.* sp. nr. *lutea* (L), *T.* sp. nr. *mwanzai* (L), *T.* sp. nr. *lutea* (H) and *T. bruni* (H). Additionally, *T.* sp. nr. *lutea* (M) had a higher intrinsic rate of increase than *T.* sp. nr. *mwanzai* (L).

At 25°C, *T.* sp. nr. *mwanzai* (M) had the highest intrinsic rate of increase. The intrinsic rate of increase for *T.* sp. nr. *lutea* (M) and *T.* sp. nr. *mwanzai* (L) were higher than for *T.* sp. nr. *lutea* (L), *T.* sp. nr. *lutea* (H) and *T. bruni* (H) while at 30°C, the intrinsic rate of increase for *T.* sp. nr. *lutea* (M) was higher than for *T.* sp. nr. *lutea* (L), *T.* sp. nr. *lutea* (H), *T. bruni* (H) and *T.* sp. nr. *mwanzai* (M). *Trichogramma* sp. nr. *mwanzai* (L) and *T.*

sp. nr. *lutea* (M) had higher intrinsic rate of increase than *T.* sp. nr. *lutea* (L) and *T.* sp.nr. *mwanzai* (M) at 35°C.

At the lower relative humidity level (40-50%), at 10°C, *T.* sp. nr. *mwanzai* (M) and *T.* sp. nr. *mwanzai* (L) had higher intrinsic rates of increase than *T.* sp. nr. *lutea* (L), *T. bruni* (H) and *T.* sp. nr. *lutea* (M) while at 15°C; *T.* sp. nr. *mwanzai* (M) had a higher intrinsic rate of increase than *T.* sp. nr. *lutea* (L), *T.* sp. nr. *lutea* (H) and *T. bruni* (H). Similarly, the intrinsic rate of increase for *T.* sp. nr. *mwanzai* (L) was higher than for *T. bruni* (H).

At 20°C, *T.* sp. nr. *mwanzai* (M) had a higher intrinsic rate of increase than *T.* sp. nr. *mwanzai* (L)and *T.* sp. nr. *lutea* (H) while *T.* sp. nr. *lutea* (L), *T. bruni* (H) and *T.* sp. nr. *lutea* (M) had higher intrinsic rates of increase than *T.* sp. nr. *lutea* (H). At 25°C, *T.* sp. nr. *mwanzai* (L), *T.* sp. nr. *mwanzai* (M) and *T.* sp. nr. *lutea* (M) did not differ but had higher intrinsic rates of increase than *T.* sp. nr. *lutea* (L), *T.* sp. nr. *lutea* (H) and *T. bruni* (H).

At 30°C, *T.* sp. nr. *mwanzai* (M) had the highest intrinsic rate of increase. The intrinsic rates of increase for *T.* sp. nr. *lutea* (L) and *T.* sp. nr. *lutea* (M) were higher than for *T. bruni* (H) while at 35°C, *T.* sp. nr. *mwanzai* (L) had a higher intrinsic rate of increase than *T.* sp. nr. *lutea* (L), *T.* sp. nr. *lutea* (H) and *T.* sp. nr. *mwanzai* (M).

At both humidity levels, the intrinsic rates of increase among the parasitoid strains increased with increasing temperature until 30°C and then decreased. The finite rate of increase also followed the same trend. It was also observed that prevailing temperature negatively affected generation time of parasitoid strains regardless of the relative humidity. The effect of temperature on generation time for the six parasitoid strains at the two humidity levels is graphically illustrated in Figure 5.1. The net reproductive rate and

intrinsic rate of increase were generally found to be higher at the lower relative humidity than at the higher humidity (Tables 5.3 and 5.4).

Table 5.1: Mean (± S.E) developmental period (days) of strains at six temperatures at 70-80% RH.

Temperature (°C)	*T.* sp.nr. *lutea* (L)	*T.* sp.nr. *mwanzai* (L)	*T.* sp.nr. *lutea* (H)	*T. bruni* (H)	*T.* sp.nr. *mwanzai* (M)	*T.* sp.nr. *lutea* (M)	F	df	P
10	11.3±0.1ab A	11.2±0.1ab A	11.2±0.1ab A	11. 7±0.2a B	11.7±0.1a A	11b A	5.7	5,42	0.0004
15	9.9±0.1c B	9.7±0.1c B	10.0±0.3c B	12.3±0.1a A	10.4±0.1b B	9.7±0.1c B	126	5,192	< 0.0001
20	9.1±0.1c DE	8.6±0.1d D	8.7±0.1d D	10.2±0.1a D	9.4±0.1b D	8. 8±0.1d B	73	5,202	< 0.0001
25	9.5±0.1b C	9.2±0.1c C	9.2±0.1c C	11.3±0.1a C	9.6±0.1b CD	9.3±0.1c C	122.9	5,279	< 0.0001
30	9.3±0.1b CD	8.5±0.1c D	8.8±0.1c CD	10.0a D	8.8±0.1c E	8.7±0.1c C	17.9	5,110	< 0.0001
35	8.9±0.1bc E	9.2±0.1b C	9.7±0.3a B	0	9.7±0.1a C	8.5±0.2c C	17.7	5,36	< 0.0001
F	39.7	61.3	58	121.8	110.7	31.1			
df	5,143	5,140	5,146	5,103	5,193	5,136			
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001			

Means followed by the same lower case letter in each row and those followed by the same upper case letter in each column are not significantly different at P=0.05 (Student-Newman-Keuls test).

Table 5.2: Mean (± S.E) developmental period (days) of strains at six temperatures at 40-50% RH.

Temperature (°C)	*T.* sp.nr. *lutea* (L)	*T.* sp.nr. *mwanzai* (L)	*T.* sp.nr. *lutea* (H)	*T. bruni* (H)	*T.* sp.nr. *mwanzai* (M)	*T.* sp.nr. *lutea* (M)	F	df	P
10	11.1±0.1a A	11.3±0.1a A	11.1±0.1a A	11a BC	11.4±0.2a A	11a A	2.6	5,24	0.05
15	9.9±0.1c B	9.7±0.1d B	9.7±0.1d B	12.3±0.1a A	10.3b B	9.6±0.1d B	138.2	5,257	< 0.0001
20	8.8±0.1cd D	8.8±0.1cd D	8.9±0.1c D	10.5a C	9.3b E	8.6d B	135.2	5,597	< 0.0001
25	9.7±0.1c BC	9.5c BC	9.6±0.1c B	11.4±0.1a B	10±0.1b C	9.6±0.1c C	78.1	5,408	< 0.0001
30	9.3±0.1c C	9.0±0.1d CD	8.9±0.1e D	11.3±0.1a B	9.7b D	8.7±0.1e C	85.1	5,179	< 0.0001
35	8.9±0.1bc D	9.5±0.2a BC	9.3±0.1ab C	0	9.4±0.1a E	8.7±0.1c C	8.0	5,47	< 0.0001
F	47	43.3	41	81.7	78.5	77.4			
df	5,238	5,269	5,235	5,121	5,371	5,278			
P	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001			

Means followed by the same lower case letter in each row and those followed by the same upper case letter in each column are not significantly different at P=0.05 (Student-Newman-Keuls test).

Table 5.3: Net reproductive rate (R_o), intrinsic rate of increase (r_m), mean generation time (GT) in days and finite rate of increase (λ) of six indigenous trichogrammatid species/strains at different temperatures at 70-80% RH .

Temperature (°C)	Parameter	*T.* sp.nr. *lutea* (L)	*T.* sp.nr. *mwanzai* (L)	*T.* sp.nr. *lutea* (H)	*T. bruni* (H)	*T.* sp.nr. *mwanzai* (M)	*T.* sp.nr. *lutea* (M)
10	R_o	0.96±0.03abc	0.91±0.65ab	1.87±0.35bcd	1.75±0.77abcd	5.66±1.67d	0.29±0.29a
	r_m	-0.01±0.02ab	0.027±0.06b	0.046±0.02b	0.046±0.03b	0.09±0.02b	-0.118a
	GT	18.2	23.12	13.73	14.49	17.762	18.44
	λ	1.00	1.00	1.05	1.04	1.1	0.94
15	R_o	4.18±1.49abc	9.37±2.41cde	1.89±0.96a	7.17±2.71abcd	31.09±3.37f	3.62±0.99ab
	r_m	0.088±0.02ab	0.157±0.01cde	0.052±0.04a	0.144±0.03abcd	0.224±0.02f	0.107±0.02abc
	GT	16.789	14.33	14.871	14.24	15.338	12.205
	λ	1.09	1.17	1.04	1.14	1.25	1.11
20	R_o	7.6±2.24b	2.31±0.93a	6.6±1.7b	6.72±2.55ab	10.81±2.28b	7.58±2.93ab
	r_m	0.154±0.02ab	0.078±0.04a	0.156±0.02abc	0.168±0.03abcd	0.252±0.02e	0.209±0.04bcde
	GT	12.74	11.75	12.234	11.63	9.43	10.074
	λ	1.17	1.07	1.17	1.18	1.29	1.22
25	R_o	8.29±2.51abc	15.88±3.99bcd	5.58±1.23a	6.64±1.38ab	25.68±3.09d	16.87±4.38cd
	r_m	0.199±0.02abc	0.266±0.01de	0.179±0.02ab	0.187±0.02a	0.35±0.01f	0.282±0.01d
	GT	10.74	10.4	9.732	10.216	9.28	10.02
	λ	1.22	1.3	1.19	1.2	1.41	1.33
30	R_o	15.97±4.39abcde	8.53±2.85ab	8.9±2.52abcd	6.84±2.45a	8.55±1.55abc	22.8±4.6e
	r_m	0.304±0.02abcd	0.309±0.05abcde	0.292±0.04ab	0.274±0.05a	0.295±0.03abc	0.413±0.02e
	GT	9.154	7.12	7.6	7.275	7.342	7.6
	λ	1.35	1.35	1.33	1.3	1.34	1.51
35	R_o	1.67±0.34b	4.67±1.12c	3.39±1.54bc	0a	2.22±2.22bc	4.86±4.86c
	r_m	0.076±0.03a	0.211±0.03c	0.195±0.07abc	.	0.104±0.04ab	0.225±0.03c
	GT	7	7.42	6.86		8.28	7.15
	λ	1.07	1.23	1.19		1.1	1.25

Means ± SE followed by the same letter in each row are not significantly different (P = 0.05) (Student-Newman-Keul's test).

Table 5.4: Net reproductive rate (R_o), intrinsic rate of increase (r_m), mean generation time (GT) in days and finite rate of increase (λ) of six indigenous trichogrammatid species/strains at different temperatures at 40-50% RH .

Temperature (°C)	Parameter	*T.* sp.nr. *lutea* (L)	*T.* sp.nr. *mwanzai* (L)	*T.* sp.nr. *lutea* (H)	*T. bruni* (H)	*T.* sp.nr. *mwanzai* (M)	*T.* sp.nr. *lutea* (M)
10	R_o	0.60±0.22abc	1.3±0.87c	0.79±0.42abc	0.172±0.17a	1.26±0.44c	0.21±0.08ab
	r_m	-0.025±0.03bc	0.023±0.04d	-0.002bcd	-0.135a	0.021±0.03d	-0.072±0.02b
	GT	16.49	16.08	16.19	20	13.369	19.65
	λ	0.97	1.02	0.99	0.916	1.02	0.925
15	R_o	6.90±1.64abc	9.26±2.6abcd	5.73±1.44ab	3.2±1.09a	18.09±3.21d	9.2±3.79abcd
	r_m	0.131±0.02abc	0.160±0.02bcd	0.130±0.02ab	0.089±0.02a	0.203±0.01d	0.153±0.03abcd
	GT	14.926	14.09	13.52	13.67	14.076	15.041
	λ	1.14	1.17	1.14	1.09	1.23	1.16
20	R_o	20.75±3.92abcd	14.4±3.82abc	13.4±3.16a	13.88±3.87ab	32.37±4.53d	21.59±3.86abcd
	r_m	0.210±0.01bc	0.188±0.02ab	0.174±0.01a	0.220±0.02bc	0.257±0.02c	0.222±0.01bc
	GT	14.49	14.339	15	12	13.539	13.832
	λ	1.232	1.2	1.19	1.24	1.29	1.25
25	R_o	8.38±2.62abc	31.77±4.82de	9.69±3.2ab	6.5±1.95a	39.67±4.51e	23.23±3.81d
	r_m	0.186±0.03ab	0.301±0.01d	0.204±0.02abc	0.173±0.03a	0.29±0.01d	0.275±0.01d
	GT	11.59	11.51	11.268	11.08	12.66	11.46
	λ	1.2	1.35	1.22	1.18	1.34	1.32
30	R_o	23.37±5.38de	5.81±1.99a	11.21±1.4bc	6.73±3.12ab	46.74±4.63f	16.25±3.34cd
	r_m	0.334±0.02bcd	0.237±0.05ab	0.291±0.01abc	0.2±0.05a	0.401±0.01f	0.341±0.03bcde
	GT	9.48	7.676	8.31	10.184	9.576	8.23
	λ	1.39	1.26	1.34	1.21	1.494	1.4
35	R_o	3.35±0.85bc	11.1±2.17f	1.59±0.77ab	0a	4.2±1.08bcd	4.4±1.17bcde
	r_m	0.169±0.03ab	0.267±0.02d	0.079±0.07a	.	0.190±0.19bc	0.206±0.04abcd
	GT	7.32	9.07	7.657		7.72	7.35
	λ	1.18	1.3	1.06		1.2	1.22

Means ± SE followed by the same letter in each row are not significantly different (P = 0.05) (Student-Newman-Keul's test).

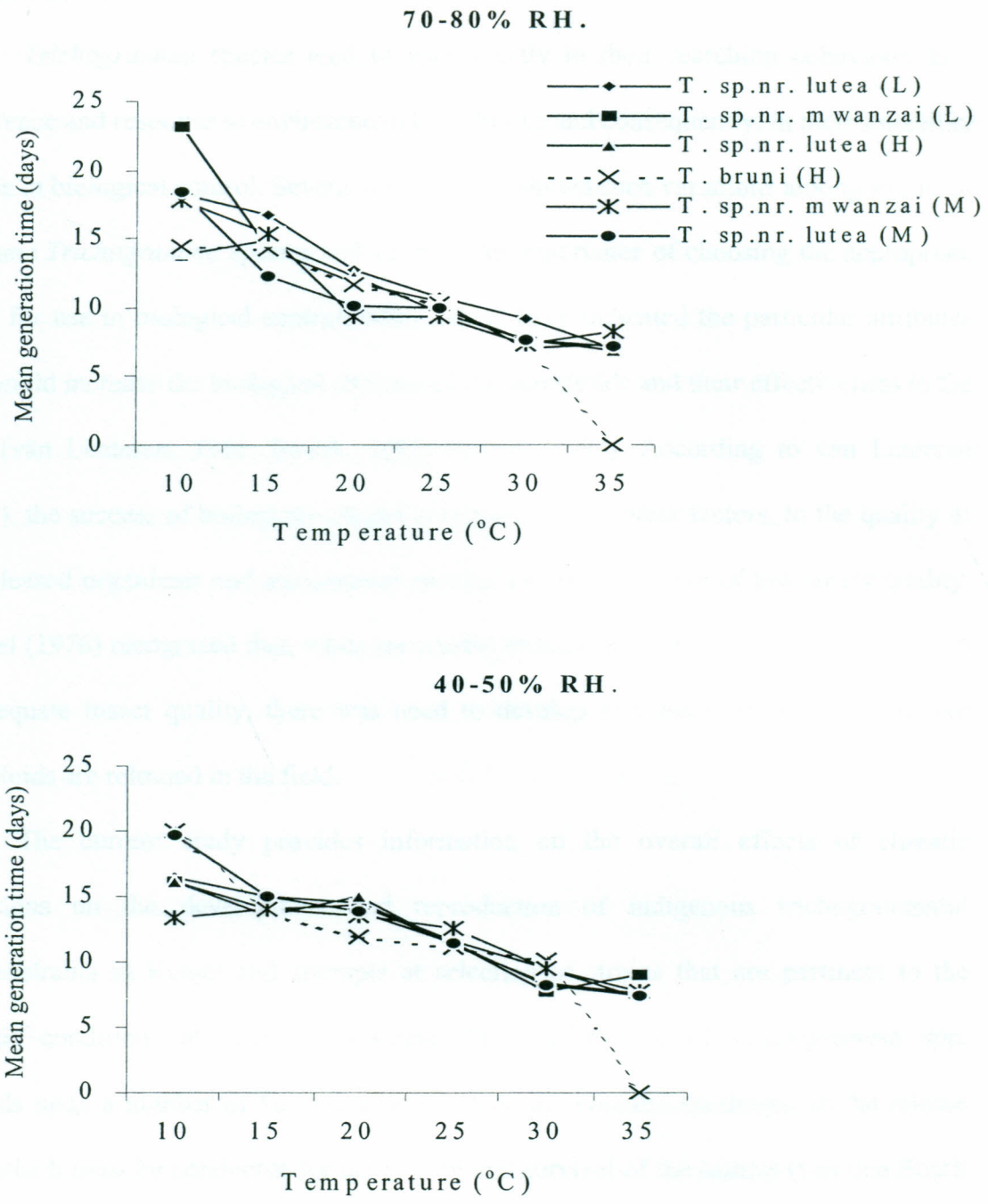

Fig. 5.1. Mean generation time of six trichogrammatid species/strains at six temperatures and two humidity regimes.

