THE BIOLOGY OF A FACULTATIVE HYPERPARASITOID, *TETRASTICHUS* *HOWARDI* OLLIFF (HYMENOPTERA: EULOPHIDAE), AND ITS POTENTIAL AS A BIOCONTROL AGENT OF LEPIDOPTEROUS STEM BORERS

THESIS

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CONTENTS

ABSTRACT

The gregarious pupal endoparasitoid, *Tetrastichus howardi* Olliff (Hymenoptera: Eulophidae), was introduced into South Africa as a biocontrol agent against the maize and the sorghum stem borers, *Busseola fusca* Fuller and *Chilo partellus* Swinhoe. Preovipositional behaviour, ovipositional behaviour, development, fertility, sex-ratio, and longevity were studied in the laboratory.

A complex courtship behaviour was observed, however 35.3% of females were mated before emergence from the host pupa. Preoviposition period ranged from 100 mins up to 5 days. Host searching time in Petri dishes was shorter for lepidopteran pupae than for their parasitoid pupae, and shortest when *T. howardi* had previously experienced the host. Duration of oviposition was significantly longer in the lepidopteran pupae than in the smaller tachinid puparia.

T. howardi showed no difference in preference for hosts of different ages. The lepidopteran hosts were preferred to their parasitoids. If *T. howardi* had previously experienced a certain host its pereference for that host tended to increase, but not significantly. When reared on a certain host, the preference for that host did increase.

The parasitoid was able to discriminate between parasitzed and unparasitzed pupae although this ability developed only 2 days after the pupa was parasitized. *Cotesia sesamiae* Cameron, the main indigenous parasitoid of *B. fusca* and *C. partellus*, was not attacked by *T howardi*.

The total duration of development from egg deposition to the adult stage ranged from 18 to 26 days at 24°C and 60% RH. Emergence of adults began after first light, mean emergence time in winter being 09h00. Emergence rate of _T_. _howardi_ from parasitized hosts, and mortality rate of parasitzed hosts, was higher for _C_. _partellus_ and _H_. _armigera_ than for _Eldana_ _saccharina_ Walker and _Palexorista laxa_ Curran. This decreased for _C_. _partellus_ and _H_. _armigera_ when superparasitized. A strong correlation existed between total parasitoids emerging from a host and percentage of females. When a lepidopteran pupa was parasitized by a single _T_. _howardi_ female, 55 progeny emerged of which 94% were females. Larger females showed greater fertility and also produced a higher percentage of females. Younger hosts were more suitable for development of _T_. _howardi_.

Females lived for 5.4 to 52.5 days, and males lived for 3.1 to 28.6 days, depending on presence or absence of food, water and hosts.

Reasons for releasing _T_. _howardi_ in the field are discussed. Only 2 recoveries of parasitized _C_. _partellus_ pupae were made from the field.

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CHAPTER 1

INTRODUCTION

Since 1950, maize has become one of the most important agricultural crops in South Africa, with a production exceeding 10 million tons in favourable years (Van Rensburg et al. 1987). Grain sorghum is also an important crop with an average production of about half a million tons (Van Rensburg & Van Hamburg 1975).

Of all the pests attacking these two summer grain crops in South Africa, the lepidopteran stem borers, _Busseola_ _fusca_ Fuller (Noctuidae) and _Chilo_ _partellus_ Swinhoe (Pyralidae) are by far the most deleterious (Skoroszewski & Van Hamburg 1987). _B_. _fusca_ which is indigenous, is the major pest in the colder regions of South Africa at higher altitudes (Van Hamburg 1987). It causes serious losses to both maize and grain sorghum (Skoroszewski & Van Hamburg 1987). _C_. _partellus_ invaded Africa from the Indian sub-continent (Mohyuddin & Greathead 1970), and was first reported in South Africa in 1958 (Van Hamburg 1979). _C_. _partellus_, which prefers to attack grain sorghum but may also severely damage maize (Skoroszewski & Van Hamburg 1987), has become the most destructive pest of maize and grain sorghum in the coastal regions of Natal and the lower lying parts of the Transvaal (Van Hamburg 1979). _C_. _partellus_ is however, rapidly spreading to the Highveld region (Van Rensburg & Bate 1987; Bate _et al._ 1991).

Estimated yield losses from borer damage range between 10% and 100% (Mally 1920; Matthee 1974; Van Rensburg & Bate 1986). Chemical control of borers is often not only uneconomical, as profit margins are low and pest control expenses can equal 56% of the gross margin above cost for an average maize yield (Van Hamburg 1987), but it is also often difficult. The timing of insecticidal applications is crucial as control measures are effective against young borer larvae only. Older larvae penetrate the stalks and are difficult to control with insecticides. In addition, the overlapping generations of the borers, especially *C. partellus*, result in reinfestations throughout the season (Van Hamburg 1987; Kfir 1988; Kfir *et al.* 1989).

Consequently alternative methods of control have been sought. A biological control approach to pest management in grain crops has been recommended (Van Hamburg 1987; Kfir 1990a). Thirteen different species of indigenous natural enemies were found from *Chilo* and *Busseola* borers in the Transvaal (Kfir 1990b). These included one egg parasitoid, two egg-larval parasitoids, five larval parasitoids, three pupal parasitoids, and two larval hyperparasitoids. However, these indigenous natural enemies are obviously not sufficient on their own to maintain borer populations below economic thresholds.

A braconid larval parasitoid, *Cotesia flavipes* Cameron, was introduced into South Africa from Pakistan for biological control of stem borers, but failed to become established (Skoroszewski & Van Hamburg 1987). Subsequently, several other natural enemies were introduced into South Africa for the same purpose. These were the egg parasitoids, *Trichogramma chilonis* Ishii and *T. ostriniae* Pang & Chen (Hymenoptera: Trichogrammtidae) from Taiwan; the hymenopteran larval parasitoids *Allorhogas pyralophagus* Marsh (Bracondidae) and *Mallochia pyralidis* Wharton (Ichneumonidae) from Mexico, and the dipteran larval parasitoid *Paratheresia claripalpis* Van der Wulp (Tachinidae) from Brazil; and the hymenopteran pupal parasitoid *Xanthopimpla stemmator* Thunberg (Ichneumonidae) from Mauritius. None of these introduced natural enemies appear to have become established.

Finally, Tetrastichus howardi Olliff (Hymenoptera: Eulophidae), a gregarious pupal endoparasitoid, was introduced into South Africa from the Philippines (Kfir et al. 1993), initially for control of C. partellus. The idea was that if it became established it could be beneficial also as a biological control agent against B. fusca, and Eldana saccharina Walker (Lepidoptera: Pyralidae), an indigenous borer on sugarcane.

BACKGROUND ON Tetrastichus howardi

Euplectus howardi Olliff was first described in 1893, in New South Wales, Australia, from a pupa of the noctuid sugarcane borer, Bathytricha trunctata Walker (Boucek 1988). Girault changed the name to Euplectrus and pointed out a similarity to Tetrastichus.

In 1921 Rohwer described Tetrastichus ayyari from a pupa of the pyralid borer Chilo zonellus (= partellus) Swinhoe (Ayyar 1927; Cherian & Subramaniam 1940). Mani and Kurian described Aprostocetus israeli in 1953, from pupae of Argyria sp. in India (Boucek 1988). All these names are synonymous with what is now known as Tetrastichus howardi Olliff.

T. howardi is fairly widely distributed across Asia, listed as occurring in China, Philippines (Rao 1965; Mohyuddin 1990), Malaysia (Ooi and Kelderman 1979a & 1979b), India (Ayyar 1927; Cherian & Subramaniam 1940; Rudriah & Sastry 1959; Rao 1965), and from Pakistan and Mauritius to Taiwan and eastern Australia (Boucek 1988).

Although it has been found that T. howardi attacks a wide range of Lepidoptera, and even pupae and puparia of other orders, namely Diptera, Coleoptera and Hymenoptera, in the laboratory (Cherian & Subramaniam 1940; Rudriah & Sastry 1959; Bennett 1965; Kfir et al. 1993), it has been recorded almost exclusively from stem borers in the field. T. howardi was reared in very small numbers from diamondback moth (Plutella xylostella L. (Plutellidae)) in Malaysia, such that it was considered as no more than an incidental parasitoid, insignificant in controlling the pest (Ooi & Kelderman 1979a).

Little work has been done on the biology of T. howardi. Cherian & Subramaniam (1940) looked at basic behavioural and reproductive biology of the parasitoid. Rudriah & Sastry (1959) made a more detailed study of developmental stages, longevity, adult habits, sex ratio, fertility, oviposition behaviour and preference, and host range. Kfir et al. (1993) made the most detailed biological study to date.

Cherian & Subramaniam (1940) were the first to discuss the potential of T. howardi as a biological control agent. In 1950 it was mass reared and released in South India against sugarcane borers, especially Scirpophaga nivella Fabricius, however no recoveries were made (Rudriah & Sastry 1959).

T. howardi was first introduced for biocontrol in 1963, into the West Indies island of Trinidad (Bennett 1965). After discovering that it is a facultative hyperparasitoid, attacking puparia of the tachinid genera, Lixophaga and Paratheresia, which are parasitic of Diatraea and other sugarcane borers, it was decided against release. However, T. howardi was considered for possible later release in Barbados and Grenada if tachinids did not become established there.

T. howardi was introduced to South Africa for possible release against *C. partellus*, *B. fusca*, and *E. saccharina*, borer pests of sorghum, maize and sugarcane (Kfir *et al.* 1993). The parasitoid was obtained from the Philippines where it attacks the Asiatic rice borer, *Chilo suppressalis* Walker.

CHAPTER 2

GENERAL MATERIALS AND METHODS

LABORATORY REARING OF *Tetrastichus howardi*

The laboratory culture of *T. howardi* was established from parasitoids emerging from parasitised *C. suppressalis* pupae which had been sent to South Africa from the International Rice Research Institute in the Philippines (Kfir *et al.* 1993). The culture was maintained mainly on pupae of the bollworm *Heliothis armigera* Hübner (Lepidoptera: Noctuidae) but also on *C. partellus* pupae, and kept in the laboratory in ventilated wooden cages (300 X 430 X 340 mm) at 24 ± 2°C and 60 ± 10% RH. Each cage housed approximately 7800 adult parasitoids, which were fed regularly with honey and water.

Hosts were presented to *T. howardi* in the cages periodically for several hours, for the maintenance of the culture. Pupae, once parasitised, were removed and placed in ventilated glass vials (25 mm diameter X 100 mm long) with a droplet of honey, until emergence of parasitoids.

Unless mentioned otherwise, all experiments were carried out with parasitoids approximately 2 days old (so females would have passed their preoviposition period), under the same conditions as the laboratory culture. The wasps were usually contained either in the vials or in small plastic Petri dishes (65 X 12 mm) during experimentation.

STATISTICAL METHODS

Several different statistical methods were used in this study to analyze data.

Analysis of Variance (ANOVA)

ANOVA is a parametric technique which requires the following assumptions (Elliott 1983):

1. The data must follow a normal distribution.
2. The variance of the sample must be independent of the mean.
3. The components of the variance must be additive.

As these conditions are rarely fulfilled, transformations are necessary before ANOVA is applied. The purpose of an ANOVA is to determine if there are significant differences between means of samples.

Transformations

The choice of the correct transformation depends upon the original frequency distribution of the counts. If the number of sampling units is too small for the counts to be arranged in a frequency distribution, then the relationship between variance and arithmetic mean can be used to choose a suitable transformation. For example, if variance is greater than mean and there are some zero counts, then x should be replaced by $\log(x + 1)$ (Elliott 1983).