5.4. Discussion

Trichogramma species tend to vary greatly in their searching behaviour, host preference and response to environmental conditions and consequently, in their suitability for use in biological control. Several authors have shown such variations among strains of the same *Trichogramma* species and stressed the importance of choosing the appropriate strain for use in biological control, while others have indicated the particular attributes that would increase the biological abilities of the parasitoids and their effectiveness in the field (van Lenteren, 1986; Pavlik, 1993; Hassan, 1994). According to van Lenteren (1991), the success of biological control is related, among other factors, to the quality of the released organisms and sub-optimal success can be indicative of low insect quality. Huettel (1976) recognised that, while successful biological control was a clear indication of adequate insect quality, there was need to develop and assess performance before parasitoids are released in the field.

The current study provides information on the overall effects of climatic conditions on the development and reproduction of indigenous trichogrammatid species/strains in Kenya and attempts at selection of strains that are pertinent to the climatic conditions of target ecosystems. The performance of *Trichogramma* spp. depends upon a number of factors, which include the climatic conditions in the release area, which must be conducive for the growth and survival of the insects (van den Bosch and Messenger, 1973; Dent, 1991). While it has been recognised that insects develop faster at higher temperatures (Wagner *et al.*, 1984), optima, maxima and minima differ among species and an understanding of these traits has important consequences for parasitoids performance in the field.

The variations observed in the life table parameters among the parasitoid species/strains at the different temperatures and relative humidities in the present study point to the importance of environmental conditions in the choice of strains for satisfactory performance of parasitoids in the field. Between the two factors, temperature appeared to play a more significant role in influencing parasitoid development and reproduction, as the parasitoids exhibited comparable response pattern across the different temperatures, regardless of the humidity level. Other authors have also found that among the physical factors, temperature exerts the strongest influence on the biological characteristics of *Trichogramma* (Noldus, 1989; Pratissoli, 1995) and the present study tends to confirm this.

Most of the strains had faster development and higher intrinsic and net reproductive rates between 25 and 30°C at both humidity levels. This may suggest that the optimum temperature for these parasitoids lies in this range. Although *T.* sp. nr. *mwanzai* (M) took relatively longer to develop at 30°C, the intrinsic and net reproductive rates were also higher at this temperature. Because the majority of strains had their highest r_m values at 30°C at both humidity levels, the optimal temperature for the native trichogrammatid parasitoids may be around 30°C. According to Pak and Oatman (1982), the true influence of temperature on population growth is reflected in the r_m values, which are a net result of the combined effects of development, survival and reproduction. At the extreme low temperature (10°C), at both humidity levels, some strains exhibited negative r_m values (Figures 5.3 and 5.4), probably as a result of prolonged development (Tables 5.1 and 5.2), and the populations had the lowest growth rate. This may also mean that the populations of these strains at this temperature will decrease to extinction unless the

climatic factor eventually changes or immigration occurs. The highest temperature of 35°C also appeared to affect most strains, resulting in lower r_m values. Indeed, *T. bruni* (H) was so adversely affected that it resulted in no development and subsequent reproduction. This temperature was apparently detrimental to the strain indicating that it cannot survive to reproduce in such high temperature. Higher temperatures tended to induce faster development and a shorter generation time in most of the strains. The intrinsic rate of natural increase is a good indicator of the temperature at which growth of a population is most favourable because it reflects the overall effects of temperature on development, reproduction and survival characteristics of a population (Tsai and Wang, 1999).

The r_m values (0.266 and 0.301) for *T.* sp. nr. *mwanzai* (L) and (0.35 and 0.29) for *T.* sp. nr. *mwanzai* (M), at 70-80 and 40-50%RH, respectively at 25°C, in the present study compare well with 0.309 obtained by Haile *et al.* (2002b) for the same species at 26±1°C and 70±10%RH. Any differences may be because of different strains of the same species and/or relative differences in test conditions. Such biological differences among strains in environmental interactions may reflect adaptations in populations to the environment (Messenger and van den Bosch, 1971). Considering the six species/strains, *T.* sp. nr. *mwanzai* (M) and *T.* sp. nr. *mwanzai* (L) were superior. The two strains were more favourably adapted to both low and high temperatures at the two humidity levels as they had higher intrinsic and net reproductive rates and also developed relatively faster. In an earlier study, the same two strains had exhibited higher survival, fecundity and progeny production than the other strains. In this study, a relationship between the

performance (development and reproduction) of the strains and the climate of their origin could not be established.

Several authors have used life tables to compare strains and /or species of *Trichogramma*. Bleicher and Parra (1990), for example, evaluated the development of three populations of *Trichogramma* using life tables, while Maceda *et al.* (1994) compared *Trichogramma pretiosum* and *Trichogramma annulata,* using the finite increase ratio (λ). Similarly, Nagaraja (1988) evaluated for differences between six species of *Trichogramma* and one *Trichogrammatoidea* hybrid using life tables, while Jalali and Singh (1993) used a similar approach to select superior strains from collections coming from different agroclimatic zones. More recently, Haile *et al.* (2002b), using the intrinsic and net reproductive rates established there were no differences between *T.*sp. nr. *mwanzai* and *T. bournieri* from Kenya, although the two differed in their parasitisation potential.

Based on this approach, the present study has demonstrated that the two strains *T.* sp. nr. *mwanzai* (M) and *T.* sp. nr. *mwanzai* (L) were superior to the others as is indicated by their higher performance and adaptation to the range of temperatures and humidities tested.

CHAPTER SIX

6.0. HOST PREFERENCES AND SUITABILITY OF DIFFERENT LEPIDOPTERAN PESTS FOR THE INDIGENOUS TRICHOGRAMMATID SPECIES/STRAINS.

6.1. Introduction

Trichogrammatid egg parasitoids are extensively used as biological control agents because they attack eggs of several lepidopteran species that cause serious damage to crops in many different plant families (Smith, 1996). Although most trichogrammatids are polyphagous, there is a tendency for most of them to show strong preference to certain host (s), crop (s) and climatic conditions (Hassan and Gou, 1991). Some species also appear to be host selective, for instance *T. nubilale* has only been collected from eggs of *Ostrinia nubilalis* in corn fields in the eastern United States (Ertle and Davis, 1975) while in the laboratory, it preferred eggs of *O. nubilalis* over other American lepidopterans (Curl and Burbutis, 1978). An understanding of these preferences among trichogrammatid species/strains would therefore be useful in visualizing their potential impact on the target lepidopteran pests in the presence of non-target hosts in the crop ecosystem.

The process leading to successful parasitism is divided into host-habitat location, host location, host acceptance and host suitability (Doutt, 1959). Upon reaching the vicinity of a host (host's habitat), the parasitoid must locate its host. However, the hosts encountered by the parasitoids may differ in their quality for development of the young ones, in time required for their attack and the probability of female mortality during

oviposition (Godfray, 1994). The choice of suitable parasitoid candidate species or strains is important for successful biological control programs, because the host specificity characteristics of candidate species are generally considered among the factors determining their potential as control agents (Pak, 1988). Their effectiveness is likely to be diminished if certain host ages or species are preferred over others, because non-preferred stages or host species have an increased probability of remaining unparasitised (Ehler and Van den Bosch, 1974).

According to De Bach (1964), the host(s) should also be suitable for the development of the parasitoid and this should form one of the primary criteria in selecting a biological control program. Host suitability is defined as the degree to which the environment within the host provides the requisites for successful development and emergence of fertile adult parasitoids (Salt, 1938). Host suitability is concerned with factors affecting the development of the parasitoid within potential hosts (Vinson and Iwantsch, 1980). Upon successful host finding and host selection, host suitability represents the last step in the host parasite relationship towards successful parasitism (Vinson, 1976). Physiological suitability is an absolute necessity for successful development of parasitoid progeny (Hailemichael *et al.*, 1997). The success of parasitoid development may depend on factors such as the nutritional adequacy of the host, its immune system, toxins and competition (Vinson and Iwantsch, 1980). It is important that suitability of the target pest is investigated before directing the biological control program towards them.

Several studies on host preference of trichogrammatids have been carried out in the past (Yu *et al.*, 1984; Bournier, 1982; Torreno and Cadapan, 1984). In Kenya, Ochiel (1989) and Guang and Oloo (1990) studied the host preference and suitability of *Trichogramma*sp. nr. *exiguum* Pinto and Platner and *T.*sp. nr. *mwanzai* Schulten and Feijen, respectively. Abera (2001) determined the association of both host plants and host insects on the natural levels of parasitism by two *Trichogramma* species *T.*sp. nr. *mwanzai* and *T. bournieri*. The present study was undertaken to investigate the relative preference of six trichogrammatid species/strains for the target pest, *Helicoverpa armigera* and its suitability in comparison to other lepidopteran hosts that commonly occur. This would allow selection of candidate strains for augmentative biological control of *H. armigera*.

6.2. Materials and Methods

Eggs of the target pest, *Helicoverpa armigera* (Hubner) and five other lepidopteran hosts namely, *Busseola fusca* Fuller, *Sesamia calamistis* Hampson (Noctuidae), *Chilo partellus* Swinhoe, *Eldana saccharina* Walker (Pyralidae) and *Plutella xylostella* (L.) (Yponomeutidae), were used in the tests with six species/strains of *Trichogramma* and *Trichogrammatoidea* (Table 1). The host eggs were obtained from laboratory colonies maintained at the International Centre of Insect Physiology and Ecology (ICIPE), Nairobi, Kenya. The parasitoids were from isofemale lines, each established from the offspring of females emerging from *H. armigera* eggs collected from the field. The strains were reared in the laboratory using the factitious host, *Corcyra cephalonica* (Pyralidae). The conditions in the laboratory were maintained at 25±2°C, and 50±10% RH.

The acceptance of the six host species was tested in a no-choice experiment. The number of eggs parasitised per adult female during 24 hours was taken as the parameter for host acceptance as well as a measure of host preference level. A host was considered accepted if it contained at least one parasitised egg. To examine the relative preference of trichogrammatid species/strains among host species, 40 eggs of *H. armigera* were offered simultaneously with equal numbers of eggs of the other hosts to a one-day-old naive and mated female parasitoid of each of the strains in a glass vial (1.00 cm diameter by 3.5 cm height). The glass vial was covered with cotton wool. Minute streaks of diluted honey were provided as food to the parasitoid. The egg batches were exposed to the parasitoid for 24h after which the parasitoid was killed and eggs kept at 25±2°C and 60-70%RH until the parasitoids emerged. Parameters for suitability included the proportion of progeny that emerged from parasitised eggs and the progeny sex ratio (proportion of females).

6.2.1. Data analysis

Data on host preference, total progeny emerged and sex ratio was analysed and compared using chi-square (SAS Institute, 2000). Analysis of variance (ANOVA), using the general linear model (PROC GLM), was performed on host acceptance data to examine the effects of strain and host and their interaction on parasitism, progeny production and sex ratio. Acceptance data were Log (x+1) transformed, while percent data were arcsine transformed before statistical analysis (Sokal and Rohlf, 1981). When ANOVA was significant, mean separation was done using Student Newman-Keul's (SNK) test (Zar, 1996).

6.3. Results

6.3.1. Host acceptability

All the strains accepted all the six host species with significant differences between strains ($F = 7.33$; $df = 5, 144$; $P < 0.0001$) as well as the hosts ($F = 9.04$; $df = 5, 140$; $P < 0.0001$). The degree of host acceptance by each species is shown in Figure 6.1. There was no significant difference in acceptance levels between host species for *T.* sp. nr. *lutea* (H), *T.* sp. nr. *lutea* (L), *T.* sp. nr. *mwanzai* (L), *T.* sp. nr. *lutea* (M) and *T. bruni* (H) ($F = 1.16$; $df = 5, 24$; $P = 0.36$, $F = 2.61$; $df = 5, 24$; $P = 0.05$, $F = 0.56$; $df = 5, 24$; $P = 0.73$, $F = 2.68$; $df = 5, 24$; $P = 0.05$, $F = 1.1$; $df = 5, 24$; $P = 0.38$, respectively) while *T.* sp. nr. *mwanzai* (M) showed significant differences in acceptance between the host species ($F = 17.30$; $df = 5, 24$; $P < 0.04$).

6.3.2. Host preference

The relative number of eggs parasitised (%) by the species/strains on the different hosts in duo-choice with *H. armigera* is shown in Table 6.1. *T.* sp. nr. *lutea* (H) did not show significant difference in preference between *H. armigera* and *B. fusca* ($\chi^2 = 0.02$; $P = 0.886$) and between *H. armigera* and *S. calamistis* ($\chi^2 = 1.92$; $P = 0.17$). *T.* sp. nr. *lutea* (H), however, preferred *H. armigera* to *C. partellus* and *P. xylostella*. On the other hand, it preferred *E. saccharina* to *H. armigera*. *T.* sp. nr. *lutea* (L) did not show significant difference in preference between *H. armigera* and *B. fusca* and between *H. armigera* and *S. calamistis* ($\chi^2 = 3.33$; $P = 0.07$ and $\chi^2 = 1$; $P = 0.32$, respectively), while it showed higher preference for *H. armigera* compared to *E. saccharina* ($\chi^2 = 15.39$; $P < 0.0001$). *T.* sp. nr. *lutea* (L) also showed a higher preference of *H. armigera*.to *C. partellus* and *P.*

xylostella. *Trichogramma* sp. nr. *mwanzai* (L) preferred *H. armigera* to *C. partellus* and *E. saccharina,* but showed no significant difference in preference between *H. armigera* and *B. fusca* (χ^2 = 3.36; P = 0.07). The strain, however, showed a higher preference for *S. calamistis,* when compared to *H. armigera* (χ^2 = 6.13; P = 0.01). *H. armigera* was also preferred to *P. xylostella* (χ^2 = 5.83; P = 0.02). *T.* sp. nr. *lutea* (M) did not show significant preference between *H. armigera* and *B. fusca* (χ^2 = 0.02; P = 0.9) and *H. armigera* and *P. xylostella* (χ^2 = 1.39; P = 0.24). It however appeared to prefer *H. armigera* over *C. partellus*. *T.* sp. nr. *lutea* (M) also showed higher preference for *H. armigera* when in choice with *E. saccharina* and *S. calamistis* (χ^2 = 33.39; P < 0.0001 and χ^2= 23.44; P < 0.0001, respectively). Whereas *T. bruni* (H) exhibited preference for *H. armigera* over *P. xylostella* and *E. saccharina,* it did not show significant difference in preference between *H. armigera* and *C. partellus* and *H. armigera* and *S. calamistis* (χ^2 = 1.29; P = 0.26 and χ^2 = 0.67; P = 0.41, respectively). *Helicoverpa armigera* was preferred to *B. fusca* (χ^2 = 4; P = 0.05). *T.* sp. nr. *mwanzai* (M) did not show distinct preference between *H. armigera* and *B. fusca* (χ^2 = 0.13; P = 0.72, but preferred *H. armigera* to *C. partellus* and also to *P. xylostella* (χ^2 = 64.22; P < 0.0001). However, the strain showed a higher preference for *E. saccharina* and *S. calamistis* compared to *H. armigera* (χ^2 = 8.07; P = 0.005 and χ^2 = 4.57; P = 0.03, respectively).

6.3.3. Host suitability

6.3.3.1. Progeny production

Progeny production was affected significantly by the strain as well as the host (F = 8.85; df = 5, 140; P < 0.0001; F = 9.75; df = 5,140; P < 0.0001, respectively). The percentage of progeny produced by the different species/strains on the different hosts in

choice with *H. armigera* is shown in Table 6.2. *H. armigera* and *B. fusca* did not differ significantly in progeny production by *T.* sp. nr. *lutea* (H), but more progeny was produced on *H. armigera* than on *C. partellus, E. saccharina* and *P. xylostella. H. armigera,* however, had less progeny when compared to *S. calamistis* (χ^2 = 16.07; P < 0.0001). *T.* sp. nr. *lutea* (L) produced significantly more progeny on *H. armigera* than on *B. fusca*, *P. xylostella* or *C. partellus,* while no significant differences were observed between *H. armigera* and *E. saccharina* and between *H. armigera* and *S. calamistis* (χ^2 = 0.29; P = 0.59 and χ^2 = 0.07; P = 0.8, respectively).

Trichogramma sp. nr. *mwanzai* (L) produced more progeny on *H. armigera* than on *P. xylostella, E. saccharina* or *C. partellus,* but less progeny on *B. fusca* or *S. calamistis* (χ^2 = 34.77; P < 0.0001 and χ^2 = 18.75; P < 0.0001, respectively). Whereas there was no significant difference in progeny production between *H. armigera* and *B. fusca* (χ^2 = 3.37; P = 0.07) for *T.* sp. nr. *lutea* (M), there was more progeny produced on *H. armigera* compared to the other hosts.

For *T. bruni* (H), progeny production did not differ significantly between *H. armigera* and *B. fusca* or *C. partellus* or *S. calamistis* (χ^2 = 0.03; P = 0.86; χ2 = 1.8; P = 0.18 and χ^2 = 0.47; P = 0.49, respectively). More progeny was produced on *H. armigera* than on *E. saccharina* or *P. xylostella. Trichogramma* sp. nr. *mwanzai* (M) produced more progeny on *H. armigera* than on *C. partellus* or *P. xylostella,* while *H. armigera* and *S. calamistis* did not differ significantly (χ^2 = 0.76; P = 38). Less progeny was produced on *H. armigera* compared to *B. fusca* or *E. saccharina.*

6.3.3.2 Sex ratio

The proportion of females produced by the different species/strains on the different hosts in choice with *H. armigera* is shown in Table 6.3. *T.* sp. nr. *lutea* (H) produced a female biased sex ratio on *S. calamistis* compared to *H. armigera* ($\chi^2 = 5.4$; P = 0.02). Sex ratio for *T.* sp. nr. *lutea* (L) did not differ significantly between *H. armigera* and *E. saccharina* ($\chi^2 = 0.08$; P = 0.78) and between *H. armigera* and *S. calamistis* ($\chi^2 = 0.5$; P = 0.48). *Trichogramma* sp. nr. *mwanzai* (L) had a higher female biased sex ratio on *B. fusca* or *S. calamistis* than on *H. armigera* ($\chi^2 = 29.54$; P < 0.0001 and $\chi^2 = 14.24$; P = 0.0002), but there was no significant difference between *H. armigera* and *P. xylostella*. *Trichogrammatoidea* sp. nr. *lutea* (M) had a higher female biased sex ratio on *H. armigera* for all the hosts except *B. fusca,* where it was more male biased ($\chi^2 = 9.38$; P = 0.002). For *T. bruni* (H), the sex ratio did not differ between *H. armigera* and most of the other host species, except *P. xylostella* or *E. saccharina*. *Trichogramma* sp. nr. *mwanzai* (M) produced significantly more females in progeny on *B. fusca* than on *H. armigera* ($\chi^2 = 11.95$; P = 0.0005), but the progeny sex ratio between *H. armigera* and *S. calamistis* did not differ appreciably ($\chi^2 = 0.5714$; P = 0.45).

Table 6.1: Relative preference (%) of trichogrammatid species/strains for *H. armigera* eggs in duo-choice with five different lepidopteran host species.

Trichogrammatid species/Strain	*H. armigera*	*C. partellus*	*H. armigera*	*P. xylostella*	*H. armigera*	*B. fusca*	*H. armigera*	*E. saccharina*	*H. armigera*	*S. calamistis*
T. sp. nr. *lutea* (H)	100	0	100	0	49	51	0	100	40.4	59.6
T. sp. nr. *lutea* (L)	100	0	100	0	33.3	66.7	88.5	11.5	60	40
T. sp. nr. *mwanzai* (L)	100	0	72.4	27.6	38.8	61.2	100	0	28.1	71.9
T. sp. nr. *lutea* (M)	100	0	58.7	41.3	50.9	49.1	4.9	95.1	87.8	12.2
T. bruni (H)	28.6	71.4	100	0	63.5	37.5	100	0	41.7	58.3
T. sp. nr. *mwanzai* (M)	100	0	97.2	2.8	52.1	47.9	13.3	86.7	32.4	67.6

Table 6.2: Progeny production (%) by six trichogrammatid species/strains on *H. armigera* eggs in duo-choice with five different lepidopteran host species.

Trichogrammatid species/Strain	*H. armigera*	*C. partellus*	*H. armigera*	*P. xylostella*	*H. armigera*	*B. fusca*	*H. armigera*	*E. saccharina*	*H. armigera*	*S. calamistis*
T. sp. nr. *lutea* (H)	100	0	100	0	40.4	59.6	0	100	23.2	76.8
T. sp. nr. *lutea* (L)	100	0	100	0	100	0	57.1	42.9	53.3	46.7
T. sp. nr. *mwanzai* (L)	100	0	75	25	18.4	81.6	100	0	18.8	81.8
T. sp. nr. *lutea* (M)	100	0	69.8	30.2	39.5	60.5	96.2	3.8	95.7	4.4
T. bruni (H)	20	80	100	0	50.8	49.2	100	0	42.1	57.9
T. sp. nr. *mwanzai* (M)	100	0	100	0	27.1	72.9	0	100	42.4	57.6

Table 6.3: Sex ratio (% female) produced by six trichogrammatid species/strains on *H. armigera* eggs in duo-choice with five different lepidopteran host species.

Trichogrammatid species/Strain	*H. armigera*	*C. partellus*	*H. armigera*	*P. xylostella*	*H. armigera*	*B. fusca*	*H. armigera*	*E. saccharin*	*H. armigera*	*S. calamistis*
T. sp. nr. *lutea* (H)	100	0	100	0	72	28	0	100	30.6	69.4
T. sp. nr. *lutea* (L)	100	0	100	0	100	0	46.2	53.8	62.5	37.5
T. sp. nr. *mwanzai* (L)	100	0	33.3	66.7	19.2	80.8	0	100	17.7	82.3
T. sp. nr. *lutea* (M)	100	0	73.3	26.7	72.3	27.7	94.4	5.6	100	0
T. bruni (H)	50	50	100	0	52.7	47.3	100	0	50	50
T. sp. nr. *mwanzai* (M)	100	0	100	0	27.9	72.1	0	100	42.9	57.1

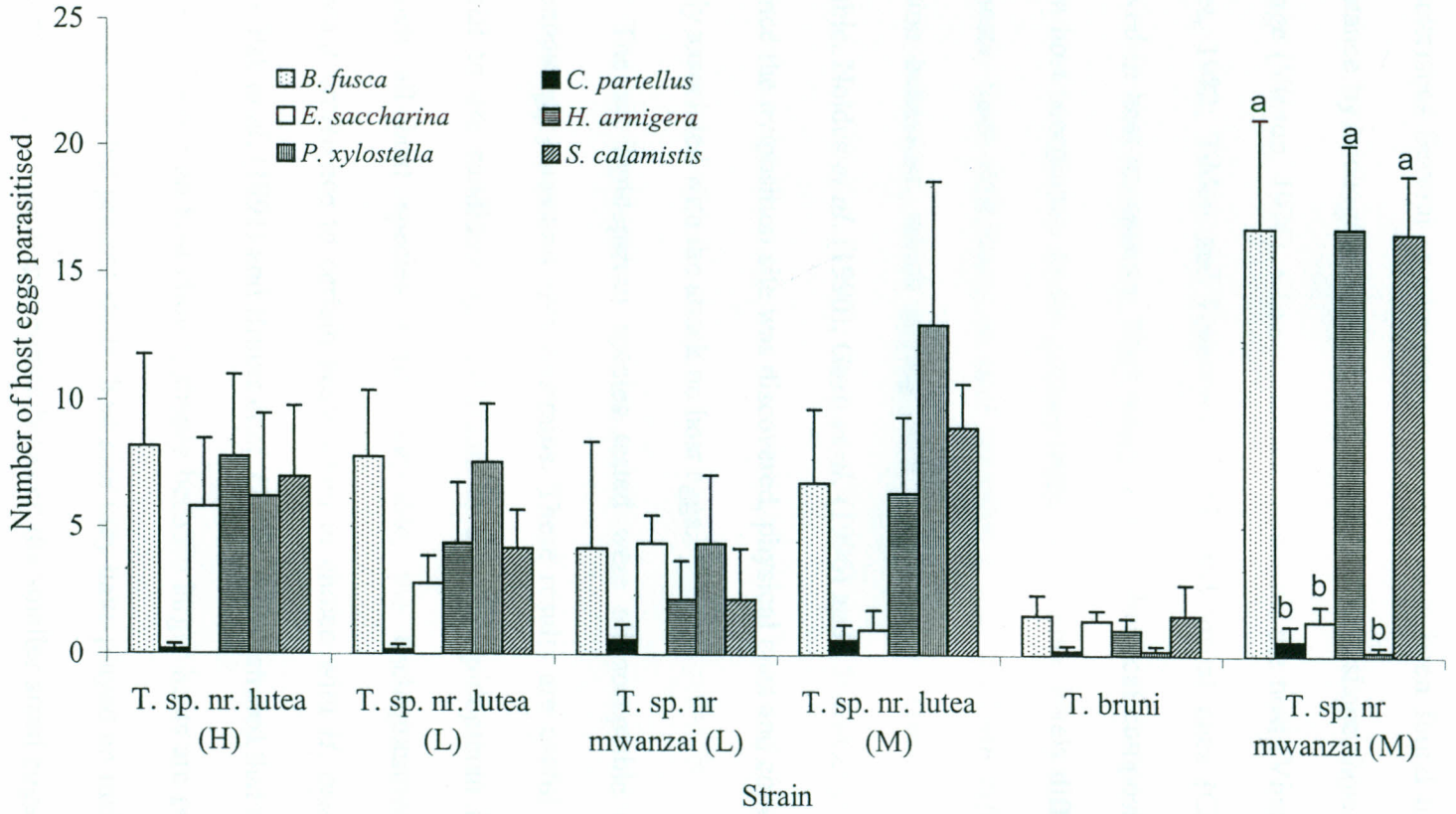

Fig.6.1. Host acceptance (number of host eggs parasitised) among six trichogrammatid species/strains for different hosts. Bars caped with different letters are significantly different, SNK, P = 0.05

6.4. Discussion

Before accepting a host, it should be evaluated by the parasitoid (van Driesche and Bellows, 1996). The decision by a parasitoid to accept a host depends on several characteristics Several species-specific factors have been found to play a role in host acceptance by trichogrammatids and include host egg shape, host size and colour and host age (Vinson, 1976). Chemicals associated with the host (Vinson, 1976; Mudd and Corbet, 1982; Tilden and Ferkovich, 1988) and visual cues (Casas, 1988) may be involved in host acceptance. Host odour and its chemical composition also play a key role in host acceptance. In the present study, acceptance levels differed between strains and hosts. Such differences in host acceptance may indicate differences in the host selection behaviour, which among other factors, may depend on the hosts that are available. Noldus *et al.* (1990), Gazit *et al.* (1996) and Chabi-Olaye *et al.* (2001b) noted that once the oviposition site was discovered, physical cues and contact kairomones were directly associated with the attack on host eggs.

The six lepidopteran species tested were all acceptable as hosts of the six indigenous egg parasitoid species/strains. These results are useful as they indicate the potential to use candidate species/strains to control lepidopteran key pests in Kenya. Although all host species were acceptable, the trichogrammatid strains showed significant preference to certain hosts when in choice with *H. armigera*. Monje *et al.* (1999), Pak *et al.* (1991) and Bruins *et al.* (1994) demonstrated that relative host size can have an influence on host choice, simply because larger hosts are perceived earlier than smaller ones. In the present study, host size may have played an insignificant role, since most strains did not show preference between the smaller sized eggs of *H. armigera* and

the larger sized eggs of *B. fusca, S. calamistis* and *E. saccharina*. *Trichogrammatoidea* sp. nr. *lutea* (L), *T.* sp. nr. *mwanzai* (L), *T.* sp. nr. *lutea* (M), *T. bruni* (H), for example, showed higher preferences for *H. armigera* over *E. saccharina*. Although not tested in this study, olfactory cues may be considered as possible factors responsible for the variations in host preferences. It is possible that, between *H. armigera* and either *B. fusca* or *S. calamistis*, differences in olfactory cues are perhaps more critical than between *H. armigera* and either *C. partellus* or *P.xylostella*.

The observation that most trichogrammatid species/strains showed higher preferences for oviposition in eggs of *H. armigera, B. fusca* or *S. calamistis* (all noctuids) as compared to others, further supports the finding of Kot (1964) that members of the family Noctuidae appear to be universally acceptable hosts of *Trichogramma*. Chabi-Olaye *et al.* (2004), in a similar study on host preference and suitability for *Lathromeis ovicida* Risbec (Hymenoptera: Trichogrammatidae), an egg parasitoid of cereal stemborers in Africa found significant preference for noctuids over pyralids.

Helicoverpa armigera, *B. fusca* and *S. calamistis* did not differ for most strains in terms of progeny production and sex ratio indicating that the three hosts are equally suitable for the development of the indigenous parasitoids. According to van Alphen and Janssen (1982) and van Alphen and Vet (1986), parasitoid females accept their hosts according to their suitability for progeny development. Sex ratio differences observed in the progeny could also be due to differences in the relative suitability of hosts. Waage (1982) and Ramadhan *et al.* (1995) reported host suitability to have a strong influence on sex ratio in arrhenotokous parasitoids. Unsuitable hosts or poor quality hosts may result

in a male-biased sex ratio (Charnov and Skinner, 1985). A female wasp may also lay more unfertilized eggs in hosts perceived to be of lower quality (Samira, 2003).

The male-biased sex ratio in the progeny may also be due to a high mortality of immature females in the host. Differential parasitoid mortality in which females suffered higher mortality than males has been reported (Rotary and Gerling, 1973). Godfray (1994) also reported that host suitability has a strong effect on parasitoid fitness. According to Jervis and Copland (1996), the growth and development of the parasitoid (not tested in this study) may also vary in relation to different host species, given that different host species are likely to constitute different resources in both quantitative and qualitative aspects.

The likelihood of occurrence of more than one host species in a given crop, may therefore suggest the use of a strain, which will accept the target host and any other hosts. This would be particularly relevant in smallholder-mixed crop-systems that are a common practice in sub-Saharan Africa. In such systems, a generalist parasitoid may be more advantageous when the interest is to provide general impact on all lepidopterans. However, in an inundative program, a host specific parasitoid may be more important apparently because releases are targeted against a single pest species (van Lenteren, 1980). *Trichogramma* sp. nr. *mwanzai* (L) and *T.* sp. nr. *mwanzai* (M), however, preferred *S. calamistis* and *E. saccharina* respectively to *H. armigera*. The two hosts were also more suitable for the development of parasitoids. These two strains may be good candidates, where *H. armigera* and the two pests co-exist and where *H. armigera* is the target pest of the control program.

In vegetable-based cropping systems, however, all the strains would be good candidates for *H. armigera,* since there is less likelihood that the other noctuids will be present. It

would be safer, however, to target *H. armigera* with strains that show more preference for it over the other lepidopteran pests in a particular agroecosystem. Conversely, where a strain prefers the other lepidopteran pests to *H. armigera*, it may not be ideal to deploy it and if need be, suitable adjustment in dosage to make impact for *H. armigera* should be considered.

According to the results of the present study, it is evident that the six species/strains tested are good candidates for use against *H. armigera*. Other selection criteria would be important to take into consideration in addition to preference and suitability studies.

CHAPTER SEVEN

7.0. RELATIVE PARASITISM OF *H. ARMIGERA* ON TWO HOST PLANTS BY DIFFERENT TRICHOGRAMMATID SPECIES/STRAINS.

7.1. Introduction

Host selection by many parasitoids is determined by habitat preference, and is subject to the influence of ecological factors such as characteristics of the host's habitat, climatic conditions, and physiological and experimental state of the parasitoids (Vinson, 1976; van Alphen and Vet, 1986). *Trichogramma* are known to be more habitat specifc than host specifc (Smith, 1996). The host plant is an important factor that may influence the parasitoid's host selection and therefore effectiveness in biological control. Different rates of egg parasitism are reported for the same host species on different host plants, by several *Trichogramma* spp. (Lopez *et al.*, 1982). One of the various ways that plants exert this influence is through the production of cues (synomones) that guide natural enemies to infested plants (Dicke, 1996). Plant odors have been found to play a role in host habitat location by *Trichogramma* (Altieri *et al.*, 1982; Nordlund *et al.*, 1985). A plant may also influence apparent host preference by providing the parasitoid orientation or lack of orientation to the host habitat (Vinson, 1976). Host plant adaptations probably interact with host-species adaptations, and their combined effect results in strains of *Trichogramma* being specialized and released in different crops.For example, *Trichogramma maidis* against the corn borer, *O. nubilalis* and *T. pretiosum* against the cotton bollworm, *Heliothis zea*. Morphological features of plants such as dense trichomes and waxy leaves and other plant characteristics may also represent a constraint to

foraging parasitoids and as such impair host encounters by parasitoids (Gingras *et al.*, 2003). It has also been recognized that *Trichogramma* spp. are not able to use stimuli derived from their hosts because insect eggs do not emit long-range volatiles (Kaiser *et al.*, 1989; Noldus, 1989). These egg parasitoids rely on sex pheromones and plant volatiles to detect presence of host eggs on plants. Noldus (1989) listed several examples of *Trichogramma* spp. responding to host sex pheromones, and a number of studies have shown that *Trichogramma* spp. are attracted/arrested or repelled by plant volatiles (Nordlund *et al.*, 1985; Kaiser *et al.*, 1989).