Taylor's Power Law (Elliott 1983) can also be used to determine if transformation of data is necessary by calculating the distribution of the sample counts. When log of variance is plotted against log of mean, the angle (Θ) between the regression line and the X-axis (mean) indicates what the distribution is. If Θ is greater than $45°$, the distribution is contagious and data must be transformed.

Least Significant Difference (LSD) multiple range tests

ANOVA should be followed by a multiple range test to determine where the significant differences are. In this study the LSD multiple range test was used.

Generalised Linear Model (GLM)

GLM is a non-parametric method of analysis, meaning that the assumptions necessary for an ANOVA, which is a parametric method, do not apply here. The basic distribution used in dealing with discrete events rather than with continuously varying quantities is the Poisson distribution (McCullagh & Nelder 1985). In a Poisson series model variance is approximately equal to mean.

After the GLM is applied, means are separated using a multiple comparison Bonferroni Least Significant Difference (LSD) test.

Odds ratio

The calculation of odds ratios follows GLM analysis. It is an approximate method based on appropriate conditional likelihood functions (Dobson 1983). The odds ratio is a measure of the relative likelihood of an event occurring for each of 2 different groups, expressed as e.g. 2.5 times more likely for group A than for group B.

Chi-square (x^2) tests

Chi-square tests can be used to test for agreement between observation and hypothesis and can be extended to any situation where a basic hypothesis specifies the proportions or probabilities of a series of observations falling into several groups (Elliott 1983). The usual hypothesis is that all expected values are the same.

Contingency tables

This method is similar to the Chi-square test. When individuals are classified in 2 directions with 2 or more categories in each classification, the data are arranged in a contingency table (Elliott 1983). Observed and expected values for each cell are compared by the usual Chi-square test.

t-test

A t-test is a parametric method, therefore its basic requirement is that the underlying parent distribution is at least approximately normal with variance independent of mean. So counts from contagious distributions must be transformed before the t-test is applied.

The t-test is used to compare 2 means for significant difference.

Regression analysis

A regression line describes the average change in value of one variable (dependent variable y) for a unit change in another related variable (independent variable x) (Elliott 1983). The regression analysis determines if there is any significant correlation between two related variables.

The specific techniques used are indicated in the materials and methods of each experiment.

CHAPTER 3

PREOVIPOSITIONAL BEHAVIOUR

MATING BEHAVIOUR

Materials and Methods

As adult female T. howardi emerged from the host pupa, they were captured and isolated in vials from any contact with males. After the preoviposition period of about 2 days, each female was presented with a C. partellus pupa, which was then removed after 2 days. These parasitized pupae were kept in vials until emergence of parasites. The purpose of this experiment was to determine if any mating took place inside the host before emergence, and if so, what proportion of females were mated. This could be determined because unmated females reproduce parthogenetically, producing only males.

To observe mating behaviour, parasitised host pupae were dissected approximately a day before the expected emergence of parasitoids, and male and female T. howardi pupae were removed and separated. This was to make certain that no mating had taken place. Once the adults emerged, females were placed individually in vials, usually with more than one male. Behaviour was observed through a light microscope.

Results and Discussion

Sexual dimorphism occurs in T. howardi, females being noticeably larger than males. Females possess spear-shaped antennae and males, club-shaped antennae. Colouration of antennae and prothoracic legs also differs for the sexes: female antennae are black whereas male antenne are yellow with only the apical club being black; female prothoracic legs are black whereas those of the male are yellow.

Of 34 H. armigera and C. partellus pupae which were successfully parasitized by T. howardi females, 12 produced both male and female parasitoids, and 22 produced only males. This means that 35.29% of females were mated in the host pupa before they emerged.

Mating behaviour of T. howardi did not show a consistent pattern, however it was possible to separate the behaviour into sequential categories after observing mating conduct with 15 separate females (Fig.1). Initially males would wander around slowly. When closer to a female, the male would sometimes stop and turn towards her, responding to either visual or pheromonal stimulation. The male would sometimes pursue the female. If the female remained standing, the male would approach her from the side or back, but usually from the side, touching her with his antennae. The male would then raise his wings and vibrate them, after which mounting would occur if the female submitted. During the main courtship behaviour the male bent forwards, lifted up his abdomen, vibrated his wings, and took the antennae of the female between the basal segments of his own, stroking them upwards. This behaviour was repeated several times until he attempted mating, or eventually dismounted. If the female was receptive to the male, insertion of the aedeagus would take less than a second. If not, then the male would attempt copulation for up to a few seconds, after which time he would either resort back to the main courtship ritual or dismount. Immediately succeeding successful mating, the male would dismount. Mating was actually rarely observed even though the entire courtship behaviour was a

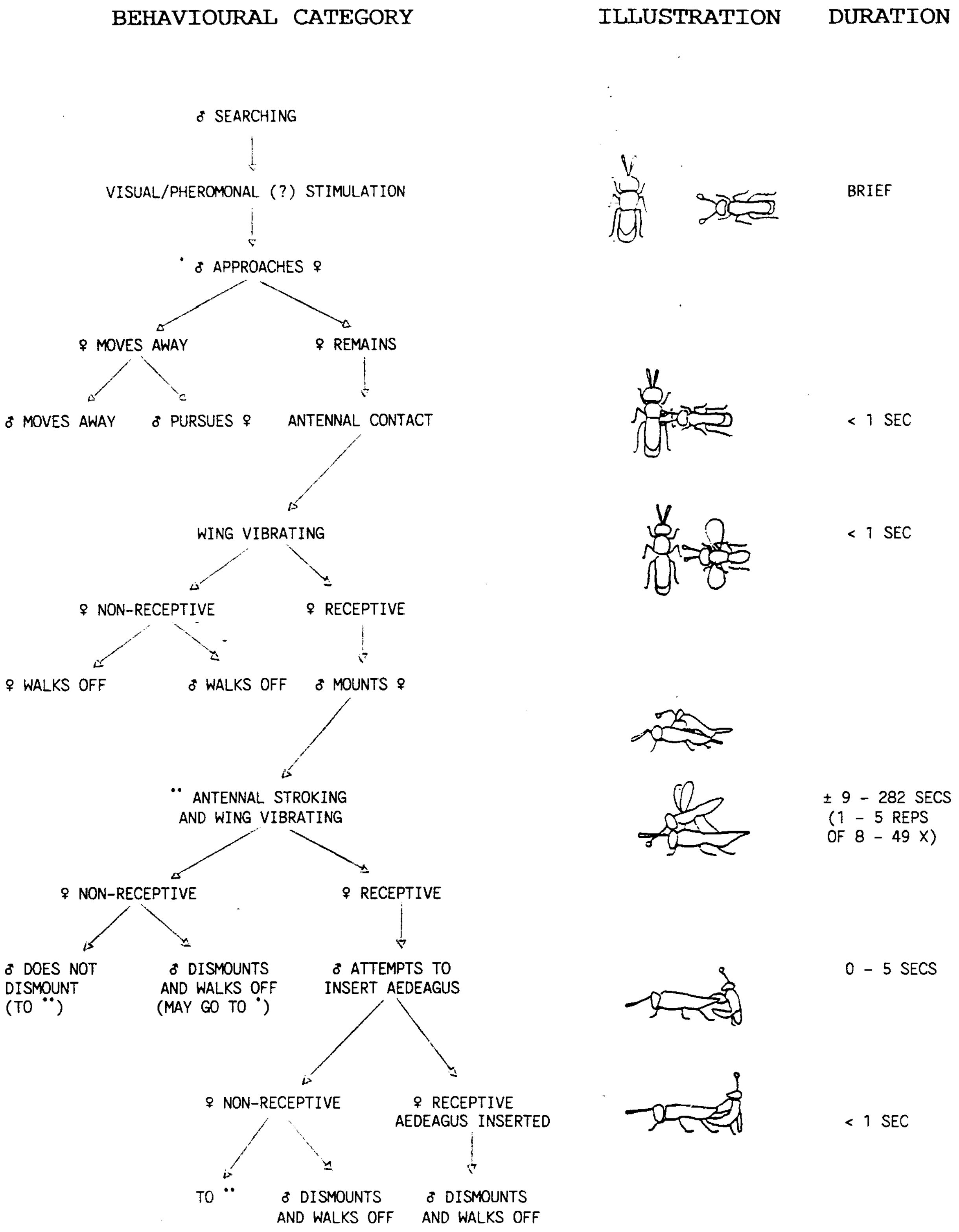

Fig.1 Diagrammatic representation of mating behaviour in <u>T.howardi</u>.

common occurrence. This was probably influenced by the fact that more than a third of the females were mated before emergence.

Similar courtship was observed by Rudriah & Sastry (1959) for *T. howardi*. They however, observed copulation to last for about 5 seconds, and females copulated with up to 6 males. In the current study no female was observed to be mated more than once, but was courted many times by different males.

PREOVIPOSITION PERIOD

Materials and Methods

Immediately after emergence and mating, 20 *T. howardi* females were placed individually in glass vials, each with a droplet of honey. Two *H. armigera* pupae were placed in each vial. They were removed after 24 hrs and replaced with fresh pupae. For the purpose of determining the duration of the preoviposition period, this was continued until all female parasitoids had oviposited. Removed pupae were placed in vials and marked. All emergence of parasitoids was recorded.

In another test, 10 newly emerged and mated *T. howardi* females were placed singly in small Petri dishes. Four *H. armigera* pupae were placed in each Petri dish. They were observed every 10 mins and if any female was seen ovipositing, the time between mating and oviposition was noted. Three replications of this trial were conducted, each one lasting for 8 - 9 hrs.

Results and Discussion

In the first test 7 out of 20 females oviposited in the first 24 hours; 9 females oviposited for the first time during the second day, 1 during day 3, 1 during day 4 and 1 during day 5. Only one female *T. howardi* did not oviposit at all. This means that 35% of females oviposit within the first 24 hours (Fig.2). After 48 hours 80% of all females had oviposited.

The results of the second test showed that 40% of females oviposited within the first 8 hours after mating. Minimum preoviposition period observed was 100 mins. Consequently preoviposition period of *T. howardi* ranges from 100 minutes to 5 days.

Certain members of the subfamily Tetrastichinae are known to oviposit immediately on emergence, whereas others may have a preoviposition period of up to 12 days (Clausen 1940). *T. howardi* is therefore quite typical of the subfamily. Cherian & Subramaniam (1940) noted that eggs are laid, in some cases, on the day of emergence.

HOST SEARCHING TIME

Materials and Methods

The data obtained for this experiment were from the short-term host preference tests (Chap.4). Mated *T. howardi* females were placed

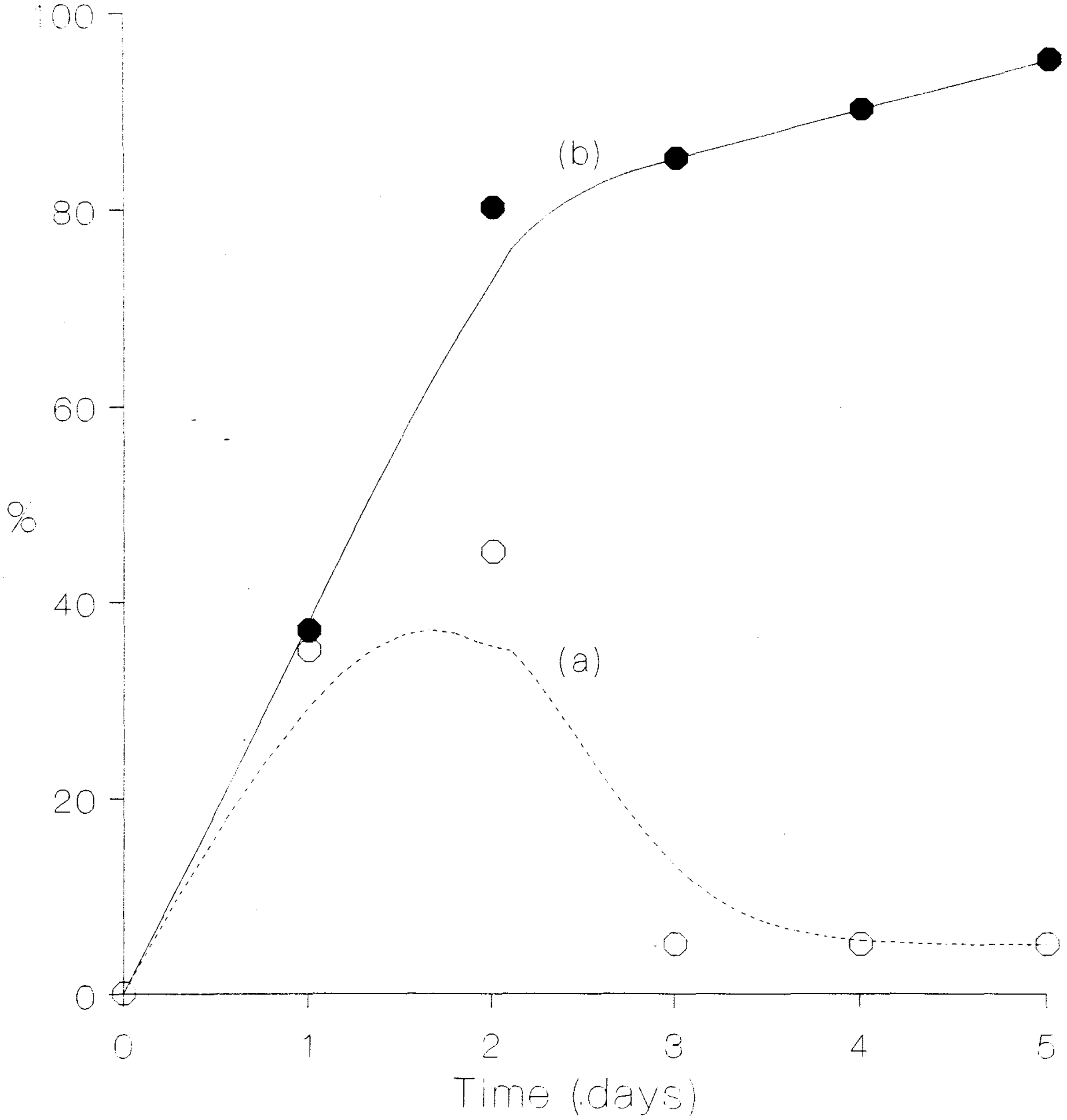

Fig.2 Preoviposition period of T.howardi (a) daily and (b) cumulative.

singly in Petri dishes with a choice of 2 different hosts, from *C. partellus*, *H. armigera*, *Palexorista laxa* Curran (Diptera: Tachinidae), and *X. stemmator*. All parasitoids had passed their preoviposition period. Time elapsed between exposure to the hosts and start of oviposition was recorded. These times were separated according to which host was chosen and what the previous experience of the parasitoid was (i.e. which host the parasitoid was previously exposed to), if any.

Data was analyzed by an ANOVA and means separated by an LSD multiple range test.

Results and Discussion

For all hosts, searching time was greatest if *T. howardi* had no previous experience (Fig.3). Searching time for *C. partellus* and *H. armigera* was least when *T. howardi* had previous experience with the same host which it chose. This did not prove to be the case with *P. laxa* and *X. stemmator*, but probably only because sample size was too small (Table 1). Over all, searching time was shortest for *H. armigera*, followed by *C. partellus*, *P. laxa*, and *X. stemmator*, in that order. This was probably influenced by the parasitoids having been reared from *H. armigera*.

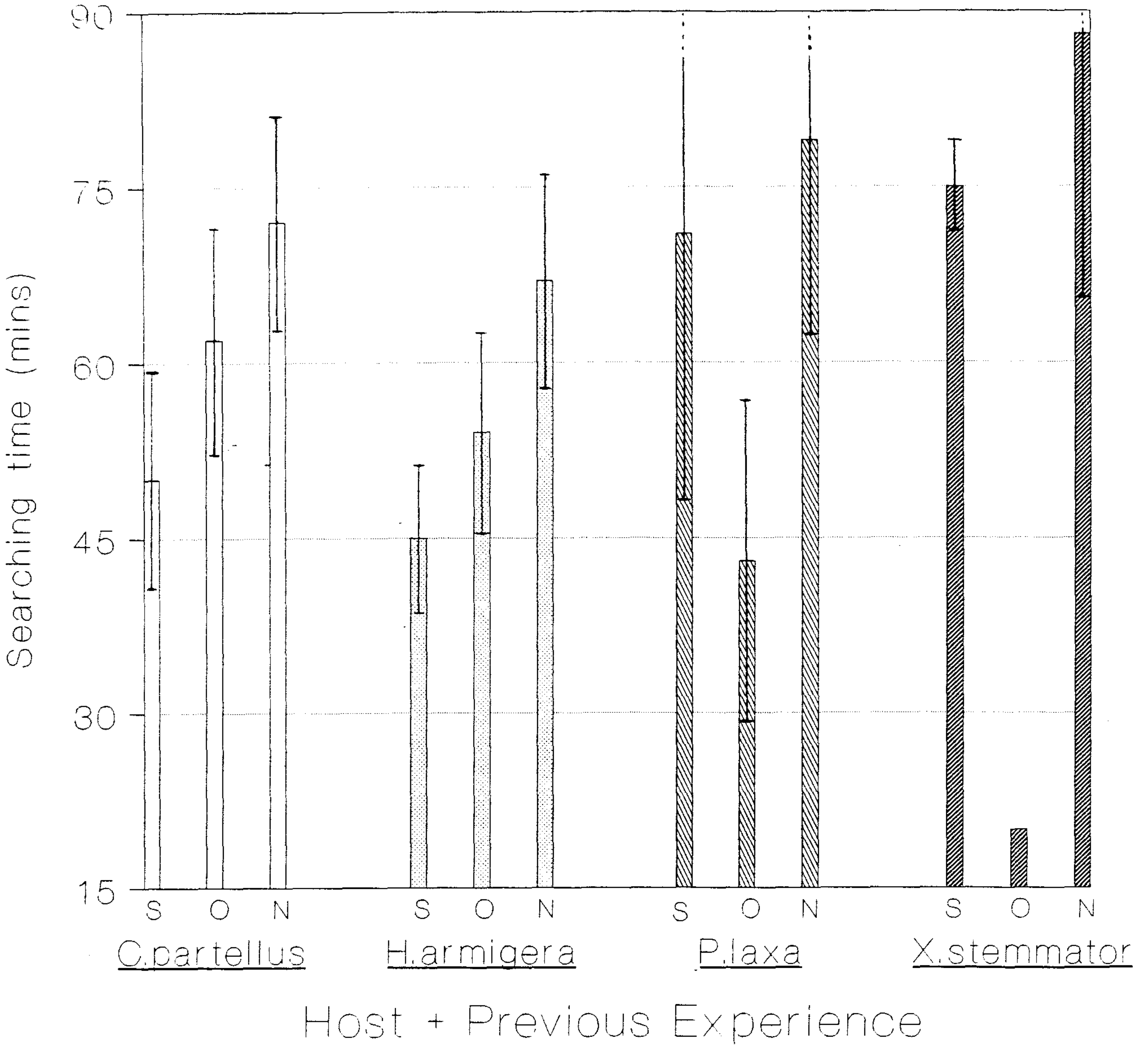

Fig.3 Searching time of T.howardi on various hosts, with previous experience indicated (S = same host, O = other host, N = no previous experience).

Table 1. Host searching time of _T_. _howardi_ for 4 different hosts, and the influence of previous experience.

Host	Previous Experience	n	Mean Searching Time (mins.)	SE
_C_._partellus_	–	67	61.64	6.80
	_C_._p_.	23	49.56	11.51
	Other	18	61.67	12.13
	None	26	72.31	11.52
_H_._armigera_	–	76	55.79	5.81
	_H_._a_.	25	44.80	7.49
	Other	22	53.64	10.25
	None	29	66.90	11.31
_P_._laxa_	–	19	70.53	14.11
	_P_._l_.	7	71.43	28.40
	Other	3	43.33	16.67
	None	9	78.89	20.31
_X_._stemmator_	–	9	77.78	18.77
	_X_._s_.	2	75.00	5.00
	Other	1	20.00	0
	None	6	88.33	26.63

_C_._p_. = _C_. _partellus_, _H_._a_. = _H_. _armigera_, _P_._l_. = _P_. _laxa_, _X_._s_. = _X_. _stemmator_.

An ANOVA showed no significant differences between searching times. This was probably due to the high variation in the data.

Four distinct and consecutive processes of selection, limiting the natural host list of a parasitoid are believed to occur (Flanders 1947; Doutt 1959). These are: host habitat finding, host finding, host acceptance, and host suitability. Here we dealt only with host finding as all hosts proved acceptable.

Reflection of these results upon searching time in nature is limited as initially host habitat must be found, often by plant odour (Arthur 1962; Streams _et al._ 1968; Read _et al._ 1970; Camors & Payne 1972; Shahjahan & Streams 1973; Vinson 1976; Inayatullah 1983; Vinson & Piper 1986), and once this has been achieved, structural complexity of host habitat can affect the success of parasitoid search (Andow & Prokrym 1990).

These results indicate little about _T. howardi_'s searching time in nature but they could be interpreted as indications of difference in preference between hosts. _T. howardi_ on encountering a less favourable host could refuse it and continue searching for a more acceptable host. This may depend on how long the parasitoid has already been searching for a suitable host.

CHAPTER 4

OVIPOSITION BEHAVIOUR

DURATION OF OVIPOSITION

Materials and Methods

Thirty mated *T. howardi* females of ± 2 days old were placed singly in petri dishes. Two hosts were placed in each petri dish, 10 petri dishes with *H. armigera*, 10 with *C. partellus*, and 10 with *P. laxa*. Observations were made every 10 mins and if the position of a parasitoid was on a host, it was recorded. Duration of oviposition was thus determined. Three replications of the trial were conducted with both *H. armigera* and *C. partellus* pupae, and two replications with *P. laxa* pupae.

Mean oviposition times were checked for normal distribution to determine if transformation of data was necessary for statistical analysis. After transformation, data was subjected to an ANOVA to determine if there were significant differences between mean durations of oviposition. The LSD multiple range test was then applied to determine where the significant differences were.

Results and Discussion

In total 12 of the females oviposited in *H. armigera*, 12 in *C. partellus*, and 7 in *P. laxa*. Mean oviposition time in *H. armigera* was 188.33 ± 26.88 mins (x ± SE). In *C. partellus* it was 182.50 ± 20.49 mins (x ± SE), and in *P. laxa* oviposition time was 100 ± 5.34

mins (x ± SE).

By plotting means against variances it was determined that distribution was not normal. Data was transformed so it could be subjected to parametric statistical analysis. Because variance was greater than the mean and there were no zero counts, the transformation used was log x (Elliott 1983).

An ANOVA revealed a significant difference in the mean duration of oviposition in the three hosts (F-ratio = 5.25; $P < 0.01$). An LSD multiple range test indicated that oviposition times in C. partellus and H. armigera were not significantly different, but that oviposition in P. laxa was significantly shorter than that in the other two hosts.