The present study aimed at determining the relative preference among trichogrammatid egg parasitoid species/strains for *H. armigera*, when occurring on two host plants, tomato and okra both in the laboratory and also in the field.

7.2. Materials and Methods

Four trichogrammatid species/strains namely *T.* sp. nr. *mwanzai* (L), *T.* sp. nr. *lutea* (H), *T.* sp. nr. *mwanzai* (M) and *T.* sp. nr. *lutea* (M)) were chosen for this study out of the original six because of their relatively higher performance, adaptation and higher reproductive rates (Chapters 3, 4 and 5).

7.2.1. Bioassay

The role of host plants and host insects on parasitism by different *Trichogramma* spp. can be determined by laboratory studies under choice and no choice situations (Bjorksten and Hoffmann, 1998) and field tests (Romeis *et al.*, 1997, 1998, 1999). In the present study, choice tests were carried out on two host plants of *H. armigera* (tomato and okra) both in laboratory and in field cage experiments. Laboratory tests involved use

of fresh leaves of the two plants, while field cage experiments involved use of potted plants.

To determine if the parasitoids showed preference between tomato and okra, 30 eggs of *H. armigera* were placed singly on leaves of the two host plants in a petri dish (7 cm diameter by 2 cm height). The leaves were placed in such a way that each of the host plant leaves covered half of the petri dish. The leaves were of the same age. A single one-day-old naïve female was then released in the center of the petri dish. The petri dish was then kept in an environmental chamber at 25°C and 70-80% RH. Seventeen replicates were used for each treatment. After 24h, the female parasitoid was killed and eggs monitored for blackening. Five days after exposure, the number of parasitised (black) eggs was counted under a stereo compound microscope. The experiment was also conducted with potted plants of the two host crops placed in glass cages measuring 48 cm length by 65 cm width by 100 cm height, at the Federal Institute of Biological control, Darmstadt, Germany. Single eggs of *H. armigera* on small pieces of paper were glued to the leaf surfaces of the plants using traganth-pulver (adhesive gum). The eggs were placed at equal distances from each other. Female parasitoids (one day old) were then released into the cage in a ratio 1:1 parasitoid to the host eggs.

7.2.2. Data analysis

Two-way Analysis of Variance (ANOVA) (Proc GLM, SAS Institute, 2000) was used to examine main effects (strains and host plant) and their interactions on parasitism performance. Choice comparisons of the number of eggs parasitised by each strain on the two host plants was analysed using chi-square (SAS Institute, 2000).

7.3. Results

Parasitism was significantly affected by strain ($F = 2.8$; $df = 3,7$; $P = 0.048$), while host plant and its interaction with strain did not significantly affect parasitism ($F = 0.7$; $df = 3,72$; $P = 0.421$ and $F = 0.2$; $df = 3,72$; $P = 0.914$, respectively). The relative parasitism of *H. armigera* by the four-trichogrammatid species/strains on the two plants is indicated in Figure 7.1.

In the Laboratory, choice tests showed significant preference for okra to tomato by *T.* sp. nr. *mwanzai* (L) ($\chi^2 = 0.11.6$; $P = 0.0006$). The other strains did not show significant differences in preference between the two plants (; $\chi^2 = 0.1$; $P = 0.8$ for *T.* sp. nr. *lutea* (H); $\chi^2 = 1.5$; $P = 0.3$ for *T.* sp. nr. *mwanzai* (M) and $\chi^2 = 0.5$; $P = 0.5$ for *T.* sp. nr. *lutea* (M)).

In the field cages, no significant differences in parasitism were observed between the two host plants among the four species/strains ($\chi^2 = 0.6$; $P = 0.4$ for *T.* sp. nr. *mwanzai* (L); $\chi^2 = 0.3$; $P = 0.1$ for *T.* sp. nr. *lutea* (H); $\chi^2 = 0.4$; $P = 0.5$ for *T.* sp. nr. *mwanzai* (M) and $\chi^2 = 1.7$; $P = 0.2$, respectively).

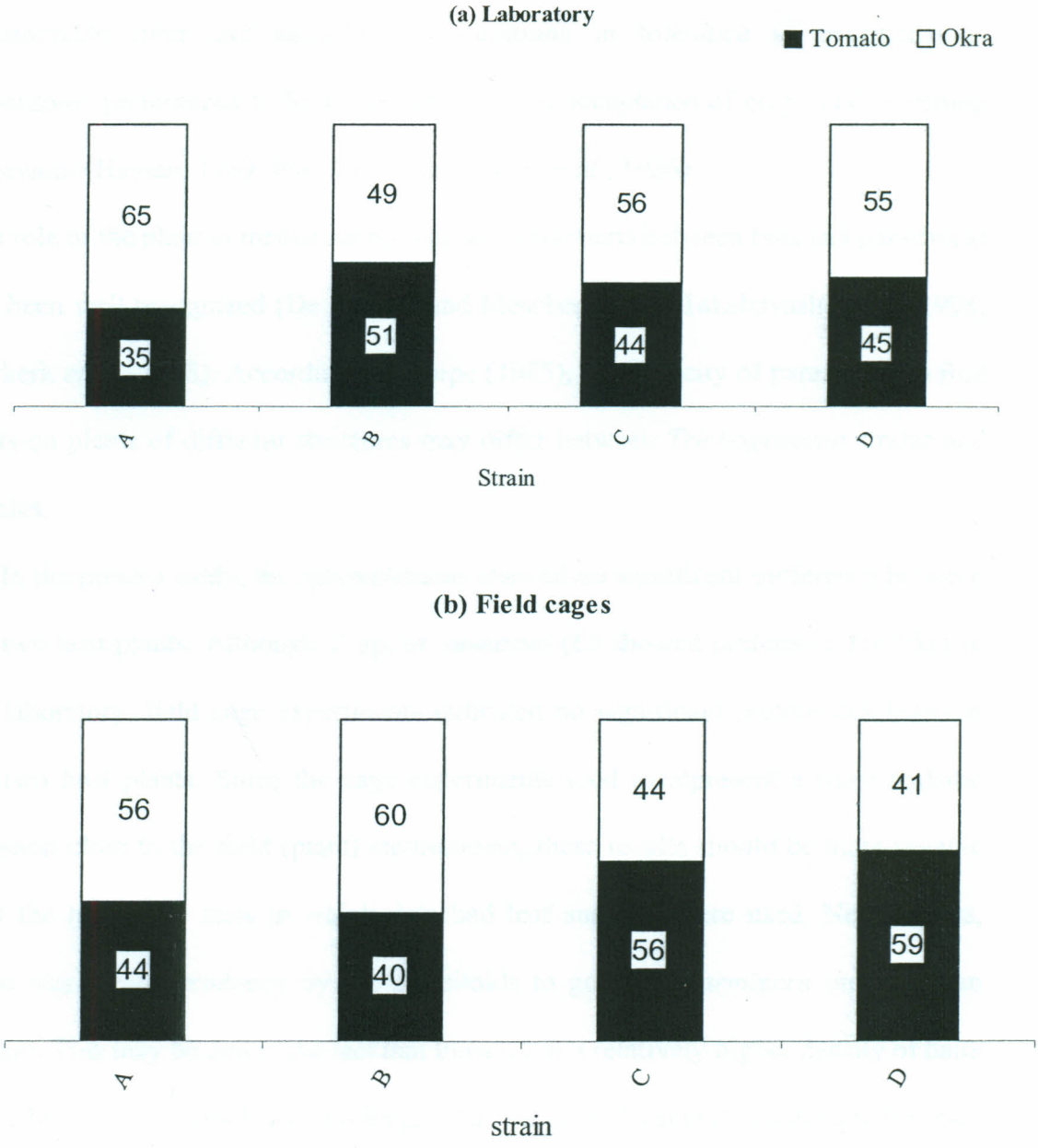

Fig. 7.1. Relative choice preference (%) of *H. armigera* on tomato and okra by four trichogrammatid species/strains in the laboratory and field cages. (A = *T.* sp. nr. *mwanzai* (L), B =*T.* sp. nr. *lutea* (H), C =*T.* sp. nr. *mwanzai* (M), D = *T.* sp. nr. *lutea* (M)).

7.4. Discussion

The egg parasitoids, *Trichogramma* are efficient biological control agents, commercially used to control lepidopterous pests in many crops (Li, 1994; Pak, 1992;

Smith, 1996). However, their suitability as biological control agents may vary due to considerable inter and intra-specific variations in tolerance to environmental conditions, preferences to hosts, recognition and acceptance of crops and searching behaviour (Hassan, 1989; Pak, 1988; van Dijken *et al.*, 1986).

The role of the plant in mediating ecological interactions between host and parasitoids has been well recognized (De Moraes and Mescher, 1999; Takabayashi *et al.*, 1998; Verkerk *et al.*, 1998). According to Thorpe (1985), the capacity of parasitoids to find hosts on plants of different structures may differ between *Trichogramma* strains and species.

In the present study, the species/strains showed no significant preference between the two host plants. Although *T.* sp. nr. *mwanzai* (L) showed preference for Okra in the laboratory, field cage experiments indicated no significant preferences between the two host plants. Since the cage experiments tend to represent a more realistic situation close to the field (plant) environment, these results should be more reliable than the laboratory tests in which detached leaf surfaces were used. Nevertheless, there was greater tendency by the parasitoids to go for *H. armigera* on okra than tomato. This may be due to the fact that tomato has a relatively higher density of hairs on its leaf surface, which are also longer than on okra. Such plant characteristics may represent a constraint to foraging parasitoids, and as such impair host encounters by parasitoids. Morphological features of plants such as dense trichomes (Kauffman and Keneddy, 1989; van Lenteren *et al.*, 1995), waxy leaves (Carter *et al.*, 1984; Kareiva and Sahakian, 1990), leaf surface area (Knippling and McGuire, 1968), and complex plant structure (Andow and Prokrym, 1990) have all been shown to reduce the

effectiveness of predators or parasitoids. The differences in parasitism should reflect differences in ability and efficiency with which parasitoids searched on plants of different structures (Franzer and McGregor, 1994; Gardner and Dixon, 1985).

Other studies have shown that a host species can experience different risk of parasitism when on different plant species or varieties (Gross and Price, 1988; Noldus, 1989;), or on different parts of the same plant (Gardner and Dixon, 1985). Plant odors, plant preference, or plant structure may account for these differences (Gardner, 1982). The present study did not test for plant odors, but it is assumed that differences in the type of volatiles are smaller between the two host plants as (van Etten and Tookey, 1979). Trichogrammatids have the ability to respond to a different array of plant synomones and vary in their degree of olfactory response and it is such differences that are responsible for the reported habitat-preference of *Trichogramma*.

The ability of the wasps to parasitise *H. armigera* on the two host plants suggests absence of deterrent properties in the tested host plants and also gives an indication that *Trichogramma* can be used in the two crop systems. Probably for tomato, a relatively higher dose of *Trichogramma* would need to be applied to enhance the impact.

CHAPTER EIGHT

8.0. GENERAL DISCUSSION, CONCLUSIONS AND RECOMMENDATIONS

8.1. General discussion

Use of trichogrammatid egg parasitoids has received great importance in applied biocontrol in different parts of the world, because of their large-scale use in release programmes against various pests. The use of trichogrammatid egg for control of pests in Africa is limited to a few countries such as South Africa and Egypt. Although commercially used in other areas of the world, relatively little information exists on the impact of these indigenous egg parasitoids in eastern Africa. This study focussed on assessing locally existing egg parasitoid species/strains collected from different agroecologies for potential impact against *H. armigera* and also establishing their relative adaptation to a arrange of temperature and humidity regimes representative of the tomato growing season. Assessments of parasitoid species/strains were conducted based on functional response and life table parameters (fecundity, progeny production, longevity, sex ratio, developmental time). The suitability of common lepidopteran pests for progeny development by the strains as well as their relative preferences for the host species when in choice with *H. armigera* was also investigated. The relative performance of species/strains on two *H. armigera* host plants was also investigated. The overall objective was to select promising species/strains for augmentative biocontrol of *H. armigera* in Kenya.

Climatic tolerance affects the survival and reproduction of a natural enemy in a given environment and plays an important role in determining the effect of the biological agents under ambient climatic conditions. Climatic adaptation and the ability of a natural

enemy to colonize in a given environment with extreme conditions is important (Chapter3 and 4). In the present study, tolerance to different temperature and humidity conditions was one selection criterion.

Functional response of indigenous parasitoids was mainly affected by temperature. Type I was most common, followed by type III, while type II was rare. The presence of a linear response is important for the continued interaction of the host and parasitoid, while the type III functional response can lead to density dependence, when host densities are low and thus can potentially stabilize host-parasitoid interactions (Berryman, 1999; Bernstein, 2000). The prevalence of type I responses showed that the parasitoids were able to find and attack hosts even at the lowest host density, which indicates a high searching ability of the parasitoids.

The number of host eggs successfully parasitised by the adult female parasitoid within her lifetime (fecundity) after release in the field, is a key attribute for selecting the appropriate species/strains. Such performance of a parasitoid (chapter 4) in its intended role after release is an important attribute of its quality (Bigler, 1994; Smith, 1996). Life history traits (progeny production/sex ratio) are secondary and are important only as efficiency parameters in mass production and hence of limited importance in selecting for an inundative system. Different researchers have used different biological attributes in evaluating performance. It is, however, essential that longevity is matched by greater fecundity, if a generational response is desired. The interaction of temperature and humidity, however, implies that to achieve high efficacy, not only temperature but also other climatic factors such as relative humidity need to be considered. In the current study, *Trichogramma* sp. nr. *mwanzai* (L), *T.* sp. nr. *mwanzai* (M) and *T.* sp. nr. *lutea*

(M) were shown to be the most promising candidates for use in augmentative biocontrol programmes against *H. armigera* in warmer areas where the pest is a serious problem. The other strains like *T. bruni* (H) may not have much impact due to poor parasitism performance and poor adaptation to the range of temperature and humidity regimes.

The potential reproductive capacity of a natural enemy, usually expressed by the intrinsic rate of natural increase, which combines the development time, emergence rate, survival and fecundity into population growth rate (Pak and Oatman, 1982) was also evaluated (Chapter 5). A strain with higher reproductive capacity is more likely to be a suitable strain in biological control. For inundative biological control, the reproductive potential does not appear to be a useful selection criterion, because a limited parasite fecundity can, in theory, be adjusted by releasing more parasites. However, Smith and Hubbes (1986) did find that the differences in field performance between various indigenous geographical strains of *T. minutum*, in the parasitism of spruce budworm eggs, *C. fumiferana*, corresponded to differences in reproductive capacity between strains determined in the laboratory.

Host specificity concerns the range of host species accepted by a parasitoid. As a selection criterion, host specificity is related to other criteria, such as environmental risks (narrow host range and low propensity of hyperparasitism and host suitability as well as density responsiveness (high host finding capacity). For inundative and seasonal inoculative releases, host specificity is not considered an important selection criterion, apparently due to the assumption that releases are directed against a single pest species (van Lenteren, 1980). However, if two or more related species are to be controlled, the

use of a single effective parasitoid is probably more economical (e.g. costs of the development of the program) than the use of different parasites for each host species (chapter 6). The ability of wasps to attack the target host species is considered more important for inoculative than for seasonal inoculative and inundative biological control. In the current study, all species/strains, however, showed good preference levels for *H. armigera*. This host was also suitable for progeny development.

Host plant attributes tend to interact with host-species adaptation and their combined effect results in some strains of *Trichogramma* being specialised and released in different crops. In the present study, tomato and okra did not differ significantly (chapter 7) and hence trichogrammatids can be used suitably in the two crop systems.

8.2. Conclusions

- *Trichogramma* sp. nr. *lutea* from high altitude, *Trichogramma* sp. nr. *mwanzai* from low altitude and *T.* sp. nr. *mwanzai* from medium altitude showed higher parasitism across the widest temperature range and hence are promising candidates for augmentative biocontrol of *H. armigera*.
- The highest parasitism at 40-50 % and 70-80% relative humidities was at 30°C for all strains.Survival followed a type I survivorship curve at lower temperatures and a type III survivorship curve at higher temperatures.Intrinsic rate of increase and finite rate of increase were found to increase with increasing temperature up to 30°C.
- Both net reproductiion and intrinsic rates of increase were higher at lower humidity.Temperature negatively affected generation time of parasitoids

regardless of the humidity level Most species/strains of parasitoids showed greater preference for noctuids over pyralids and yponomeutids.The different species/strains did not show significant differences in parasitising eggs of H. armigera when placed on tomato and okra.

8.3. Recommendations for future work

- Carry out functional response studies of species/strains under field conditions.
- Verify whether the parameters estimated in the laboratory at constant temperatures compare well to the field performance, where the diurnal temperature tends to vary.
- Investigate the role of morphological and chemical properties of plants in mediating host and host plant influences on parasitoid performance.
- Develop field release and impact assessment strategies for the parasitoids as a measure of their efficiency and effectiveness.

REFERENCES.

Abera, T.H. (2001). Studies on native parasitoid (*Trichogramma*) species in Kenya. PhD Thesis, Kenyatta University, Kenya. 190pp.

Abraham, C.C. (1970). Exploration of the feasibility of developing races of *Trichogramma australicum* Girault (Hymenoptera: Trichogrammatidae) suitable for different environments. PhD Thesis, IARI, New Delhi, India. 126 pp.

Alba, M.C. (1990). Utilisation of *Trichogramma* for biological control of sugarcane borers in Philippines. In: Wajnberg, E., Vinson, S.B. (Eds.), *Trichogramma and other Egg parasitoids, Proceedings of the 3rd International Symposium, San Antonio, Texas*, no. 56. INRA, Paris, pp. 161-165.

Altieri, M.A., W.J. Lewis, D.A. Nordlund, R.C. Gueldner and J.W. Todd (1981). Chemical interactions between plants and *Trichogramma* wasps in Georgia soybean fields. *Prot. Ecol.* **3**: 259-263.

Andow, D.A. and D.R. Prokrym (1990). Plant structural complexity and host finding by a parasitoid. *Oecologia* **82**: 162-165.

Andrewartha, H.G. and L.C. Birch (1954). *The distribution and abundance of animals.* University of Chicago Press, Chicago, 782pp.

Annecke, D.P. and V.C. Moran (1982). Insects and mites of cultivated plants in South Africa. Butterwoths, Durban.

Anonymous (1995). 1996 Directory of least-toxic pest control products. IPM Practitioner **17** (11/12): 1-48.

Balk, F. and J.H. Koeman (1984). Future hazards from pesticide use, with special reference to West Africa and Southeast Asia. *The Environmentalist*.**4** *suppl*.6. 99p.

Balla, A.N. (1982). Progress in research and development for *Heliothis* management in the Sudan.

Ballal, C.R. and S.P. Singh (2003). The effectiveness of *Trichogramma chilonis*, *T. pretiosum* and *T. brasiliense* (Hymenoptera: Trichogrammatidae) as parasitoids of *Helicoverpa armigera* (Lepidoptera: Noctuidae) on sunflower (*Helianthus annus*) and Redgram (*Cajanus cajan*). *Biocontrol Sci. Tech.* **13**: 231-240.

Bao, Jian-Zhang and Chen, Xiu-Hao (1989). *Research and application of* Trichogramma *in China*. Academia Books and Periodicals Science Press, Beijing, 220 pp.

Beeden, P. (1974) Bollworm oviposition of cotton in Malawi. Cotton growing Review **5**: 52-61.

Bellows, T.S., R.G Van Driesch, and J.S. Elkinton (1992). Life table construction and analysis in the evaluation of natural enemies. *Ann. Rev. Entomol.* **37**: 587-614.

Bernstein, C. (2000). Host-parasitoid models: the story of successful failure. In: Hochberg, M., Ives, A. (Eds.), *Population biology of host-parasitoid interactions*. Princeton University Press, Princeton, NJ, pp. 41-57.

Berryman, A.A. (1999). The theoretical foundations of biological control. In: Hawkins, B.A., Cornell, H.V. (Eds.), *Theoretical approaches to Biological control*. Cambridge University Press, Cambridge, UK. pp. 3-21.

Bigler, F. (1986). Mass production of *Trichogramma maidis* Pinto and Voegele and its field application against *Ostrinia nubilalis* Hubner in Switzerland. *J. Appl. Entomol.***101**: 23-29.

Bigler, F. (1989). Quality assessment and control in entomophagous insects used for biological control. *J. Appl. Entomol.* **108**: 390-400.

Bigler, F. (1994). Quality control in *Trichogramma* population. In: *Biological control with Egg Parasitoids* (Wajnberg, E. & Hassan, S.A., Eds). Biddles, Guildford & King's Lynn, pp. 93-112.

Bigler, F., F. Cerutti and J. Laing. (1991). First draft of criteria for quality control (product control) of *Trichogramma*. In: *Proceedings of 5th workshop of the IOBC Global-working Group Quality control of mass reared Arthropods* (Bigler, F. and Wageningen, N.L., Eds). pp. 200-201.

Bjorksten, T.A. and A.A. Hoffmann (1998). Plant cues influence searching behaviour and parasitism in the egg parasitoid *Trichogramma* sp. nr. *brassicae*. *Econ. Entomol.* **23**: 355-362.

Bleicher, E. and J.R.P. Parra (1990). Espieces de *Trichogramma* parasitoids de *Alabama argillacea*. II. Tabela de vida de fertilidae e parasitismo de tres populacoes. *Pesquisa Agropecuaria Brasileira* **25**: 207-214.

Bourchier, R.S and S.M. Smith (1996). Influence of environmental conditions and parasitoid quality on field performance of *T. minutum*. *Entomol. Exp. Appl.* **80**: 461-468.

Bournier, J.P. (1982). Comportement de plusieurs souches de Trichogrammes vis-avis des oeufs d'*Heliothis armigera* Hbn. (Lep. Noctuidae) et de *Chilo partellus*

Swinhoe (Lep. Pyralidae). In. *Les Trichogrammes, 1er Symposium International, Antibes, France. Les colloques de l'INRA* 9: 85-96.

Bruins, E.B.A., E. Wajnberg and G.A. Pak (1994). Genetic variability in the reactive distance in *Trichogramma brassicae* after automatic tracking of the walking path. *Entomol. Exp. Appl.* **72**: 592-598.

Burnett, T. (1958). Effect of host distribution on the reproduction of *Encarsia formosa* Gahan (Hymenoptera: Chalcidoidae). *Can. Entomol.* **90**: 179-191.

Caltigirone, L.E. (1981). Landmark examples in classical biological control. *Ann. Rev. Entomol* **26**: 213-32.

Calvin D.D., M.C. Knapp, S.M. Welch, F.L. Poston and R.J. Elzinga (1984). Impact of environmental factors on *Trichogramma pretiosum* reared on south western corn borer eggs. *Environ. Entomol.***13**: 774-780.

Carey, J.R. (1993). *Applied demography for biologists with special emphasis on insects.* Oxford University Press, New York

Carner, G.R. and W.C. Yearian (1989). Development and use of microbial agents control of *Helioths* spp. in the USA. In: King, E.G. and Jackson, R.D. eds.). *Proceedings of the workshop on biological control of Helioths: Increasing the effectiveness of natural enemies*, 11-15 November 1985, New Delhi, India, FERRO/USDA, New Delhi, India, 469-481.

Carter, M.C., D. Sutherland and A.F.G. Dixon (1984). Plant structure and searching efficiency of coccinellid larvae. *Oecologia* **63**: 394-397.

Casas, J. (1988). Analysis of searching movements of a leaf miner in a structured environment. *Physiol. Entomol.* **13**: 23-32.

Cerutti, F. and F. Bigler (1995). Quality assessment of *Trichogramma brassicae* in the laboratory. *Entomol. Exp. Appl.* **75**: 19-26.

Chabi-Olaye A., M.K. Fiaboé and F. Schulthess (2004). Host preference, suitability and thermal requirements of *Lathromeris ovicida* Risbec (Hymenoptera: Trichogrammatidae) egg parasitoid of cereal stemborers in Africa. *Biol. Control* (in press).

Chabi-Olaye, A., F. Schulthess, H.M. Poehling and C. Borgemeister (2001a). Factors affecting the biology of *Telenomus isis* (Polaszek) (Hymenoptera: Scelionidae), an egg parasitoid of cereal stem borers in West Africa. *Biol. Control* **21**: 44-54.

Chabi-Olaye, A., F. Schulthess, H.M. Poehling and C. Borgemeister (2001b). Host location and host discrimination behaviour of *Telenomus isis*, an egg parasitoid of the African cereal stemborer, *Sesamia calamistis. J. Chem. Ecol.* **27**: 663-678.

Chabi-Olaye, A., F. Schulthess, T.G. Shanower and A.N. Bosque-Pérez (1997). Factors influencing the bionomics of *Telenomus busseolae* (Gahan) (Hymenoptera: Scelionidae), an egg parasitoid of *Sesamia calamistis* Hampson (lepidoptera: Noctuidae). *Biol. Control* **8**: 15-21.

Charnov, E.L. (1976). Optimal foraging, the marginal theorem. *Theoretical Population Biol.* **17**(6): 945-951.

Charnov, E.L. and S.W. Skinner (1985). Complementary approaches to the understanding of parasitoid oviposition decision. *Environ. Entomol.*14: 383-391.

Cock, M.J.W., H. van Den Berg, G.I. Oduor, and E.K. Onsongo (1991). The population of *Helicoverpa armigera* in smallholder crops in Kenya, with emphasis on its natural enemies, Final report, Phase II. International Institute of Biological Control Ascot, UK. 179 pp.

Comins, H.N and M.P. Hassell (1979). The dynamics of optimally foraging predators and parasitoids. *J. Anim. Ecol.* **48**: 335-351.

Cook, R.M and S.F. Hubbard (1977). Adaptive searching strategies in insect parasites. *J. Anim. Ecol.* **46**: 115-126.

Curl, D.D. and P.P. Burbutis (1978). Host preference studies with *Trichogramma nubilale*. *Environ. Entomol.* **7**: 541-543.

Daly, J.C. (1988). Insecticide resistance in *Heliothis armigera* in Australia. *Pestic. Sci.* **23** (2): 165-176.

Daly, J.C. and D.A.H. Murray (1988). Evolution of resistance to pyrethroids in *Heliothis armigera* (Lepidoptera: Noctuidae) in Australia. *J. Econ. Entomol.* **81**: 984-988.

De Bach, P. (1964). *Biological control of Insect pests and weeds*. Reinhold Publ. Corp., New York, 844 pp.

De Moraes, C.M. and M.C. Mescher (1999). Interactions in entomology, plant-parasitoid interaction in tritrophic systems. *J. Entomol. Sci.***34**: 31-39.

DeBach, P. (1965). Weather and the success of parasites in population regulation.*Can. Entomol.* **97**: 848-863.

Dent, D. (1991). *Insect Pest Management.* CAB International, London.

Dent, D.R. and M.P.Walton (eds.) (1997). *Methods in Agricultural and Ecological Entomology*.CAB International, Wallingford, UK. 387pp.

Dicke, M. (1996). Crop plants affect biological control agents: Prospects for breeding for host plant resistance. *Bulletin IOBC/WPRS* **19** (5): 72-80.

Doutt, R.L. (1959). The biology of parasitic hymenoptera. *Ann. Rev. Entomol.* **4**: 161-182.

Dreyer, H. and J. Baumgartner (1996). Temperature influence on cohort parameters and demographic characteristics of the two cowpea coreids, *Clavigralla tomentosicollis* and *C. shadabi*. *Entomol. Exp. Appl.***78**: 201-213.

Dutton, A., F. Cerutti and F. Bigler (1996). Quality and environmental factors affecting *Trichogramma brassicae* efficiency under field conditions. *Entomol. Exp. Appl.* **81**:71-79.

Ebbert, M.A. (1993). Endosymbiotic sex ratio distorters in insects and mites. In: 'Evolution and diversity of sex ratio in insects and mites' (D.L. Wrensch and M.A. Ebbert, Eds.), pp. 150-191. Chapman and Hall, New York.

Ehler, L.E. (1990). Introduction Strategies in biological control of insects. In: Critical issues in Biological Control of Insects (M.Mackauer, L.E. Ehler and J.Roland, Eds.), pp. 111-134. Intercept, Andover, UK.

Ehler, L.E. and R. van den Bosch (1974). An analysis of the natural biological control of *Trichoplusia ni* (Lepidoptera: Noctuidae) on cotton in California. *Can. Entomol.* **106**: 1067-1073.

Ehler, L.E., K.G. Eveleens and R. van Den Bosch (1973). An evaluation of some natural enemies of cabbage looper on cotton in California. *Environ. Entomol.* **2**: 1009-1015.

El-Heneidy, A.H., M.S.T. Abbas, M.M. Embaby and M.A. Ewiese (1990). Utilization of *Trichogramma evanescens* Westwood to control the lesser sugar-cane borer, *Chilo agamemnon* Bles. in sugar cane fields in Egypt. 5. An approach towards large-scale release. *Proc. Int. Symp. 3rd, San Antonio, Texas*. No. 56. Paris: INRA. 187-90 pp.

Ertle, L.R. and C.P. Davis (1975). *Trichogramma nubilale* new species (Hymenoptera: Trichogrammatidae), an egg parasite of *Ostrinia nubilalis* (Hubner). *Ann. Entomol. Soc. Am.* **68**: 525-528.

Farrell, G., G.N. Kibata and J.A. Sutherland (1995). *A Review of Crop protection Research in Kenya*. KARI/ODA Crop Protection Project. National Agricultural Research Laboratories, KARI, Kenya, 165 pp.

Fenili, G. and F. Pegazzano (1971). Contributo alla conoscenza dei parassiti del *Dacus oleae* Gmelin. Ricerche eseguite in Toscana negli anni 1967 e 1968. *Redia* **52**: 1-29.

Fernandez, V.A. and J.C. Corley (2003). The functional response of parasitoids and its implications for biological control. *Biocontrol Sci. Tech.* 13(4): 403-413.

Fitt, G.P. (1989). The ecology of *Heliothis* in relation to agroecosystems. *Ann. Rev. Entomol.* **34**: 17-32.

Flanders, S.E (1929). The mass production of *Trichogramma minutum* Riley and observations in the natural and artificial parasitism of the codling moth egg. *Proceedings of the 4th International Congress of Entomology* **2**: 110-130.

Flanders, S.E. (1931). The temperature relationships of *Trichogramma minutum* as a basis for racial segregation. *Hilgardia* **5**: 395-405.

Flanders, S.E. (1965). On the sexuality and sex ratios of hymenopterous populations. *American Naturalist* **99**:489-494

Force, D.C. and P.S. Messenger (1964). Duration of development, generation and longevity of three hymopterous parasites of *Therioaphis maculata* reared at various constant temperatures. *Ann. Entomol. Soc. Am.***57**: 405-413.

Franz, J.M and J.Voegele (1974). Les Trichogrammes en vergers. Les auxiliaries en verger de pommiers. *Bull.OILB/SROP*, **3**: 201-210.

Frazer, B.D. and R.R. McGregor (1994). Searching behaviour of adult female coccinellidae (Coleoptera) on stem and leaf models. *Can. Entomol.***126**: 389-399.

Gardner, S.M. (1982). The ecology and behaviour of *Aphidius rhopalosiphi*. Ph.D. Dissertation, University of East Anglia, East Anglia, UK.

Gardner, S.M. and A.F.G. Dixon (1985). Plant structure and the foraging success of *Aphidius rhopalosiphi* (Hymenoptera: Aphidiidae). *Ecol. Entomol.* **10**: 171-179.

Gazit, Y., W. J. Lewis and T.H. Tumlison (1996). Arrestment of *Telenomus remus* (Hymenoptera: Scelionidae) by a kairomone associated with eggs of its host, *Spodoptera frugiperda* (Lepidoptera: Noctuidae). *Biol. Control* **6**: 283-290.

Gingras, D., P. Dutilleul and G. Boivin (2003). Effect of plant structure on host finding capacity of lepidopterous pests of crucifers by two *Trichogramma* parasitoids. *Bio. Control* **27**: 25-31.

Godfray, H.C.J. (1994). *Parasitoids, behavioural and evolutionary ecology*. Princeton University Press, Princeton, New Jersey, 473 pp.

Greathead, D.J. and J.K. Waage (1983). Opportunities for biological control of agricultural pests in developing countries. *World bank technical paper* **11**: 44pp.

Greenberg, S.M. (1991). Evaluation techniques for *Trichogramma* quality. *In*: F.Bigler (ed.), *Proceedings of the 5th International workshop on quality control of mass reared Arthropods*. Wageningen, The Netherlands. March 25-28, 1991, pp. 138-145.

Gross P. and P.W. Price (1988). Plant influences on parasitism of two leaf miners, a test of enemy-free space. *Ecology* **69**: 1506-1516.

Guang, L.Q. and Oloo, G.W. (1990). Host preference studies on *Trichogramma* sp. nr. *mwanzai* Schulten and Feijen (Hymenoptera: Trichogrammatidae) in Kenya. *Insect Sci. Applic.* **11**: 757-763.

Gunning, R.V., C.S. Easton, L.R. Greenup and V.E. Edge (1984). Pyrethroid resistance in *Helioths armigera* (Lepidoptera: Noctuidae) in Australia. *J.Econ. Entomol.* **77**: 1283-1287.

Guo, X. (1986). Bionomics of *Trichogramma ostriniae* Pang et Chen. *Chinese J. Bio. Control* 2: 148-152.

Gupta, B.D. (1956). Failure *of Trichogramma* in the control of sugarcane stem borer, *Chilotraea infus catellus* Snell in India. *Indian Sugar* **6**: 291-292.

Hackett and Gatehouse (1982). Diapause in *Heliothis armigera* (Hb.) and *H. fletcheri* (Hardwick) (Lepidoptera: Noctuidae) in the Sudan, Gezira. *Bull. Entomol. Res.* **72**: 1283-1287.