It is not unusual for other members of the Tetrastichinae to remain with the ovipositor inserted in the host for many hours (Clausen 1940), however T. howardi's egg laying was previously recorded to be little more than half an hour (Rudriah & Sastry 1959).

Kfir et al. (1993) found a linear relation between the number of emergent T. howardi and the volume of the host from which they emerged. Size of H. armigera pupae (18.7 x 5.5 mm) substantially exceeded that of C. partellus pupae (13.4 x 3.1 mm), which in turn was greater than that of P. laxa (7.4 x 3.4 mm).

Even though the lesser number of adult parasites emerging in smaller pupae is partly due to less food material available for the developing larvae (Rudriah & Sastry 1959), it is possible that parasitoids could lay fewer eggs in smaller hosts, as this pattern was observed in the eulophid Euplectrus kuwanae (Uematsu 1981), and hence have a shorter oviposition time on smaller hosts. In Tetrastichus israeli Mani the process of oviposition lasted longer when the parasitoid attacked large hosts and so more eggs could be laid (Nadarajan & Jayaraj 1975). In other hymenopterans, various host species are not parasitised to the same degree, probably because of difference in body-surface area of host, difference in cuticle thickness, and preference of the parasite for laying in the host species in which it had itself developed (Eisjackers & Van Lenteren 1970).

INFLUENCE OF HOST AGE ON PREFERENCE

The aim of the experiment was to determine if _T_. _howardi_ females displayed any discrimination between hosts of different ages, but the same species, when ovipositing, and if their preference could be influenced by previous experience with either old or young pupae.

Materials and Methods

Thirty females of _T_. _howardi_ were held for a day with honey only or with host pupae as well (either 1 day old or 5 day old pupae), and were placed individually in small plastic Petri dishes. Each Petri dish contained 4 hosts: 2 of 1 day old and 2 of 5 days old, which were differentiated according to their position in the Petri dish and their colour (older pupae being darker). Females were observed at 10 minute intervals for the next 200 minutes and their position noted if they were on either host. Number of females on either host as well as mean times spent ovipositing were considered as measures of preference for the host. This experiment was conducted twice with both *C. partellus* and *H. armigera* pupae.

Results and Discussion

In the preference tests, *T. howardi* showed no discrimination according to age of the host. The 1 day old *C. partellus* pupae were selected by 33 parasitoids compared to the 5 day old pupae, by 32 parasitoids. Mean oviposition time on the younger hosts was 66.06 mins compared to 68.75 mins on the older pupae.

Mean oviposition time on the 1 day old *H. armigera* pupae was 84.54 mins (n = 22) compared to 86.67 mins (n = 24) on the 5 day old pupae.

It is clear that there is no differentiation according to age of host by the parasitoid.

Hymenopteran parasitoids have often been observed to prefer certain larval instars or larval ages above others (Eisjackers & Van Lenteren 1970; Vinson 1976; Donaldson & Walter 1991; Kidd & Jervis 1991a & b; Kirsten & Kfir 1991), even amongst the genus *Tetrastichus* (Hammerski *et al.* 1990; Mushtaque 1990). However, there are no recorded cases of a hymenopteran parasitoid displaying preference for a certain age of pupa. *T. howardi* being gregarious and polyphagous, this is not surprising.

HOST PREFERENCE

Long and short term tests were carried out to establish the preference of *T. howardi* between *C. partellus* and *H. armigera*, and between these phytophagous insects and their parasitoids, *X.*

stemmator and *P*. *laxa*. Pupae of *C*. *partellus* and *H*. *armigera* were used as hosts as well as puparia of *P*. *laxa*, and pupae of *X*. *stemmator*, which normally pupate singly inside their host pupa (*C*. *partellus* in this case).

a. Long Term Tests

Materials and Methods

In the long term test, mated *T*. *howardi* females reared from *H*. *armigera* were kept singly with males in glass vials, each containing 4 pupae (2 from each host tested). All hosts were removed and replaced with fresh ones every 24 hrs for 5 days. On each of the 5 days, 10 replications of each trial were conducted. Each experiment was repeated 3 times, therefore giving a total of 30 replications each. All exposed pupae were kept singly in glass vials and emergence was recorded.

Data was analyzed using Chi-square analyses, and also using the GLM with the Poisson Distribution. A multiple comparison Bonferroni LSD test was used to determine if *T*. *howardi* showed any significant preferences. Odds ratios were also calculated to more clearly signify degrees of preference.

Table 2. Host preferences of _T. howardi_ in long term (5 day) preference tests, when presented with 2 options, and the change of preference over time.

2 Options	Host Preferred	Overall Odds ratio	Day	Odds ratio /day (relative to Day1)	P
_Cp_ _Xs_	_Cp_	1.3	1	–	< 0.20
			2	–	
			3	–	
			4	–	
			5	–	
_Ha_ _Pl_	_Ha_	23.7	1	–	< 0.10
			2	–	
			3	–	
			4	–	
			5	–	
_Cp_ _Pl_	_Cp_	3.6	1	2.0	< 0.02
			2	0.7	
			3	1.5	
			4	3.0	
			5	133252.3	
_Cp_ _Ha_	_Cp_	2.1	1	0.6	< 0.01
			2	5.6	
			3	6.7	
			4	5.0	
			5	18.4	

_C.p._ = _C. partellus_, _X.s._ = _X. stemmator_, _H.a._ = _H. armigera_, _P.l._ = _P. laxa_.

Results

T. howardi developed a significant preference for C. partellus over H. armigera over the 5 days ($x^2 = 15.05$; $P = 0.005$) (Fig.4). T. howardi also strongly preferred C. partellus pupae over P. laxa pupae ($x^2 = 10.01$; $P = 0.04$) (Fig.5). T. howardi showed a slight preference for C. partellus over its parasitoid, X. stemmator, however, this difference was not significant ($x^2 = 7.38$; $P = 0.116$) (Fig.6). The parasitoid showed a significant preference for H. armigera over P. laxa (Fig.7).

According to contingency tables using the GLM, choice of host by T. howardi was not dependent on the time in the tests involving C. partellus versus X. stemmator and H. armigera versus P. laxa, however overall preferences were calculated for C. partellus and H. armigera respectively (Table 2). T. howardi significantly preferred C. partellus to H. armigera and C. partellus to P. laxa, and in both cases the choice of the parasitoid was dependent on the time (Table 2). The odds ratios calculated indicate the degree of likeliness of one host being preferred to the other, or the extent to which a host is more likely to be preferred on one day compared to a previous day.

b. Short Term Tests

Materials and Methods

In the short term preference tests females of T. howardi reared from H. armigera were held for a day with honey only or with host pupae as well (either of the 2 hosts tested), and were placed individually

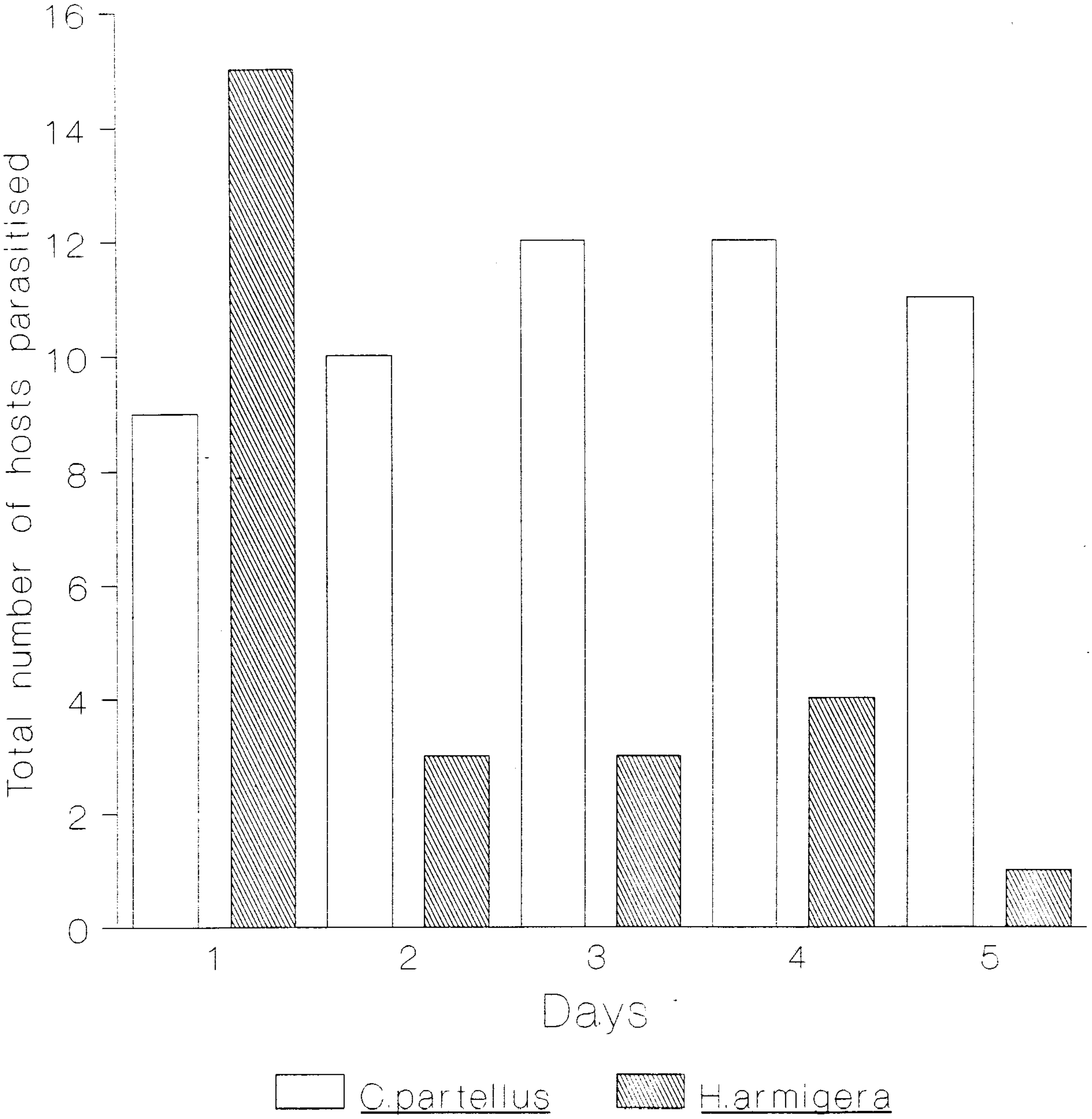

Fig.4 Host preference of T.howardi, between C.partellus and H.armigera over 5 days.