Haile, A.T., S.A. Hassan, C.P.K.O. Ogol, J. Baumgartner, S. Sithanantham, J.C. Monje and C. P.W. Zebitz (2002a). Temperature-dependent development of four egg parasitoid *Trichogramma* species (Hymenoptera: Trichogrammatidae). *Biocontrol Sci. Tech.* **12**(5), 555-567.

Haile, A.T., S.A. Hassan, S. Sithanantham, C.K.P.O.Ogol and J. Baumgartner (2002b). Comparative life table analysis of *Trichogramma bournieri* Pintureau and Babault and *Trichogramma* sp. nr. *mwanzai* Shulten and Feijen (Hymenoptera: Trichogrammatidae) from Kenya. *J. Appl. Entomol.* **126**(6): 287-292.

Hailemichael, Y., F. Schulthess.; J.W. Smith and W. A Overholt. (1997). Suitability of West African gramineous stemborers for the development of *Cotesia* species (Hymenoptera: Braconidae). *Insect Sci. Applic.* **17**: 89-95.

Hall, R.W. (1993). Alteration of sex ratios of parasitoids for use in biological control. In: *Evolution and diversity of sex ratio in Insects and Mites* (D.L.Wrensch and M.A. Ebbert, Eds.), pp. 542-547. Chapman and Hall, New York.

Harrison, W.W., E.G. King and J.D. Outzs (1985). Development of *Trichogramma exiguum* and *T. pretiosum* at five temperature regimes. *Environ. Entomol.* **14**: 118-121.

Hassan, S.A. (1980). Control of the European corn borer with one release of the egg parasitoid *Trichogramma evanescens. Nachrichtenbl. Deut. Pflanzenschutzd.* **32**: 97-99.

Hassan, S.A. (1982). Mass production of *Trichogramma*: III. Results of some research projects related to the practical use in the Federal republic of Germany. 1st *International Symposium on* les Trichogrammes, *Antibes, France*. No. 9. Paris: *Les colloques de l'INRA*. 213-19 pp.

Hassan, S.A. (1985). Mass production and utilisation of *Trichogramma*: Seven years experience with the control of the European corn borer *Ostriniae nubilalis* Hubner. *Gesunde Planzen* **37**(5): 197-2002.

Hassan, S.A. (1989). Selection of suitable *Trichogramma* strains to control the codling moth *Cydia pomonella* and the summer fruit tortrix moth *Adoxophyes orana, Pandemis heperana* (Lepidoptera: Tortricidae). *Entomophaga* **34**: 19-27.

Hassan, S.A. (1993). The mass rearing and utilization of *Trichogramma* to control lepidopterous pests: achievements and outlook. *Pestic. Sci.* **37**: 387-391.

Hassan, S.A. (1994). Strategies to select *Trichogramma* species for use in biological control. In: Wajnberg, E., Hassan,S.A. (Eds.), *Biological control with other Egg parasitoids*, CAB International, UK. pp. 55-73.

Hassan, S.A. and Guo, M.F. (1991). Selection of effective strains of egg parasites of the genus *Trichogramma* (Hymenoptera: Trichogrammatidae) to control the European corn borer *Ostinia nubilalis* Hb. (Lepidoptera: Pyralidae). *J. Appl. Entomol.* **111**: 335-341.

Hassan, S.A. and Wen Qing Zhang (2001). Variability in quality of *Trichogramma brassicae* (Hymenoptera: Trichogrammatidae) from commercial suppliers in Germany. *Biol. control* **22**:115-121.

Hassell, M.P. (1978). *The dynamics of arthropod-prey systems*. Princeton University Press, Princeton, NJ.

Hassell, M.P. (1982). Patterns of parasitism by insect parasitoids in patchy environments. *Ecol. Entomol.* **7**: 365-377.

Hassell, M.P. (2000). *The spatial and temporal dynamics of host-parasitoid interactions. Oxford Series in Ecology and Evolution*. Oxford University Press, London.

Hassell, M.P. and May, R. (1973). Stability of Insect host-parasite models. *J. Anim. Ecol.* **42**: 693-726.

Hassell, M.P., J.H. Lawton and J.R. Beddington (1977). Sigmoid functional responses by invertebrate predators and parasitoids. *J. Anim. Ecol.* **46**: 249-262.

Hassell, M.P., J.K. Waage and R.M. May. (1983). Variable parasitoid sex ratios and their effect on host parasitoid dynamics. *J. Anim. Ecol.* **52**: 889-904.

Hirashima, Y., K. Muira and T. Hasegawa (1990). Studies on the biological control of the diamondback moth, *Plutella xylostella* (Linnaeus). 4. Effect of temperature on the development of the egg parasitoids *Trichogramma chilonis* and *Trichogramma ostriniae*. *Science Bull. Faculty of Agriculture, Kyushu University* **44**: 81-87.

Hodgman, C.D., 1948. *Handbook of Chemistry and Physics*, Chemical Rubber Publ. Co., Cleveland, Ohio.

Hokkanen, H.M.T. (1985). Success in classical biological control. CRC *Critical Rev. Plant Sci.* **3**: 35-72.

Holling, C.S. (1959). The components of predation as revealed by a study of small mammal predation of the European pine sawfly. *Can. Entomol.* **91**: 293-320.

Holling, C.S. (1961). Principles of Insect predation. *Ann. Rev. Entomol.* **6**: 163-182.

Holling, C.S. (1966). The functional response of invertebrate predators to prey density. *Mem. Entomol. Soc. Can.* **48**: 1-86.

Hommay, G., C. Gertz, J.C. Kienlen, J. Pizzol and P. Chavigny (2002). Comparison between the control efficacy of *Trichogramma* evanescens Westwood (Hymenoptera: Trichogrammatidae) and two *Trichogramma cacoeciae* Marshal strains against grape moth (*Lobesia botrana* Den and Schiff.), depending on release density. *Biocontrol Sci. Tech.* **12**: 569-581.

Horn, D.J. (1988). Ecological approach to pest management. New York: Guilford Press, 285 pp.

Houck, M.A. and R.E. Strauss (1985). The comparative study of functional responses: experimental design and statistical interpretation. *Can. Entomologist* **117**: 617-629.

Huettel, M.D. (1976). Monitoring the quality of laboratory reared insects: a biological and behavioural perspective. *Environ. Entomol.* **5**: 807-814.

Huffaker, C.B., P.S. Messenger and P. DeBach (1971). The natural enemy component in natural control and the theory of biological control. In: Huffaker, C.B. (Ed.), *Biological control*. Academic Press, New York, pp. 16-67.

Hughes, R.D., L.T. Woolcock and M.A. Hughes (1992). Laboratory evaluation of parasitic Hymenoptera used in attempts to biologically control aphid pests of crops in Australia. *Entomol. Exp. Appl.* **63:** 177-185.

Hulting, F.L, D.B. Orr and J.J. Obrycki (1990). A computer program for calculation and statistical comparison of the intrinsic rate of increase and associated life table parameters. *Florida Entomologist* **73**: 602-612.

ICIPE (1997). 1996-1997 Annual Scientific Report. The International Centre of Insect Physiology and Ecology, Nairobi, Kenya.

Ikin, R; Schulten, G.G.M. and D.E. Borhegyi (1993). Report of Regional Seminar on the Development and Application of Integrated Pest Management on Vegetables in Africa, 20-30 November 1992, Dakar, Senegal. *FAO Plant Protection Bulletin,* (Rome) **41**: 155-160.

Jalali, S.K. and S.P. Singh (1993). Superior strain selection of the egg parasitoid *Trichogramma chilonis* Ishii – Biological parameters. J. Biol. Control 7 (2): 57-60.

Jervis, M.A. and M.J.W. Copland (1996). The life cycle. In: M. Jervis and N. Kidd, Eds., *Insect natural enemies, Practical approaches to their study and evaluation*. Chapman and Hall, London 63-160 pp.

Jones, W.T. 1982). Sex ratio and host size in a parasitoid wasp. *Beh. Ecol.& Socio-biology* **10**: 207-210.

Kaiser, L., M.H. Pham-Delegue, E. Bakchine and C. Masson (1989). Olfactory responses of *Trichogramma maidis* Pinto and Voegele: Effects of chemical cues and behavioural plasticity. *J. Insect Behav.* **2**: 701-712.

Kareiva, P. (1990). The spatial dimension in pest-enemy interaction. In: Mackauer, M., Ehler, L.E., Roland, J. (Eds.), *Critical issues in Biological control*, Intercept. Anover, Hants, pp. 213-227.

Kareiva, P. and R. Sahakian (1990). Tritrophic effects of simple architectural mutation in pea plants. *Nature* **345**: 433-434.

Karel, A.K. (1985). Yield loss from bean pod borers, *Maruca testulalis* (Lepidoptera: Pyralidae) and *H. armigera* (Lepidoptera: Noctuidae) and control. *J. Econ. Entomol.* **78**(6): 1323-1326.

Karel, A.K. (1997). Insect pest control: A tool for high legume yields. Tanzania Notes Record 94.

Kauffman, W.C. and G.G. Kennedy (1989). Relationship between trichome density in tomato and parasitism of *Heliothis* spp. (Lepidoptera: Noctuidae) eggs by *Trichogramma* spp. (Hymenoptera: Trichogrammatidae). *Environ. Entomol.* **18**: 698-704.

Kfir, R. (1983). Functional response to host density by the egg parasite, *Trichogramma pretiosum. Entomophaga* **28** (4): 345-353.

Kidd, N.A. and Jervis, M.A. (1996). Population dynamics. pp.293-374. In: Jervis, M and Kidd, N. (eds) *Insect natural enemies*. London, Chapman and Hall.

Kidd, N.A.C. and Jervis, M.A. (1989). The effects of host feeding behavior on the dynamics of parasitoid-host interactions and the implications for biological control. *Researches on Popul. Ecol.* **31**: 235-274.

King E.D., L.F Bouse, D.L Bull, L.R.Bouse and J.R. Phillips (1985a). Biological control of *Heliothis* spp. in cotton by augmentative releases of *Trichogramma*. *Southwest. Entomol.* **8**: 1-10 (suppl.)

King E.D., R.J Coleman, J.R. Phillips and W.A Dickerson (1985b*). Heliothis* spp. and selected natural enemy populations in cotton: A comparison of three insect control programs in Arkansas (1981-82) and North Carolina (1983). *Southwest.Entomol.* **8**: 71-98 (suppl.)

King E.D; Bouse, L.F; Bull, D.L; Coleman, R.J and Dickerson, W.A. (1986). Management of *Heliothis* spp. in cotton by augmentative releases of *Trichogramma pretiosum* Riley. *J. Appl. Entomol.* **101**: 2-10.

King, B.H. (1987). Offspring sex ratio in parasitoid wasps. *Q. Rev. Biol.* **62**: 367- 396.

King, B.H. (1993). Sex ratio manipulation by parasitoid wasps. In: *Evolution and diversity of sex ratio in Insects and Mites* (D.L. Wrensch and M.A. Ebbert, Eds.), pp. 418-441. Chapman and Hall, New York.

King, E.G., D.L. Bull, L.F. Bouse, and J.R Phillips (1985c). Biological control of bollworm and tobacco budworm on cotton by augmentative releases of *Trichogramma*. *Southwest Entomol.* (Supp). **8**: 1-198.

Klomp, H.B., B.J. Teerink and W.C. Ma (1980). Discrimination between parasitised and unparasitised hosts in the egg parasioid *Trichogramma embryophagum* (Hymenoptera: Trichogrammatidae): a matter of learning and forgetting. *Neth. J. Zool.* **30**: 254-277.

Knippling, E.F. and J.U. McGuire (1968). Population models to appraise the limitations and potentialities of *Trichogramma* in managing host insect populations. *US Department Agric. Technol. Bull.* **1387**: 1-44.

Kot, J. (1964). Experiments in the biology and ecology of species of the genus *Trichogramma* Westwood and their use in plant protection. *Ekol. Pol.* **A 12**: 243-303.

Kot, J. (1979). Analysis of factors affecting the phytophage reduction by *Trichogramma* Westw. Species. *Pol. Ecol. Stud.* **5**: 5-59.

Lawton, J.F; M.P. Hassell and J.R. Beddington (1975). Prey death rates and rate of increase of arthropod predator populations. *Nature* **255**: 60-62.

Lewis, W.J., R.L. Jones and A.N. Sparks (1972). A host-seeking stimulant for the egg parasite *Trichogramma evanescens*: Its source and a demonstration of its lab and field activity. *Ann. Entomol. Soc. Am.* **65**: 1087-1989.

Li, L.Y. (1994). Worldwide use of *Trichogramma* for biological control on different crops, a survey. In: Wajnberg, E. and S.A. Hassan (eds.). *Biological Control with Egg Parasitoids*. CAB International, UK, pp. 37-53.

Liu, F. and Smith, S.M. (2000). Measurement and selection of Parasitoid quality for mass-reared *Trichogramma minutum* Riley used in inundative release. *Biocontrol Sci. Tech.* **10**: 3-13.

Lopez, J.D. and R.K. Morrison (1980). Overwintering of *Trichogramma pretiosum* in central Texas. *Environ. Entomol.* **9**: 75-78.

Lopez, J.D. Jr., R.L. Jones and V.S. House (1982). Species of *Trichogramma* parasitising eggs of *Heliothis* spp. and some associated lepidopterous pets in central Texas. Southw. *Entomol.* **7**: 87-93.

Luck, R. F., R Stouthamer and L.P. Nunney (1993). Sex determination and sex ratio patterns in parasitic Hymenoptera. In: *Evolution and diversity of sex ratio in Insects and Mites* (D.L. Wrensch and M.A. Ebbert, Eds.), pp. 442-476. Chapman and Hall, New York.

Luck, R.F. (1985). Principles of arthropod predation. In: Huffaker, C.B., Rabb, R.L. (Eds.), *Ecological entomology*, Wiley, New York, pp.497-530.

Lund, H.O. (1934). Some temperature and humidity relations of two races of *Trichogramma minutum* Riley. *Ann. Entomol. Soc. Am.* **27**: 324-340.

Maceda A., C.L. Hohmann, H.R. Santos and H.R. Dos-Santos (1994). Comparative life table for *Trichogramma pretiosum* Riley, 1879 and *Trichogramma annulata* De Santis 1972 (Hym.: Trichogrammatidae). *Revista do Setor de Ciencias Agrarias, Curitiba* **13**: 279-281.

Madden, J.L. and D. Pimentel (1965). Density and spatial relationships between a wasp parasite and its housefly host. *Can. Entomol.* **97**: 1031-1037.

Manjunath, T.M., V.S Bhatnagar, C.S. Pawar, and S. Sithanantham (1989). Economic importance of *Helioths* in India and an assessment of their natural enemies. In: King, E.G. and Jackson, R.D. (eds). *Proceedings of the workshop on Biological control of Helioths: Increasing the effectiveness of natural enemies*,11-15 November 1985, New Delhi, India, pp.179-240.

McCaffery, A.R., A.B.S. King, A.J. Walker and H. El-Nayir (1989). Resistance to synthetic pyrethroids in the bollworm, *Heliothis armigera* from Andhra Pradesh. India. *Pestic. Sci.*, **27**: 65-67.

Messenger, P.S. (1970). Bioclimatic inputs to biological control and pest management programs. In: *Concepts of Pest Management* (Rabb, R.L. and Guthrie, F.E., Eds.) North Carolina State University, Raleigh, pp. 84-99.

Messenger, P.S. and R. van den Bosch (1971). The adaptability of introduced biological control agents. In: C.B. Huffaker (ed.). *Biological control.* Plenum, New York: 68-92.

Messenger, P.S., F. Wilson and M.J. Whitten (1976a). Variation, fitness and adaptability of natural enemies. In: C.B. Huffaker and P.S. Messenger (eds.). *Theory and practice of biological control.* Acad. Press, New York: 209-231.

Messenger, P.S; E. Biliotti and R. van den Bosch (1976b). The importance of natural enemies in integrated control. In: C.B. Huffaker and P.S. Messenger (eds.). *Theory and practice of biological control.* Acad. Press, New York: 543-563

Mohaghegh, J., P.De Clercq and L. Tirry (2001). Functional response of the predators *Podisus maculiventris* (Say) and *P.nigrispinus* (Dallas)(Het. Pentatomidae) to the beet army worm, *Spodoptera exigua* (Hübner) (Lep. Noctuidae): effect of temperature. *J. Appl. Entomol.* **125**:131-134.

Monje, J.C., C.P.W. Zebitz and B. Ohnesorge (1999). Host and host age preference of *Trichogramma galloi* and *T. pretiosum* (Hymenoptera: Trichogrammatidae) reared on different hosts. *J. Econ. Entomol.* **92**: 97-103.

Morrison, G., Lewis, W.J., Nordlund, D.A. (1980). Spatial differences in *Heliothis zea* egg density and the intensity of parasitism by *Trichogramma* spp.: an experimental analysis. *Environ. Entomol.* **9**: 79-85.

Morrison, R.K., S.L. Jones and J.D. Lopez (1978). A unified system for the production and preparation of *Trichogramma pretiosum* for field release. *Southwest. Entomol.* **3**: 62-68.

Mudd, A. and S.A. Corbet (1982). Response of the ichneumind parasite *Nemeritis canescens* to kairomones from the flour moth *Ephestia kuenhiella*. *J. Chem. Ecol.* **8**: 843-850.

Muholo, C.A. (2002). Studies on potential host range and non-target risk assessment of trichogrammatid egg parasitoids in Kenya. M. Sc. Thesis, Addis Ababa University, Ethiopia.120pp.

Munyaneza, J. and J.J. Obrycki (1997). Functional response of *Coleomegilla maculata* (Coleoptera: Coccinelidae) to Colorado potato beetle eggs (Coleoptera: Chrysomelidae). *Biol. Control.* **8**: 215-224.

Murdoch, W.W and A. Oaten (1975). Predation and population stability. *Adv. Ecol. Res.* **9**: 2-131.

Nagaraja, H. (1978). Studies on *Trichogrammatoidea* (Hymenoptera: Trichogrammatidae). *Oriental Insects* **12**: 489-530.

Nagaraja, H. (1988). Life and fertility tables of some *Trichogrammatoidea* spp. (Hymenoptera: Trichogrammatoidea) under laboratory conditions. In: Voegele, J and J.van Lenteren, Eds., *Trichogramma and other egg parasitoids* 2^{nd}

International Symposium on Trichogramma, Guangzhou, PR. China, 1986. *Les Colloques del'INRA* 43. 221-222.

Nagarkatti, S. and H. Nagaraja (1968). Biosystematic studies on *Trichogramma* species: Experimental hybridisation between *T. australicum* Girault, *T.evanescens* Westwood and *T. minutum* Riley. *CIBC Technical Bulletin, 10*: 81-96.

Nagarkatti, S. and H. Nagaraja (1971). Redescription of some known species of *Trichogramma* (Hymenoptera: Trichogrammatidae) showing the importance of the male genitalia as a diagnostic character. *Bull. Entomol. Res.61*: 13-31.

Nagarkatti, S. and H. Nagaraja (1977). Biosystematics of *Trichogramma* and *Trichogrammatoidea* species. *Ann. Rev. Entomol.* **22**: 157-176.

Nechols, J.R., J.L. Tracy and E.A. Vogt (1989). Comparative ecological studies of indigenous egg parasitoids (Hymenoptera: Scelionidae; Encrytidae) of thesquash bug, *Anasa tristus* (Hemiptera: Coreidae). *J. Kans. Entomol. Soc.* **62**: 177-188.

Newton, P.J. (1993). Increasing the use of Trichogrammatids in insect pest management: A case study from the forests of Canada. *Pestic. Sci.* **37**: 383-386.

Nicholson, A.J. and V.A. Bailey (1935). The balance of animal populations. Part I. *Proc. Zool. Soc. Lond.* **3**: 551-598.

Noldus, L.P.J.J. (1989). Semiochemicals, foraging behaviour and quality of entomophagous insects of biological control. *J. Appl. Entomol.* **108**: 425-451.

Noldus, L.P.J.J. and J.C. van Lenteren (1985b). Kairomones for the egg parasitoid *Trichogramma evanescens* Westwood. II. Effect of contact chemicals

produced by two of its hosts, *Pieris brassicae* L. and *Pieris rapae* L. *J. Chem. Ecol.* **11**: 793-800.

Noldus, L.P.J.J., W.J. Lewis and J.H. Tumlison (1990). Beneficial arthropod behaviour mediated by airborne semiochemicals. IX. Differential response of *Trichogramma pretiosum*, an egg parasitoid of *Heliothis zea*, to various olfactory cues. *J. Chem. Ecol.* **16**: 3531-3544.

Nordlud, D.A., R.B. Chalfant and W.J. Lewis (1985). Response of *Trichogramma pretiosum* females to extracts of two plants attacked by *Heliothis zea*. *Agric. Ecosystems Environ.* **12**: 127-133.

Nordlund, D.A., M.R. Strand, W.J. Lewis and S.B. Vinson (1987). Role of kairomones from host accessory gland secretion in host recognition by *Telenomus remus* and *Trichogramma pretiosum* with partial characterisation. *Entomol. Exp. Appl.* **44**: 37-43.

Nordlund, D.A., W.J. Lewis, H.R. Gross, JR., and M. Beevers (1981). Kairomones and their use for management of entomophagous insects. XII.The stimulatory effects of host eggs and the importance of egg-host density to the effective use of Kairomones for *Trichogramma pretiosum*. Riley. *J. Chem. Ecol.* **7**: 909-17.

Nordlund, D.A; R.B. Chalfant and W.J. Lewis (1985). Response of*Trichogramma pretiosum* females to extracts of two plants attacked by *Heliothis zea*. *Agric. Ecosystems Environ.* **12**: 127-133.

O'Neil, R.J. (1989). Comparison of laboratory and field measurements of the functional response of *Podisus maculiventris* (Heteroptera: Pentatomidae). *J. Kansas Entomol. Soc.* **62**: 148-155.

Ochiel, G.R.S. (1989). Biology of *Trichogramma* sp.nr. *exiguum* Pinto and Platner (Hymenoptera: Trichogrammatidae) on some lepidopteran hosts in south Nyanza, Kenya. M.Phil. Thesis, Rivers State University of Science and Technology, Nigeria, 140 pp.

Odum, E.P. (1983). *Basic ecology*. Holt-Saunders International Edition, New York.

Oduor, G.I., J.Ongaro; S. Pete; P. Karanja; J. Cooper and J. Mwaniki (1998). Pests of horticultural crops on small-scale peri-urban farms in Nyathima, Kiambu: Influence of pesticide regimes on pests and natural enemies. In: Farel, G. and Kibata, G.N. (Eds.) Crop Protection Research in Kenya, Proceedings of the 2nd Biennial Crop Protection Conference 916-17 September 1998. KARI/DFID National Agricultural Research Project II. Pp. 18-29.

Oldroyd, H. and C. Ribbands (1936). On the validity of *Trichogramma* trichiation as a systematic character. *Proc. Roy. Entomol. Soc. Lond.* **5**: 148-152.

Olkowski, W. and A. Zhang (1990). *Trichogramma*- a modern day frontier in biological control. *IPM Practitioner* **12**: 1-28.

Omer, A.M., M.W. Johnson, and B.E. Tabashnik (1996). Demography of the leaf miner parasitoid *Ganaspidium utilis* Beardsley (Hymenoptera: Eucolidae) at different temperatures. *Biol. Control* **6**: 29-43.

Orphanides, G.M. and D. Gonzalez (1971). Fertility and life table studies with *Trichogramma pretiosum* and *T. retorridum* (Hymenoptera: Trichogrammatidae). *Ann. Entomol. Soc. Am.***64**: 824-834.

Overholt, W.A. (1997). Mass rearing, release and evaluation of entomophagous insects for biological control. *Afr. J. Pl. Prot.* **7**: 1-15.

Overholt, W.A. and J.W. Smith, Jr (1990). Comparative evaluation of three exotic insect parasites (Hymenoptera: Braconidae) against the southwestern corn borer (Lepidoptera: Pyralidae) in corn. *Environ. Entomol.* **19**: 345-356.

Pak, G.A and T.G. van Heiningen (1985). Behavioural variations among strains of *Trichogramma* species: adaptability to field temperature conditions. *Entomol. Exp. Appl.***38**: 3-13.

Pak, G.A. (1988). Selection of *Trichogramma* for inundative biological control. PhD Thesis, Wageningen Agricultural University, The Netherlands, 224 pp.

Pak, G.A. (1992). Inundative release of *Trichogramma* for the control of cruciferous lepidoptera, preintroductory selection of an effective parasitoid. In: Talekar, N.S. (ed.). *Diamondback moth and other crucifer pests*. Asian Vegetable research and development center, Tainan, Taiwan, pp. 297-308.

Pak, G.A. and E.J. de Jong (1987). Behavioural variations among strains of *Trichogramma* species: host recognition. *Neth. J. Zool.* **37**: 137-166.

Pak, G.A. and J.C. van Lenteren (1988). Criteria and methods for the pre-release evaluation of different *Trichogramma* spp. strains. In: Voegele, J., Waage, J., van Lenteren, J.C. (Eds.), *Trichogramma and other egg parasitoids, Proceedings of 2nd International Symposium on Trichogramma, Guangzhou, PR China*, No. 43 *Les colloques de l'INRA*, Paris, pp. 433-442.

Pak, G.A. and R. Oatman (1982). Comparative life table, behaviour and competition studies of *Trichogramma brevicapillum* and *Trichogramma pretiosum*. *Entomol. Exp. Appl.* **32**: 68-79.

Pak, G.A., H. Berkhout and J. Klapwijk (1991). Do *Trichogramma* look for hosts? In: Wajnberg, E. and Vinson, S.B. (eds). *Trichogramma and other egg parasitoids*. 3rd International Symposium in *Les Colloques de I'INRA* **56**: 77-80.

Park, Y.K., H.P. Lee and K.S Lee (2000). Effect of temperature on the biology of *Trichogramma dendrolimi* (Hymenoptera: Trichogrammatidae) reared on a factitious host, *Antheraea pernyi* (Lepidoptera: Saturniidae) egg. *J. Asia Pacific Entomol.* **3**(2): 65-70.

Pavlik, J. (1993). Variability in the host acceptance of European corn borer, *Ostrinia nubilalis* Hbn. (Lepidoptera: Pyralidae) in strains of the egg parasitoid *Trichogramma* spp. (Hymenoptera: Trichogrammatidae). *J. Appl. Entomol.* **115**: 77-84.

Pearl, A (1928). *The rate of living*. Knopf, New York.

Pedigo, L.P. (1989). *Entomology and Pest management*. New York: Macmillan Publishing Company.

Pinto, F.P. and J. Tavares (1990). Longevity and parasitic capacity of the Azorean type of *Trichogramma cordubensis* Vargas & Cabello (Hymenoptera: Trichogrammatidae). In: '*Trichogramma and other Egg parasitoids*, (Wajnberg, E. & Vinson, S.B., Eds.), *Proceedings of 3rd International Symposium'. Les Colloques de l'INRA*, **56**: 89-91.

Pinto, J.D. (1998). *Systematics of the North American species of* Trichogramma *Westwood (Hymenoptera: Trichogrammatidae).* Memoirs of the Entomological

Society of Washington 22, The Entomological Society of Washington, Washington D.C., 287 pp.

Pinto, J.D. and R. Stouthammer (1994). Systematics of the Trichogrammatidae with emphasis on *Trichogramma* spp. *Entomol. Exp. Appl.* **66**:1-37.

Pintureau, B., M. Babault, and J. Voegele (1981) Etude de quelques factuers de variation de la fecondite chez *Trichogramma maidis* Pintureau et Voegele (Hymenoptera: Trichogrammatidae). *Agronomie* **1**: 315-322.

Pratissoli, D. (1995). Bioecologia de *Trichogramma pretiosum* Riley, 1879, nas tracas, *Scrobipalpuloides absoluta* (Meyrick, 1917) e *Phthorimaea operculella* (Zeller, 1873), em tomateiro. Piracicaba: Doutorado-Escola Superior de Agricultura "Luiz de Queiroz"/USP, 153pp.

Quednau, W. (1960). Uber die Identitat der *Trichogramma* arten und einiger ihrer okotypen. *Mutteilungen aus des Biologischen Bundesanstalt fur Land und Fortwirtschaft, Berlin-Dahlem* **100**: 11-50.

Rabb, R.L. and J.R. Bradley (1968). The influence of host plants on parasitism of eggs of the tobacco hornworm. *J. Econ. Entomol.* **61**: 1249-1252.

Ramadhan, M.M., T.T.Y. Wong and R.H. Messing (1995). Reproductive biology of *Biosteres vanderboschi* (Hymenoptera: Braconidae), a parasitoid of early instar oriental fruit fly. *Ann. Entomol. Soc. Am.* **88**: 189-195.

Raspi, A. and A. Loni (1994). Alcune note sull'allevamento massale di *Opius concolor* Szépligeti (Hymenoptera: Braconidae) e su recenti tentativi d'introduzione della specie in Toscana e liguria. *Frust Entomol.*, **30**: 135-145.

Rawat, U.S. and A.D. Pawar (1991). Field recovery of *Trichogramma chilonis* Ishii (Hymenoptera: Trichogrammatidae) from *Deudorix epijarbas* Moore (Lepidoptera: Lycaenidae) in Himachal Pradesh, India. *Entomon* **16**: 49-52.

Reed, W. (1965a). *Heliothis armigera* (Hb.) (Noctuidae) in Western Tanganyika. I. Biology, with special reference to the pupal stage; II. Ecology and natural and chemical control. *Bull. Entomol. Res.* **56**: 117-125.

Reed, W. (1965b). *Heliothis armigera* (Hb.) (Noctuidae) in Western Tanganyika. II. Ecology and natural and chemical control. *Bull. Entomol. Res.* **56**: 127-140.

Reed, W. and C.S. Pawar (1982). *Heliothis*: a global problem. In: Reed, W. and Kumble, V., Eds). *Proceedings of the International workshop on Heliothis Management, Pantacheru*, India, ICRISAT, pp. 9-14.

Romeis, J., T.G Shanover and C.P.W. Zebitz (1997). Volatile plant infochemicals mediate plant preference of *Trichogramma chilonis*. *J.Chem. Ecol.* **23**: 2455-2465.

Romeis, J., T.G. Shanover and C.P.W. Zebitz (1998). Physical and chemical plant characters inhibiting the searching behaviour of *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae). *Entomol. Exp. Applic.* **87**: 275-284.

Romeis, J; T.G. Shanover and C.P.W. Zebitz (1999). Why *Trichogramma* (Hymenoptera:Trichogrammatidae) egg parasitoids of *Helicoverpa armigera* (Lepidoptera: Noctuidae) fail on chickpea. *Bull. Entomol. Res.* **89**: 89-95.

Rotary, N. and D. Gerling (1973). The influence of some external factors upon the sex ratio of *Bracon hebetor* Say (Hymenoptera: Braconidae). *Environ. Entomol.* **2**: 134-138.

Ruberson, J.R. and T.J. Kring (1993). Parasitism of developing eggs by *Trichogramma pretiosum* (Hymenoptera: Trichogrammatidae): Host age preference and suitability. *Biol. Control* **3**: 39-46.

Rutledge, C.E. and R.N. Wieldenmann (1999). Habitat preferences of 3 congenic Braconid parasitoids: Implications for host range testing in Biological control. *Biol. Control* **16**: 144-154.

Sabelis, M.W. and K. Nagelkerke (1993). Sex allocation and pseudo arrhenotoky in phytoseiid mites. In: *Evolution and diversity of sex ratio in Insects and mites* (D.L. Wrensch and M.A. Ebbert, Eds.), pp. 512-541. Chapman and Hall, New York.

Salt, G. (1938). Experimental studies in insect parasitism. VI. Host suitability. *Bull. Entomol. Res.***29**: 223-246.

Samira, A.M. (2003). Biology, host and plant host relationships of two *Psyttalia* species (Hyemoptera: Braconidae): parasitoids of fruit flies (Diptera: Tephtritidae) in Kenya. PhD Thesis, Gezira University. 134pp.

SAS Institute (2000) SAS/STAT User's Guide, release version 8.2.SAS Institute,Cary, North Carolina.

Shen, X. and Y. Li (1987). Correlation of egg mass parasitisation and egg parasitisation of *Ostriniae furnacalis* by *Trichogramma* spp. Chinese *J. Bio. Control* **3**: 136-137.

Silva, I.M.M.S., M.M.M. van Meer, M.M. Roskan, A. Hoogenboom, G. Gort and R. Stouthamer (2000). Biological potential of *Wolbachia*-infected versus

uninfected wasps: laboratory and greenhouse evaluation of *Trichogramma cordubensis* and *T. deion* strains. *Biocontrol Sci. Tech.* **10**: 223-228.

Singer, M.C. (1986). The definition and measurement of oviposition preference in plant- feeding insects. In: *Insect plant interactions*. (J.R. Miller and T.A.Miller, Eds.). Springer-Verlag, New York pp 65-94.

Singh, H. and G. singh (1975). Biologicals tudies on *Heliothis armigera* (Hb.) in the Punjab. *Indian J. Entomol.* **37**: 154-164.

Singh, P. and M.D. Ashby (1985). Insect rearing management. *In*: Handbook of Insect rearing, Vol. 1. Ed. by Singh, P; R.F. Moore, Amsterdam: Elsevier, 185-215.

Singh, S.P. and S.K. Jalali (1994). *Trichogrammatids*. Technical Bulletin no. 9; Project Directorate of Biological control, Bangalore, India. 93 pp.

Sithanantham, S., T.H. Abera, J. Baumgartner, S.A. Hassan, B. Lohr, J.C. Monje, W.A. Overholt, A.V.N. Paul, Fang Hao Wan and C.P.W. Zebitz (2001). Egg parasitoids for augmentative biological control of lepidopteran vegetable pests in Africa: Research status and needs. *Insect Sci. Applic.* **21** (3): 189-205.