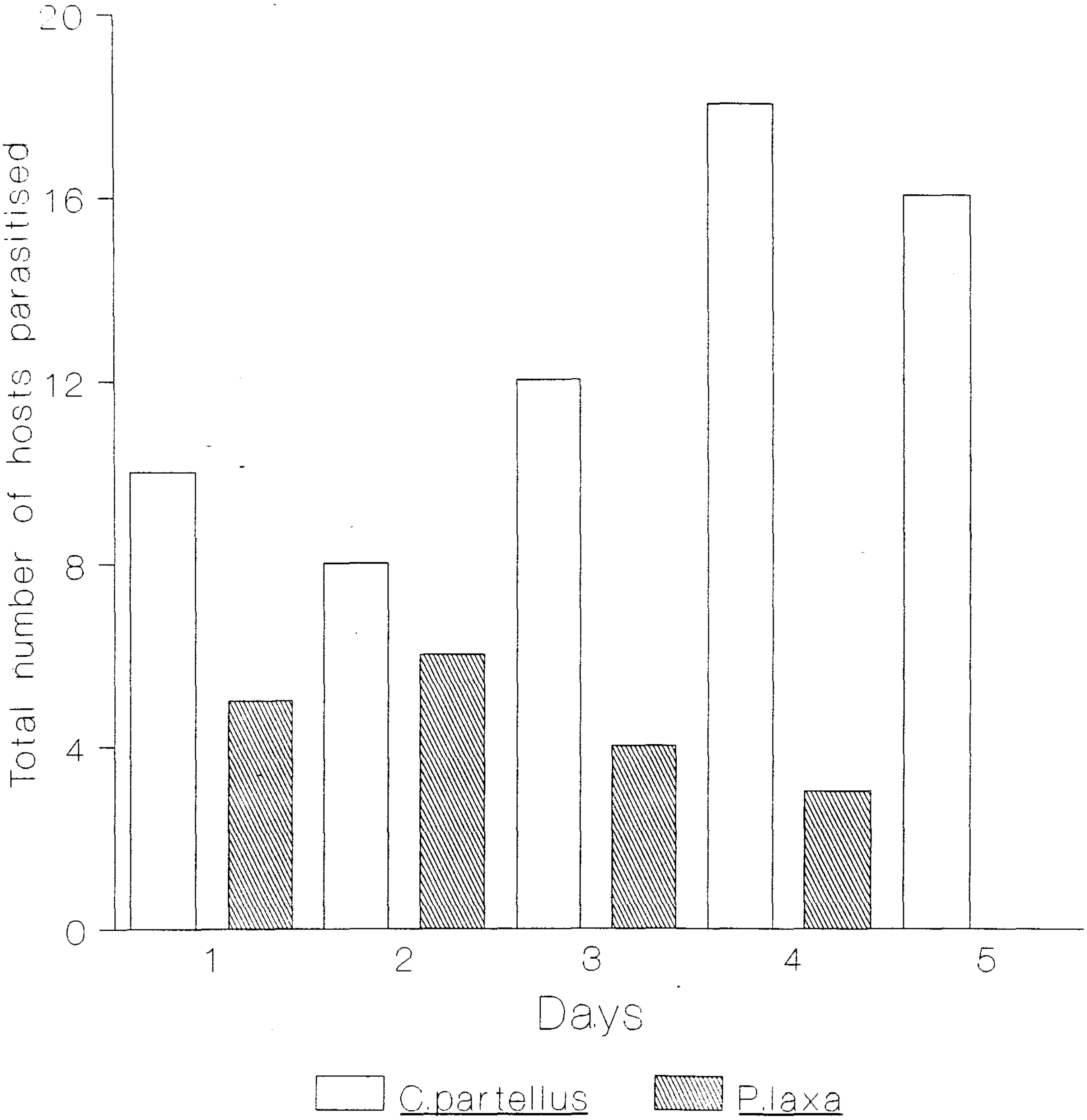

Fig.5 Host preference of *T.howardi*, between *C.partellus* and *P.laxa* over 5 days.

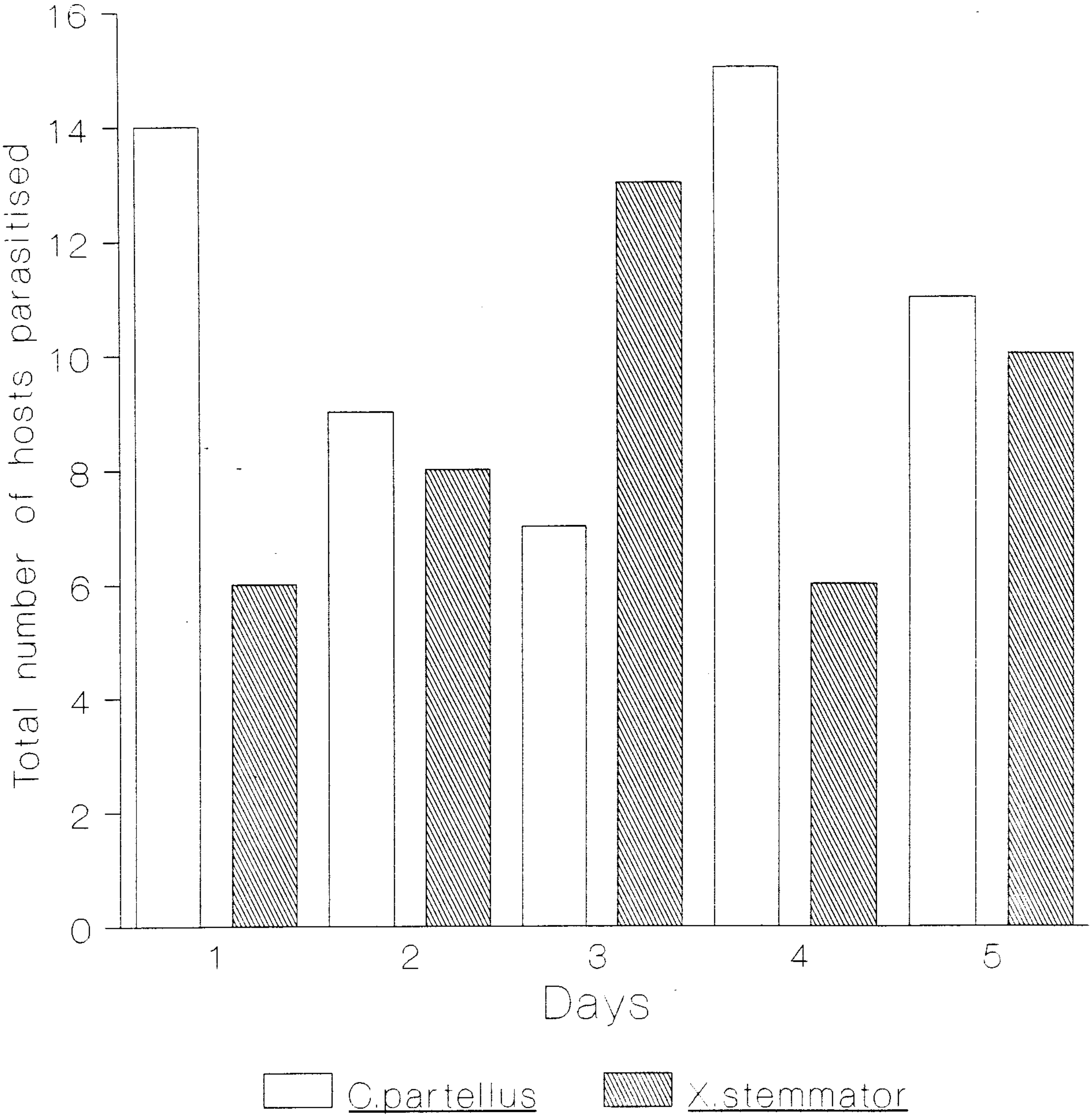

Fig.6 Host preference of _T.howardi_, between _C.partellus_ and _X.stemmator_ over 5 days.

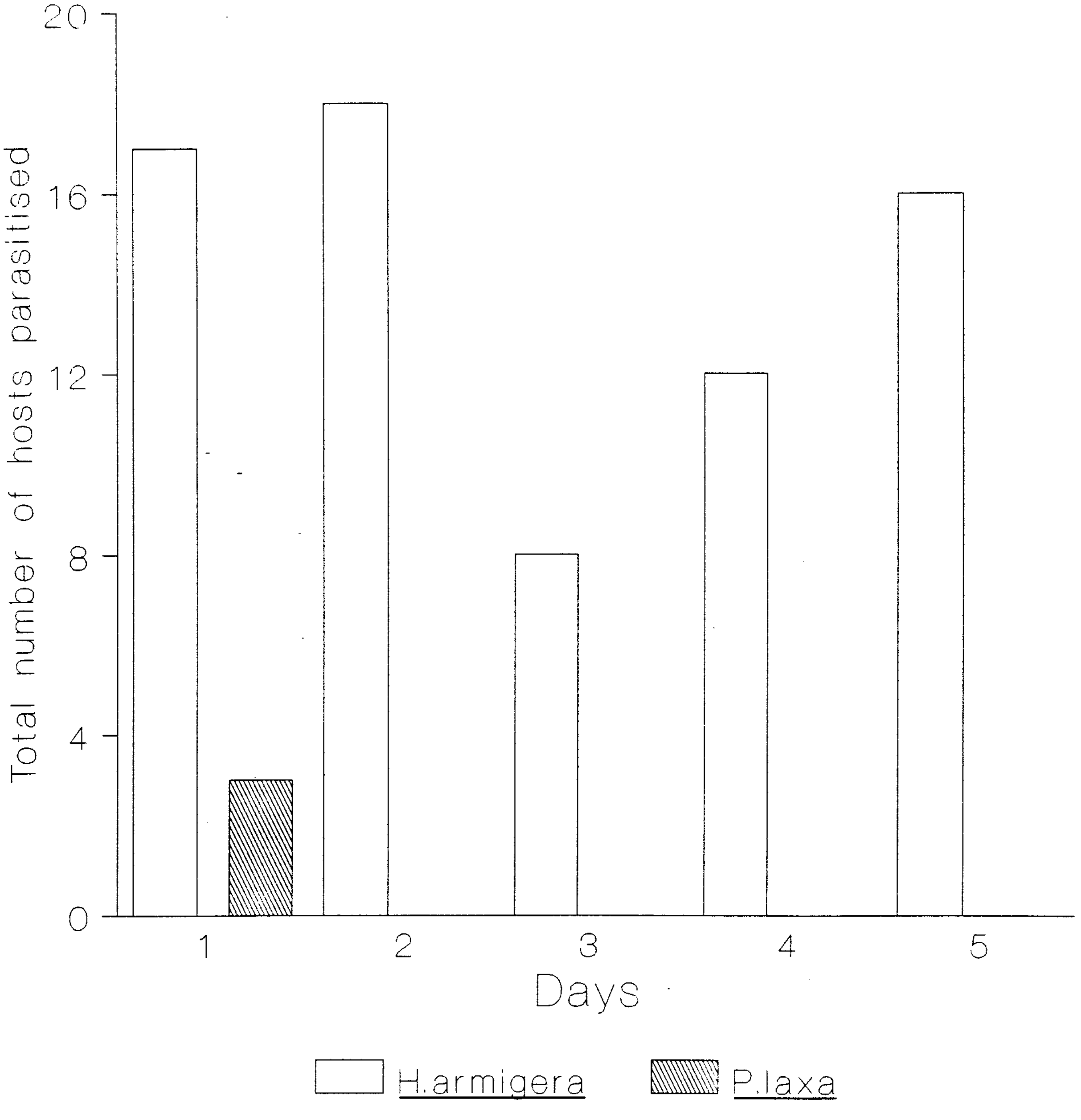

Fig.7 Host preference of T.howardi, between H.armigera and P.laxa over 5 days.

in Petri dishes. Each dish contained a total of 4 host pupae (2 from each host tested). Females were observed at 10 min intervals for the next 200 min and their position noted if they were on either host. The total number of females ovipositing in each host species as well as the total number of observations (or time spent ovipositing) for each host were taken as measures of preference for the hosts.

The preference of T. howardi females, reared from C. partellus or from H. armigera, was compared for both these hosts. Also the preference of females reared from H. armigera or from P. laxa was compared for these hosts.

Data from these tests were analyzed using Chi-square tests. Contingency tables with the GLM were used to determine significant preferences, and the odds ratio of a host being preferred were calculated. Subsequently, using oviposition time as a measure of host preference, the GLM was used, followed by a Bonferroni LSD test to separate the means.

Results

The Chi-square tests showed no significant differences in preferences of T. howardi, even though such differences were apparent (Fig.8).

Contingency tests using GLM showed preferences by T. howardi for one of the hosts in all of the tests, although this was only significant for H. armigera when it was compared with P. laxa (Table 3). The observed influence of experience on choice was in no case found to be significant.

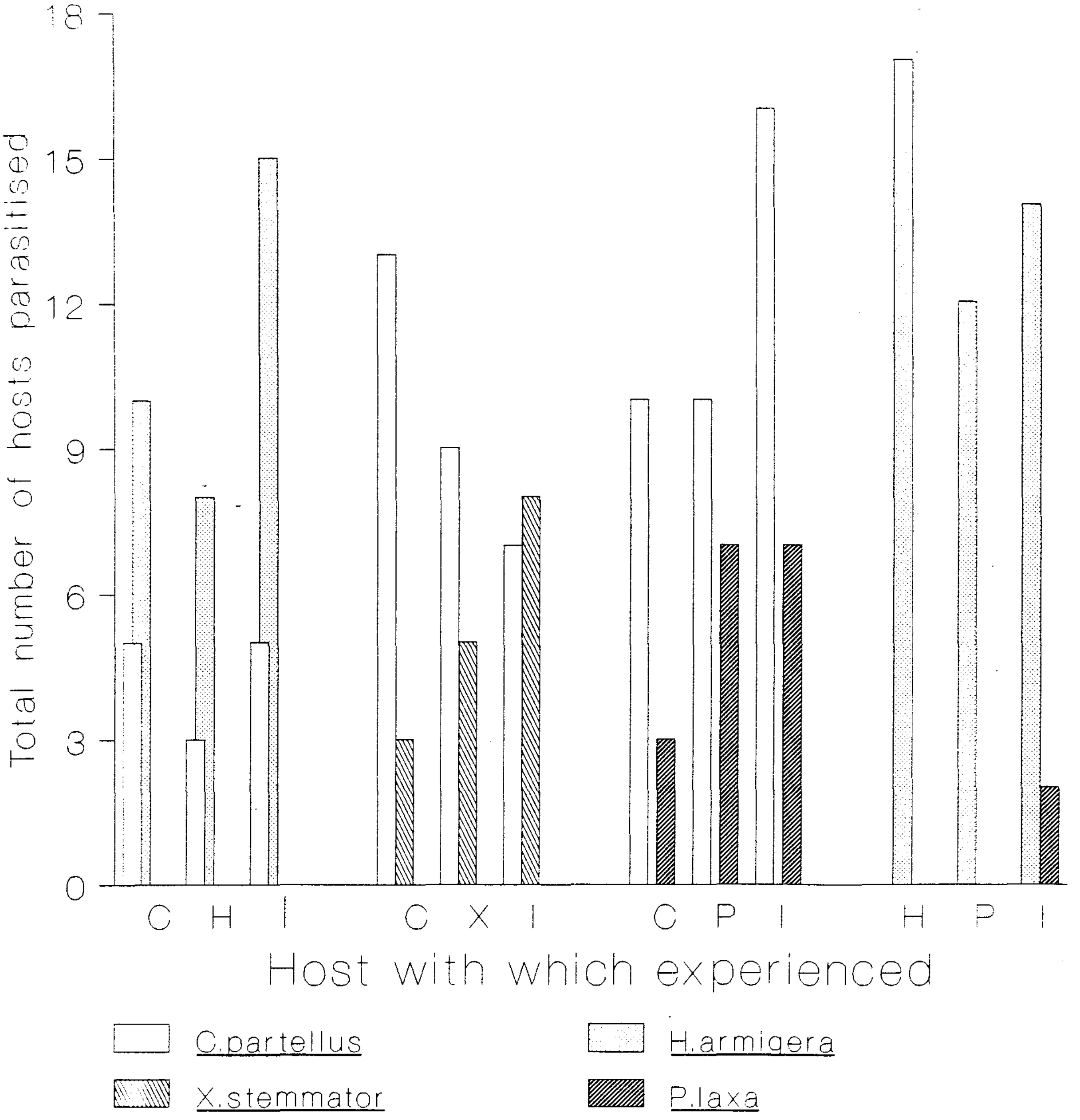

Fig.8 Short-term (200 min) host preference of *T.howardi* when given 2-way choices, and the influence of previous experience (C = *C.partellus*, H = *H.armigera*, X = *X.stemmator*, P = *P.laxa*, I = inexperienced).

Table 3. Host preferences of *T. howardi* in short term (200 min) preference tests, when presented with 2 options. The odds ratio indicates the degree of likelihood of the preferred host being selected over the alternative host.

2 Options	Host Preferred	Odds ratio	P
Cp *Xs*	*Cp*	1.8	< 0.20
Ha *Pl*	*Ha*	21.5	< 0.10
Cp *Pl*	*Cp*	2.2	< 0.20
Cp *Ha*	*Cp*	2.5	< 0.20

Cp = *C. partellus*, *Xs* = *X. stemmator*, *Ha* = *H. armigera*, *Pl* = *P. laxa*.

Regardless of which host *T. howardi* emerged from, its preference remained for *H. armigera* over *C. partellus* (although insignificant when emerged from *C. partellus*) (Fig.9), and for *H. armigera* over *P. laxa* (Fig.10). However, there was a large difference in odds ratio in both cases, which indicated that the host from which the parasitoid emerged had a significant influence on its resultant choice (Table 4).

Oviposition times were generally not an effective means of determining the parasitoid's preference of host. According to this method *T. howardi* only significantly preferred *C. partellus* over *X. stemmator* ($P = 0.027$), and *H. armigera* over *P. laxa* ($P = 0.005$), but previous experience had no significant influence on choice.

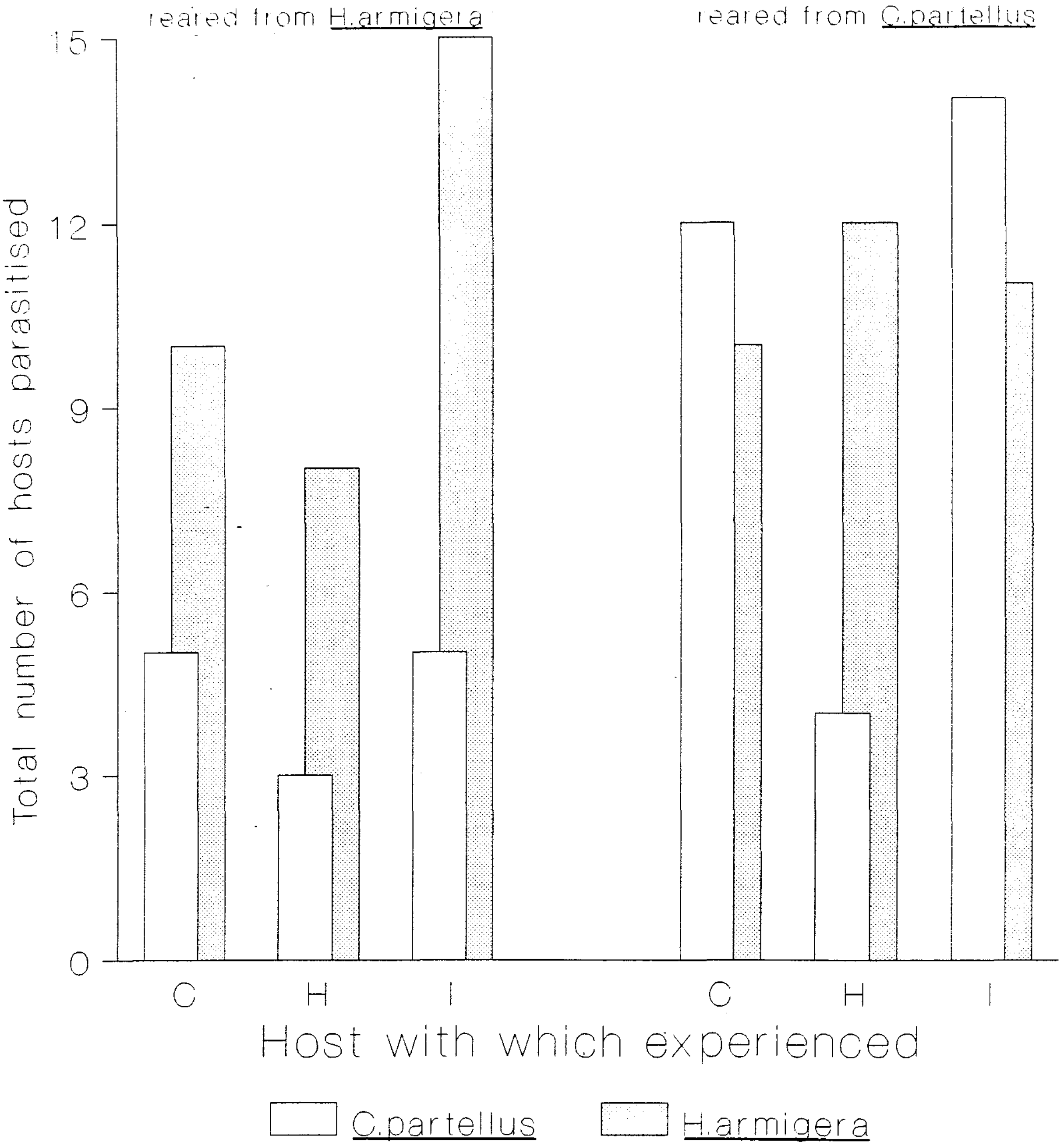

Fig.9 Short-term host preference of T.howardi, between C.partellus (C) and H.armigera (H), and influence of host from which parasitoid emerged (I = inexperienced).