Smith, S.M. (1996). Biological control with *Trichogramma*: advances, successes and potential for their use in biological control. *Ann. Rev. Entomol.* **41**:375-406.

Smith, S.M. and M. Hubbes (1986). Strains of the egg parasitoid *Trichogramma minutum* Riley. II. Utilisation for release against he spruce budworm. *J. Appl. Entomol.* **102**: 81-93.

Smith, S.M., J.R. Carrow and J.E. Laing (1990). Inundative release of the egg parasitoid, *Trichogramma minutum* (Hymenoptera: Trichogrammatidae) against Forest Insect pests such as the Spruce Budworm, *Choristoneura fumiferana*

(Lepidoptera: Tortricidae): The Ontario Project 1982-1986. *Mem.Entomol. Soc. Can.* **153**: 1-87.

Smits, P.H. (1982). The influence of kairomones of *Mamestra brassicae* on the searching behaviour of *Trichogramma evanescens*. *Proceedings of the first International Symposium on Trichogramma* (Antibes, 20-23 April 1982, *Colloques de l'INRA* **9**: 139-150.

Sokal, R.R. and F.J. Rohlf (1981). *Biometry*: *The principles and Practices of Statistics in Biological Research*. Second Edition, Freeman, New York, pp. 859.

Solomon, M.E. (1949). The natural control of insect populations. *J. Anim. Ecol.* **18**: 1-35.

Southwood, T.R.E. (1978). *Ecological methods*. Wiley, New York.

Stiling, P.D. (1987). The frequency of density dependence in insect-parasitoid systems. *Ecology* **68**: 844-856.

Stouthamer, R. (1997). *Wolbachia* induced parthenogenesis. In: S.L.O'Neill J.H.Warren and AA. Hoffmann (eds), *Influential passengers: Inherited microorganisms and Arthropod reproduction*. Oxford University press, New York, pp.102-124.

Stouthamer, R., R.F. Luck and W.D. Hamilton (1990). Antibiotics cause parthenogenetic *Trichogramma* to revert to sex. *Proc. Nat. Acad. Sci.* USA, **87**: 2424-2427.

Strand, M.R. (1986). The physiological interactions of parasitoids with their hosts and their influence on reproductive strategies. In: *Insect Parasitoids*, (Waage, J. & Greathead, D., Eds), Academic Press, London pp. 97-136.

Suzuki, Y. and K. Hiehata (1985). Mating systems and sex ratios in the egg parasitoids, *Trichogramma dendrolimi* and *T. papilionis* (Hymenoptera: Trichogrammatidae). *Animal Behaviour* **33**: 1223-1227.

Suzuki, Y., H. Tsji, and M. Sasakawa (1984). Sex allocation and effects of super parasitism on secondary sex ratios in the gregarious parasitoid, *Trichogramma chilonis* (Hymenoptera: Trichogrammatidae). *Animal Behaviour* **32**: 478-484.

Takabayashi, J., Y. Sato, M. Horikoshi, R. Yamaoka, S. Yano, N. Ohsaki and M. Dicke (1998). Plant effects on plant foraging, differences between two tritrophic systems. *Biol. Control* **11**: 97-103.

Takayabashi, J. and M. Dicke (1996). Plant-carnivore mutualism through herbivore-induced carnivore attracts. *Trends Plant Sci.* **1**: 109-113.

Taylor, F. (1981). Ecology and evolution of physiological time in insects. *Am. Nat.* **117**: 1-23.

Thompson, W.R. (1924). La theorie mathematique de l'action des parasites entomophages et le factor du hazard. *Annls. Fac. Sci. Marseille* **2**: 69-89.

Thomson, L.J. and A.A. Hoffmann (2002). Laboratory fecundity as predictor of field success in *Trichogramma caverae* (Hymenoptera: Trichogrammatidae). *J. Econ. Entomol.* **95**(5): 912-917.

Thorpe, K.W. (1985). Effects of height and habitat type on egg parasitism by *Trichogramma minutum* and *T. pretiosum* (Hymenoptera: Trichogrammatidae). *Agric. Ecosyst. Environ.* **12**: 117-126.

Tilden, R.L. and Ferkovich, S.M. (1988). Kairomonal stimulation of oviposition into an artificial substrate by an endoparasitoid, *Microplitis croceipes* (Hymenoptera: Braconidae). *Ann. Entomol. Soc. Am.* **81**: 152-156.

Tillman, P.G. and J.E. Powell (1991). Development time in relation to temperature for *Microplitis creceipes, M. demolitor, Cotesia kazak* (Hymenoptera: Braconidae) and *Hyposotor didymator* (Hymenoptera: Ichneumonidae), endoprasitoids of the tobacco budworm (Lepidoptera: Noctuidae). *Environ. Entomol.* **20**: 61-64.

Torreno, H.S. and H.E. Cadapan (1984). Laboratory studies on two *Trichogramma* spp. against the eggs of *Helicoverpa (Heliothis) armigera* (Hubner). *Phillipine Entomol.* **6**(2): 151-159.

Tran, L.C. and S.A. Hassan (1986). Preliminary results on the utilization of *Trichogramma evanescens* Westwood to control the Asian corn borer, *Ostrinia furnacalis* (Guenee) in the Philippines. *J. Appl. Entomol.* **101**: 18-23.

Tran, L.C., R. Bustamente and S.A. Hassan (1986). Release and recovery of *Trichogramma evanescens* Westwood in corn fields in the Philippines. *Proceedings of 2nd International Symposium (Guangzhou, China, 1986). Les Colloques de l'INRA* no 43. INRA, Paris, 597-607.

Trexler, J.C., C.E. McCulloh and J. Travis (1988). How can functional response best be determined? *Oecologia* **76**: 206-214.

Tsai, J.H. and K. Wang (1999). Life table study of brown citrus aphid (Homoptera: Aphididae) at different temperatures. *Environ. Entomol.* **28**(3): 412-419.

Turlings, T.C.J., F.L. Wackers, L.E.M.Vet, W.J. Lewis and J.H. Tumlinson (1993). Learning host finding cues by hymenopterous parasitoids. In: '*Insect learning*' (D.R. Papay and A.C. Lewis, Eds.), Chapman and Hall, New York.

Van Alphen, J.J.M. and A.R.M. Janssen (1982). Host selection by *Asobara tabida* Nees (Braconidae: Alysiinae), a natural parasitoid of fruit inhabiting *Drosophila* spp. II. Host species selection. *Neth. J. Zool.* 32: 215-231.

Van Alphen, J.J.M. and L.E.M. Vet (1986). An evolutionary approach to host finding and selection. In: *Insect parasitoids* (Waage, J. and Greathead, D, Eds.), Academic Press, London, pp. 169-200.

Van den Berg (1993). Natural control of *Helicoverpa armigera* in smallholder crops in East Africa. PhD Thesis, Wageningen Agricultural University, The Netherlands. Pp 233.

Van den berg, H., J.K. Waage, and M.J.W. Cock, (1988). Natural enemies of *H. armigera* in Africa-a review. C.A.B. International Institute of Biological Control Ascot, UK. 81 pp.

Van Den berg, H., M.J.W. Cock, G.I.Oduor and E.K.Onsongo (1993). Incidence of *Helicoverpa armigera* (Lepidoptera: Noctuidae) and its natural enemies on smallholder crops in Kenya. *Bull. Entomol. Res.* **83**: 321-328.

Van den Bosch, R.V.D. and P.S. Messenger (1973) *Biological control.* International Text Book Company Limited, Aylesbury, UK.

Van Dijken, M.J., M. Kole, van Lenteren J.C. and A.M. Brand (1986). Host preference studies with *Trichogramma evanescens* Westwood (Hymenoptera:

Trichogrammatidae) for *Mermestra brassicae, Pierris brassicae* and *Pierris rapae*. *J. Econ. Entomol.***101**: 64-85.

Van Driesch, R.G., T.S. Bellows, J.S. Elkinton, J.R. Gould, and D.N. Ferro (1991). The meaning of percentage parasitism revisited: Solutions to the problem of accurately estimating total losses from parasitism. *Environ. Entomol.* **20**: 1-7.

Van Driesche, R.G. and T.G. Bellows (1996). *Biological control*. Chapman and Hall, New York, 539 pp.

Van Etten, C.H. and H.L. Tookey (1979). Chemistry and biological effects of glucosinolates. In: Rosenthal, G.A., Janzen, D.H. (Eds.), *Herbivores, their interaction with secondary plant metabolites*. Academic Press, New York, pp. 471-501.

Van Lenteren, J.C. (1980). Evaluation of control capabilities of natural enemies: Does art have to become science? Neth. J. Zool. 30: 369-381.

van Lenteren, J.C. (1983). The potential for entomophagous parasites for pest control. *Agric. Ecosyst. Environ.* **10**: 143-158.

van Lenteren, J.C. (1986). Evaluation, mass production, quality control and release of entomophagous insects. In: Franz, J.M. (Ed.), *Biological Plant and Health Protection*, Fischer, Stuttgart. Germany. pp.31-56.

Van Lenteren, J.C. (1991). Quality control of natural enemies: hope or illusion? In: Bigler, F. (ed.) *Fifth Workshop of the IOBC Global Working Group. Quality Control of Mass Reared Arthropods*. Wageningen, The Netherlands, pp. 1-14.

van Lenteren, J.C. (2000). Success in biological control of arthropods by augmentation of natural enemies. In: *Biological control*: *Measures of success*, (Gurr, G. & Wratten, S., Eds.), Kluwer Academic Publishers, Hingham/USA, pp. 77-103.

Van Lenteren, J.C., Z.H. Li, J.W. Kamerman and R. Xu (1995). The parasite host relationship between *Encarsia formosa* (Gahan) (Hymenoptera: Aphenelidae) and *Trialeurodes vaporarorium* Westwood (Homoptera: Aleyrodidae) XXVI. Leaf hairs reduce the capacity of *Encarsia* to control greenhouse whitefly on cucumber. *J. Appl. Entomol.* **119**: 553-559.

Varley, C.G., G.R. Gradwell, and M.P. Hassell (1973). *Insect population ecology: An analytical approach*. Blackwell Scientific Publications, 212 pp.

Verkerk, R.H.J., S.R. Leather and D.J. Wright (1998). The potential for manipulating crop-pest natural enemy interactions for improved insect pest management. *Bull. Entomol. Res.* **88**: 493-501.

Vet, L.E.M.and M. Dicke (1992). The ecology of infochemical use by natural enemies by herbivores in a tritrophic context. *Ann. Rev. Entomol.* **37**: 141-172.

Viggiani, G. (1981). Note su alcune specie di *Ologisita* Walker (Hymenoptera: Trichogrammatidae) e descrizione di Quattro nuove specie. *Bollettino del Laboratorio di Entomologia Agraria 'Filippo Silvestri' di Portici* **38**: 125-132.

Vinson, S.B. (1976). Host location by insect parasitoids. *Ann. Rev. Entomol.* **21**: 109-133.

Vinson, S.B. and G.F. Iwantsch (1980). Host suitability for insect parasitoids. *Ann. Rev. Entomol.* **25**: 397-419.

Voegele, J. (1988). Reflections upon the last ten years of research concerning *Trichogramma* (Hymenoptera: Trichogrammatidae). In: *Trichogramma and other Egg parasitoids. 2nd International Symposium on Trichogramma, Guangzhou, PR. China. Les Colloques I'INRA* **43**: 17-33.

Voronin, K.E. (1982). Biocenotic aspects of *Trichogramma* utilization in integrated plant protection control. 1st *International Symposium on les* Trichogrammes, Antibes, France. Paris: *Les colloques de I'INRA*. **9**: 269-75 pp

Waage, J.K. (1982). Sex ratio and population dynamics of natural enemies: Some possible interactions. *Ann. Appl. Biol.* **101**: 159-164.

Waage, J.K. (1986). Family planning in parasitoids: adaptive patterns of progeny and sex allocation. In: Waage, K.K. and Greathead, D.J. (eds) *Insect Parasitoids*. Academic Press, London, pp.63-95.

Waage, J.K. and H.C.J. Godfray (1984). Reproductive strategies and population ecology of insect parasitoids. In: Sibly, R.M. and Smith, R.H. (eds) *Behavioural Ecology: the Population Consequences of Adaptive Behaviour*. Blackwell Scientific Publishers, Oxford, pp. 449-470.

Waage, J.K. and Lane, J.A (1984). The reproductive strategy of a parasitic wasp. II. Sex allocation and local mate competition in *Trichogramma evanescens*. J. *Anim. Ecol.***53**: 417-426.

Waage, J.K. and S.M. Ng (1984). The reproductive strategy of a parasitic wasp. I. Optimal progeny and sex allocation in *Trichogramma evanescens*. *J. Anim. Ecol.***53**: 401-415.

Wagner, T.L; H.I. Wu, P.J.H. Sharpe, R.M. Schoolfield and R.N. Coulson (1984). Modeling insect development rates: a literature review and application of a biophysical model. *Ann. Entomol. Soc. Am.* **77**: 208-225.

Wajnberg, E., and SA Hassan (1994). *Biological control with egg parasitoids*. Oxon, UK: CAB International. 286 pp.

Walde, S.J. and W.W. Murdoch (1988). Spatial density dependence in parasitoids. *Ann. Rev. Entomol.* **33**: 441-466.

Wang, B. and D.N. Ferro (1998). Functional responses of *Trichogramma ostriniae* (Hym: Trichogrammatidae) to *Ostrinia nubilalis* (Lep: Pyralidae) under laboratory and field conditions. *Environ. Entomol.* **27**(3): 752-758.

Wang, F.C., S.Y. Zhang and S.R. Hou (1988). Inoculative release of *Trichogramma dendrolimi* in vegetable gardens to regulate populations of cotton pests. 2nd International Symposium, Guangzhou, China, 10-15 September 1986. *In*: *Trichogramma and other egg parasitoids* (J. Voegele, K.K. Waage and J.C. van Leteren, Eds.). *Les Colloques de l'INRA* **43**: 613-619.

Wang, K., Tsai, J.H. and Harrison, H.A. (1997). Influence of temperature on development, survivorship and reproduction of buckthorn aphid (Homoptera: Aphididae). *Ann. Entomol. Soc. Am.* **90**: 62-68.

Werren, J.H. (1984). A model for sex ratio selection in parasitic wasps: local mate competition and host quality effects. *Neth.J. Zool.* **34**:81-96.

Wiedenmann, R.N. and J.W. Smith, Jr. (1993). Functional response of the parasite *Cotesia flavipes* (Hymenoptera: Braconidae) at low densities of the host

Diatraea saccharalis (Lepidoptera: Pyralidae). *Environ. Entomol.* **22**(4): 849-858.

Wolfenbarger, D.A., P.R. Bodegas and R. Flores, (1981). Development of resistance in *Heliothis* spp. in the Americas, Australia, Africa and Asia. *Bull. Entomol. Soc. Am.* **27**: 180-185.

Yang, P.J., J.R. Carey and R.V. Dowell (1994). Temperature influences on the development and demography of *Bactrocera dorsalis* (Diptera: Tephritidae) in China. *Environ. Entomol.* **23**: 971-974.

Young, S.S.Y. (1961). A further examination of the relative efficiency of three methods of selection for genetic gains under less restricted conditions. *Genetic Res.* **2**: 106-121.

Yousuf, M. and S.A. Shafee (1987). Taxonomy of Indian Trichogrammatidae (Hymenoptera: Chalcidoidea). *Indian J. Syst. Entomol.* **4**: 55-200.

Yu, D.S.K., E.A.C. Hagley and J.E. Laing (1984) Biology of *Trichogramma minutum* Riley collected from apples in Southern Ontario. *Enviro. Entomol.***13**: 1324-1329.

Zaborski, E., P.E.A.Teal and J.E. Laing (1987). Kairomones mediated host finding by the spruce budworm egg parasite, *Trichogramma minutum. J. Chem. Ecol.* **13**: 113-122.

Zalucki, M.P., D.A.H Murray, P.C Gregg, G.P. Fitt, P.H. Twine and C. Jones (1994). Ecology of *H. armigera* (Hb) and *Helicoverpa punctigera* (Wallengren) in the inland of Australia: larval sampling and host plant relationships during winter and spring. *Australian J. Zool.* **42**: 329-346.

Zalucki, M.P., G. Daglish, S. Firempong and P.Twine (1986). Ecology of *H. armigera* (Hb) and *Helicoverpa punctigera* (Wallengren) (Lepidoptera: Noctuidae) in Australia. What do we know? *Australian J. Zool.* **34**: 779-814.

Zar, J.H. (1996). *Biostatistical analysis*. 3rd ed. Prentice Hall, Upper Saddle River, New Jersey.

Zhang, J., J. Wang, G. Liu and Y. Yan (1983). Influences of the humidities and temperature-humidity combinations on *Trichogramma ostriniae* Pang and Chen (Hymenoptera: Trichogrammatidae). *Natural Enemies of Insects* **5**:129-134.