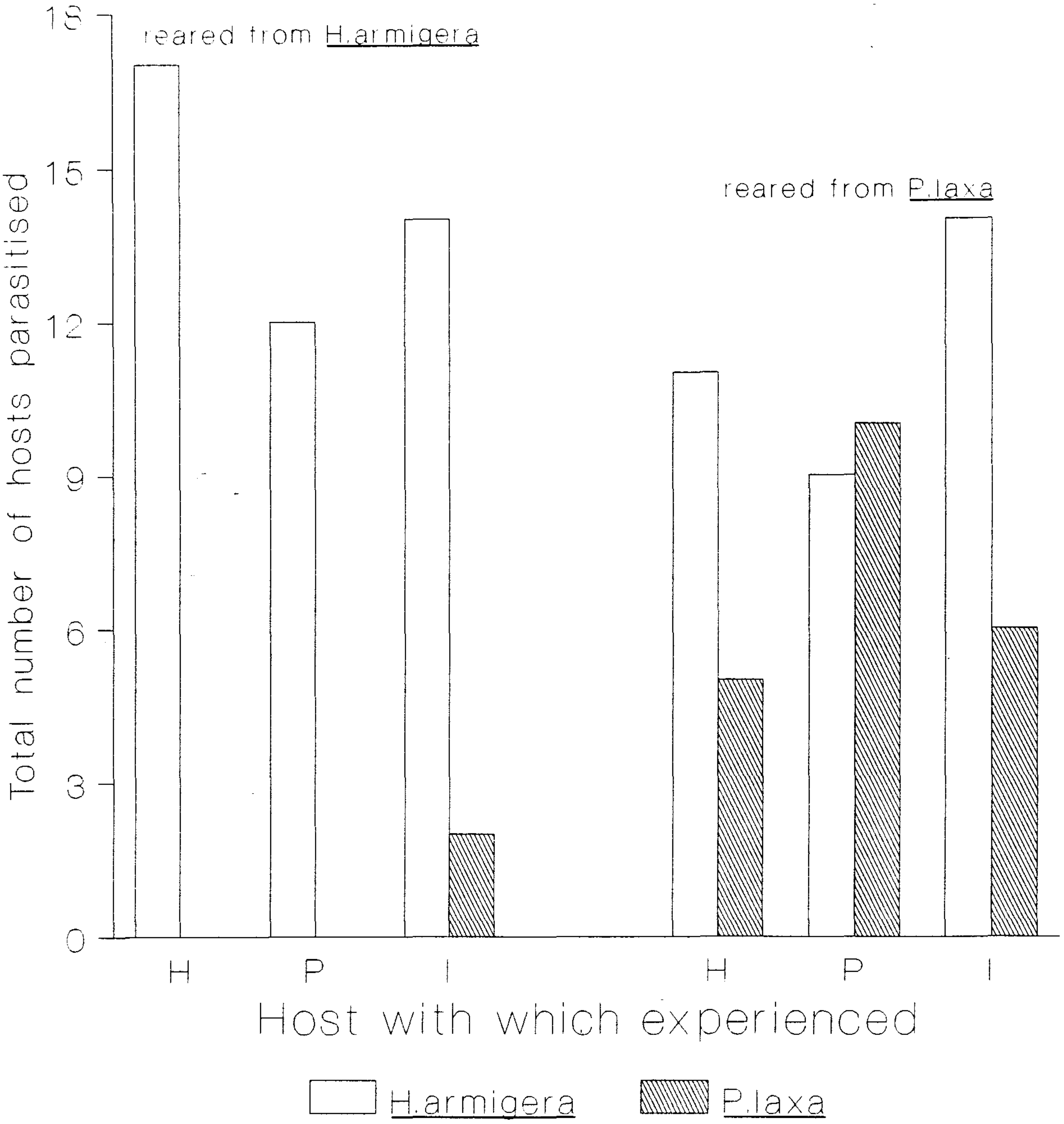

Fig.10 Short-term host preference of *T.howardi*, between *H.armigera* (H) and *P.laxa* (P), and influence of host from which parasitoids emerged (I = inexperienced).

Table 4. Influence of the host from which T.howardi emerged on its host preference.

2 Options	Host emerged from	Host Preferred	Odds ratio	P
Cp Ha	Ha	Ha	2.5	< 0.2
	Cp	Ha	1.1	> 0.5
Ha Pl	Ha	Ha	21.5	< 0.1
	Pl	Ha	1.6	< 0.3

Cp = C. partellus, Ha = H. armigera, Pl = P. laxa.

Discussion (long and short term tests)

T. howardi is polyphagous, and under laboratory conditions has a very wide host range (Kfir et al. 1993). However, no parasitoid appears to be completely indiscriminate (Doutt 1959), and under natural conditions will attack only a fraction of the species on which development is actually possible. In the artificial conditions of a laboratory one can remove the barriers which separate potential hosts and parasites in nature. Once the host has been found, its acceptability to the parasitoid is attributable to factors such as odour (Inayatullah 1983), texture, shape, size and even motion (Doutt 1959; Vinson 1976). It is these factors which probably influence the preferences of T. howardi.

Similar preferences for lepidopteran borers over tachinid parasitoids were observed for T. israeli (Bennett 1965), which is very similar in habit to T. howardi.

It has been demonstrated that prior ovipositional experience has a significant effect on a parasitoid's resultant host preference (Samson-Boshuizen et al. 1974; Williams 1991), and that conditioning (probably olfactory) is seen in the host selection of a parasitic wasp (Ohgushi 1960).

A female parasitoid with a wide host range often prefers a host species from which she has been reared (Vinson 1976). This was shown to be the case with parasitoids such as Pseudocoila bochei Weld (Eisjackers & Van Lenteren 1970), Pimpla examinator F. (Jackson 1937) and Nasonia vitripennis (Walker) (Oghushi 1960).

Although in the laboratory T. howardi showed preference for phytophagous over parasitic insects, in the field it is unlikely that females searching for hosts and encountering a parasitoid pupa would move elsewhere in search of a lepidopterous host before attacking the parasitoid (Bennett 1965; Kfir et al. 1993). On the other hand, differences in host searching time were recorded for different hosts, and this may indicate that less favourable hosts can be encountered several times before being attacked if no more favourable host is found.

HOST DISCRIMINATION

Host discrimination is the ability of an insect parasitoid to discriminate between parasitized and unparasitized hosts. This is to avoid attacking or accepting a host that has previously been parasitized (Vinson 1976). The aim of this experiment was to determine if T. howardi possesses this ability.

Materials and Methods

Short-term preference tests were conducted with T. howardi to determine if the females discriminated between parasitised and unparasitized pupae when ovipositing. The method and procedure were similar to those of the previous preference tests. H. armigera pupae were exposed to T. howardi in a cage overnight (± 20 hrs.), and then used in the experiments immediately after parasitization, 1 day after parasitization, 2 days after, 5 days after, and 12 days after parasitization. Parasitoid females were previously experienced with parasitised hosts, unparasitized hosts, or were inexperienced.

Data were transformed and analyzed using t-tests.

Results and Discussion

It was clear from data that there was no correlation between previous experience and host preference. Therefore, for the t-tests, previous experience was disregarded. With all data pooled, variance was larger than mean, and because there were also some zero counts, the transformation used was log(x + 1) (Elliott 1983). The t-tests showed that T. howardi significantly preferred the just parasitised pupae to the unparasitized pupae ($t = -2.96$; $P = 0.01$). T. howardi showed no discrimination between pupae parasitised 1 day ago and unparasitized pupae. The parasitoids significantly preferred unparasitized pupae to pupae parasitised 2 days ago ($t = 3.22$; $P = 0.009$), pupae parasitised 5 days ago ($t = 8.70$; $P = 0.000006$), and pupae parasitised 12 days ago ($t = 9.24$; $P = 0.000003$) (Fig.11). The same results were acquired in t-tests using untransformed data.

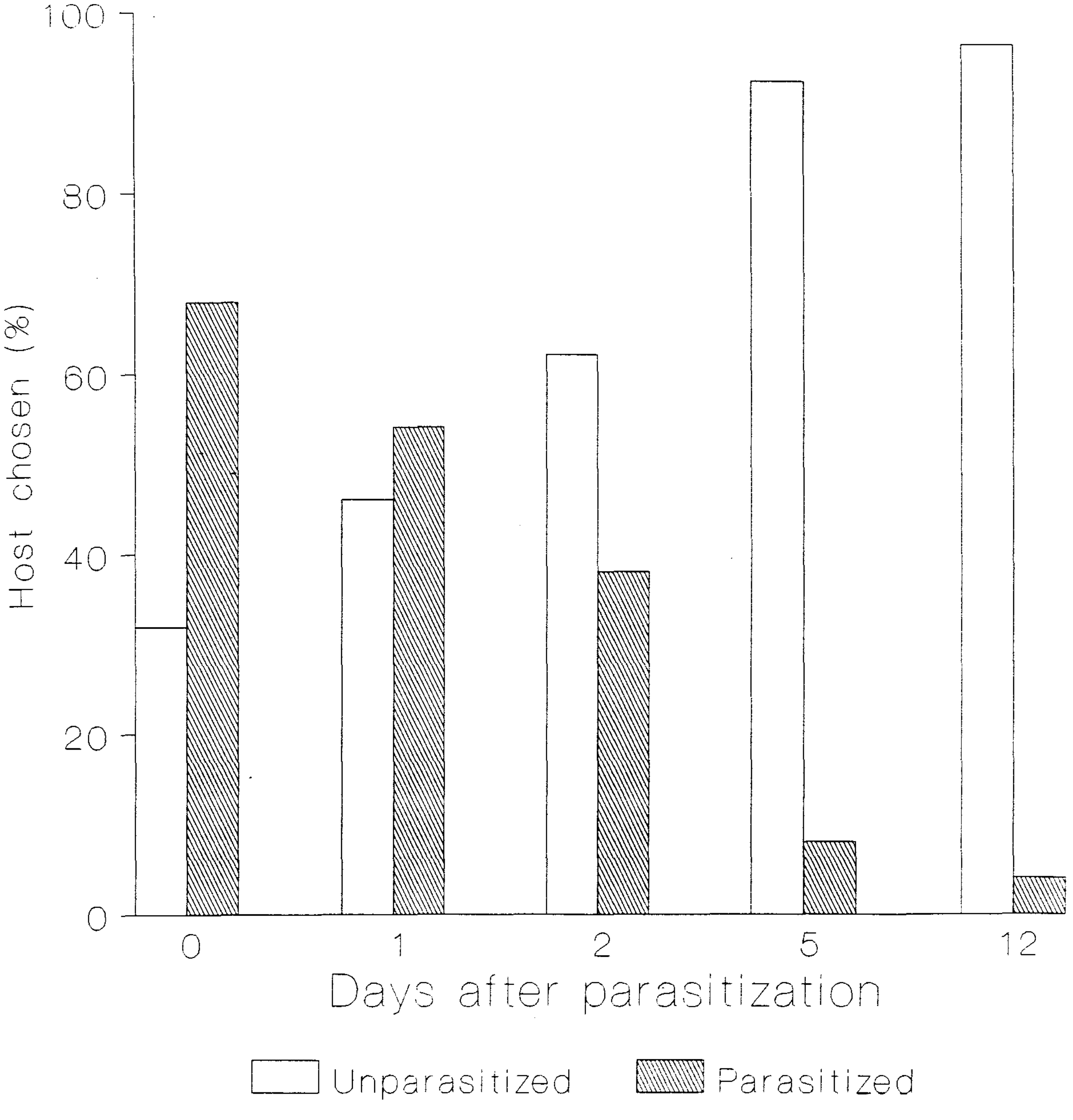

Fig.11 Discrimination between parasitized and unparasitized H.armigera pupae, by T.howardi.

Host discrimination appears to be common among the parasitic Hymenoptera (Eisjackers & Van Lenteren 1969; Vinson 1976). This differentiation may result from an odour left on the host by the parasite which first contacts it (Flanders 1951), or from an injected secretion (Vinson 1976). However, this is probably unlikely in the case of the gregarious T. howardi, as host pupae when placed in the parasitoid cages, are attacked continually and simultaneously by numerous individuals. If such an odour is transferred by the parasitoid to the host it is normally detectable within seconds to minutes (Van Lenteren 1976), whereas T. howardi appears to start discriminating against parasitised hosts only 2 days after initial parasitization.

A peculiar phenomenon is the apparent preference for parasitized hosts during the first day after parasitization. No explanation could be found for this.

RELATIONSHIP WITH Cotesia sesamiae

Because Cotesia sesamiae Cameron (Hymenoptera: Braconidae) is the most abundant and effective natural enemy against C. partellus and B. fusca in the field (Van Rensburg et al. 1988; Kfir 1990a), it was important to determine if T. howardi is able to attack C. sesamiae.

Materials and Methods

Twenty C. sesamiae cocoons were placed in a Petri dish in a T. howardi cage for approximately 24 hrs. The cocoons were removed and placed singly in glass vials. All emergence was recorded. Unemerged cocoons were dissected.

In another experiment 2 C. sesamiae cocoons were placed in each of 30 Petri dishes, with 1 mated T. howardi female in each Petri dish. Observations were made every 10 minutes for 8 hrs, and if any parasitoid was positioned on a cocoon, this was noted. This experiment was run on 2 consecutive days. The cocoons were then left with the parasitoids for a further day. Cocoons were then placed separately in glass vials and all emergence was recorded. Unemerged cocoons were dissected.

Results and Discussion

Of the 20 cocoons placed in the T. howardi cage, only 2 emerged as C. sesamiae adults. The remaining cocoons were dissected and 13 C. sesamiae adults and 4 C. sesamiae pupae were found, as well as 1 T. howardi larva in a C. sesamiae pupa.

In the first 8 hrs of observation only 3 of the 30 T. howardi females were observed ovipositing. On the second day only 2 parasitoids appeared to attack cocoons. From the 60 cocoons, 50 C. sesamiae adults emerged. Dissection showed 6 C. sesamiae adults unemerged and 4 C. sesamiae pupae.

It is possible that some of the C. sesamiae individuals may not have completed development, or emerged as adults, as a result of stinging or oviposition by T. howardi. In a cage of about 8000 T. howardi adults without any option, it is not surprising that C. sesamiae cocoons were attacked. There is an urge to lay eggs, and under such circumstances females have been seen to probe even into plastic and cotton wool. However, it appears most improbable that *C. sesamiae* will be attacked by *T. howardi* in the field.

CHAPTER 5

DEVELOPMENT

Materials and Methods

Weekly, H. armigera pupae were presented to T. howardi in cages. The hosts were removed after a few hours and placed separately in vials which were kept under laboratory conditions (24 ± 2°C and 60 ± 10% RH). Dates of parasitization and dates of emergence of adults were recorded.

One hundred H. armigera pupae were placed in a cage of T. howardi for 4 hrs from 10h00 to 14h00. On each subsequent day 5 pupae were dissected under a microscope with resolution 80 X. Observations of T. howardi development were recorded. This was continued until emergence of adult parasitoids from the pupae. This experiment was conducted twice.

Results

In total 177 host pupae were used in the first experiment. Development time ranged from 18 to 26 days, averaging at 20.7 ± 0.13 (x ± SE) days.

Daily growth and development of T. howardi from time of deposition was recorded when development time was no more than 18 days.

Day 1

Eggs were transparent elongate locules, ranging in size from 0.25 - 0.26 X 0.11 - 0.13 mm.

Day 2

More locules than were observed on the first day were seen. They ranged in size from 0.28 - 0.30 X 0.10 - 0.11 mm.

Day 3

Larvae were observed for the first time, surrounded by an extra-corporeal locule. The locule measured 0.53 - 0.65 X 0.15 - 0.19 mm, and the larval body, 0.41 - 0.53 X 0.12 - 0.15 mm.

Day 4

Larvae appeared light brown in colour, each still embodied in a locule. In the larger larvae segmentation could be seen at one end, transparent in colour. The locule measured 0.83 - 1.63 X 0.18 - 0.35 mm, and the larval body measured 0.68 - 1.25 X 0.15 - 0.33 mm.

Day 5

Extra-corporeal locules were no longer evident. Larvae measured 1.45 - 2.00 X 0.30 - 0.53 mm.

Day 6

Larvae measured 1.50 - 2.10 X 0.35 - 0.60 mm.

Day 7

Larvae were grey to light brown to pink in colour. A few larvae were colourless and transparent, and their internal organs could be seen. The size was 1.18 - 2.23 X 0.38 - 0.68 mm.

Day 8

Larvae were light brown, dark brown, pinkish, or white, and measured 1.53 - 2.50 X 0.50 - 0.68 mm.

Day 9

Mainly larvae, which were white, but also a few white prepupae. Larvae were 1.53 - 2.52 X 0.50 - 0.67 mm, whereas prepupae were a bit smaller: 1.33 - 2.10 X 0.38 - 0.60 mm.

Day 10

Almost all prepupae: 1.40 - 2.20 X 0.50 - 0.75 mm.

Day 11

Prepupae measured 1.30 - 2.03 X 0.48 - 0.68 mm.

Day 12

Prepupae became pupae at about this stage. Pupae were white with orange-red eyes. They measured the same size as the prepupae did on the previous day. A couple of larvae were observed.

Day 13

Pupae had dark red eyes and triangular red marks on their vertexes. They were the same size as the previous 2 days.

Day 14

A few pupae could be differentiated as males, by antennal shape, and were greyish in colour. These measured 1.30 - 1.45 X 0.48 - 0.52 mm. The other pupae, which must have included males and females, were 1.40 - 2.10 X 0.48 - 0.70 mm in size.

Day 15

Male pupae were black and measured 1.40 - 1.48 X 0.50 - 0.53 mm. Female pupae were white with red eyes and mouthparts, or abdominally grey with a paler head and thorax, appearing further developed than the white pupae. The females were 1.83 - 2.13 X 0.60 - 0.75 mm.

Day 16

All pupae were black, measuring approximately the same size as the previous day. One or two fully developed adults were observed.

Day 17

Adults emerged from some of the hosts.

Day 18

Adults emerged from remaining hosts.

Growth during development of the parasitoid reached a plateau around the seventh or eighth day. The size of the pupa was considerably smaller than that of the larva and there appeared to be no real growth in pupal size to adulthood (Fig.12).

T. howardi's life-cycle consists of 5 stages including incubation period, larval stage (number of instars is not known), prepupal and pupal stages, and the adult stage (Table 5).

Table 5. Developmental stages and their duration for T. howardi at 24 ± 2°C.

Developmental Stage	Approximate Duration (Days)
Incubation period	2
Larval stage	6½
Prepupal stage	2½
Pupal stage	6½
Total	17½

Discussion

Development time of Tetrastichus sokolowskii Kurdj. is from 13 to 19 days (Ooi 1988), for T. flavigaster Brothers & Moran, from 17 to 20 days (Moran et al. 1969), and for Tetrastichus sp. (near atriclavus Waterst.), 16 to 27 days (Moutia & Courtois 1952). Therefore, duration of development of T. howardi is not atypical for the genus, Clausen (1940) recording it to be between 15 and 25 days for the subfamily. Development time is dependent on temperature (Moutia &

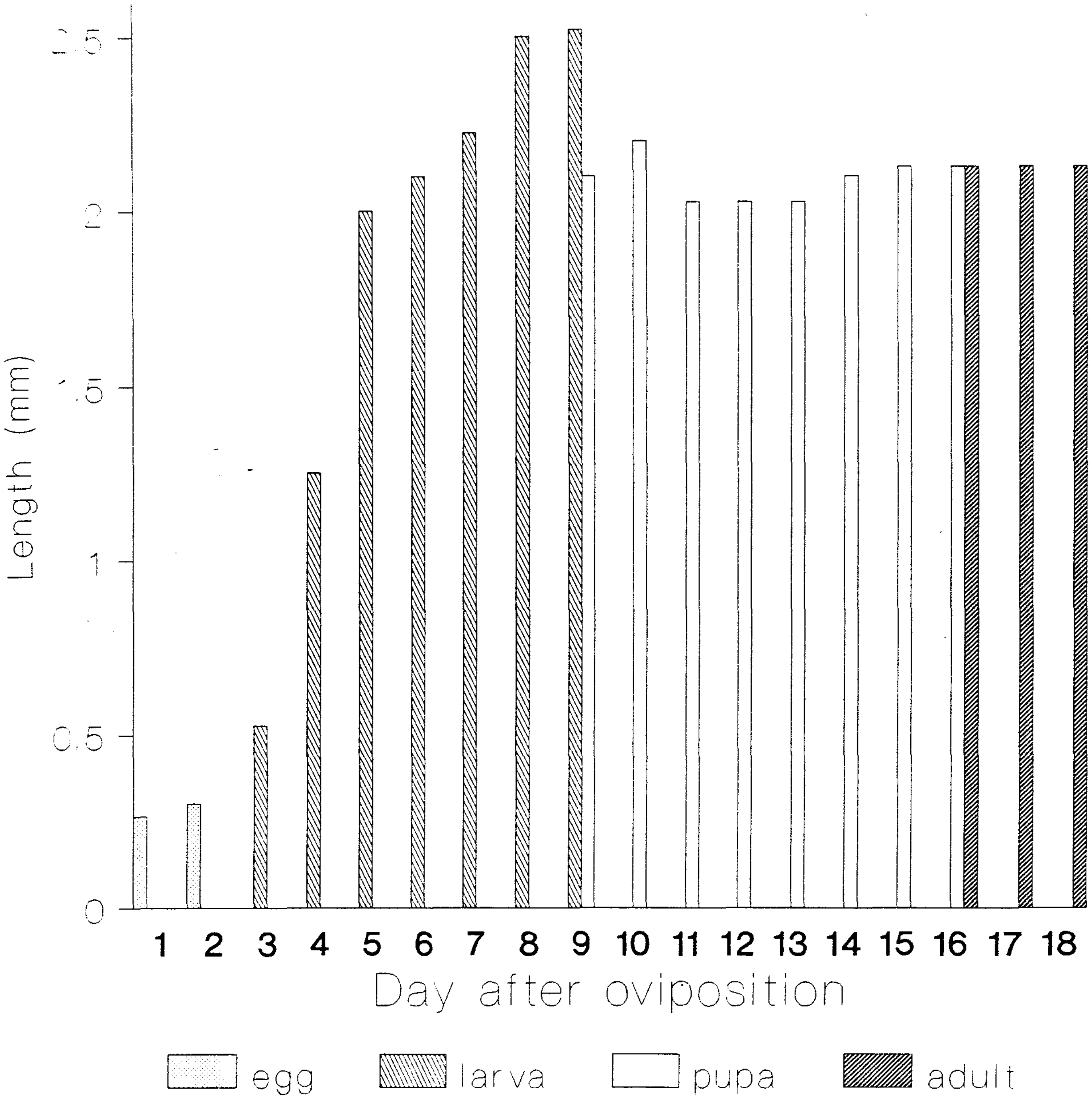

Fig.12 Growth of T.howardi, using maximum length observed.

Courtois 1952; Kfir *et al.* 1993).

Very similar developmental sequences were found for *T. howardi* in other studies (Cherian & Subramaniam 1940; Rudriah & Sastry 1959).

CHAPTER 6

EMERGENCE

EMERGENCE TIMES

Materials and Methods

H. armigera pupae were exposed to large numbers of *T. howardi* in the cages for a few hours. The parasitized hosts were then removed and placed separately in sealed glass vials and kept in the laboratory. This was repeated 2 or 3 times a week with 20 to 30 pupae each time. Every day from 07h00 to 16h00 pupae were checked at 15 to 20 minute intervals until all *T. howardi* adults had emerged. A total of 177 pupae from which *T. howardi* adults emerged were observed. Times of emergence were recorded and plotted.

To determine if light was a cue for emergence, 60 parasitized *H. armigera* pupae were placed in an incubator set at 24,5°C and at 13:11 (L:D). Change from dark to light phase occurred at 10h30 so observations could easily be made at this time. After about 2 weeks pupae were checked at 15 to 20 minute intervals until all *T. howardi* adults had emerged. Times of emergence were recorded and plotted as time elapsed between start of light phase and emergence of adults. This time was compared to that between sunrise (from 6 August to 27 September) and emergence from those pupae kept in the laboratory. Sunlight could enter the laboratory through a large window.

Results and Discussion

Time of emergence of _T. howardi_ adults ranged from before 07h00 until 15h00 (Fig.13). It was assumed that emergence only began after sunrise. Mean sunrise time between the dates during which the experiment was conducted was 06h17. Therefore the 27 pupae (15%) which gave rise to _T. howardi_ adults before 07h00 were assumed to have emerged around 06h30. Consequently, mean time of emergence was 08h59 ± 9.24 minutes (x ± SE), occurring 2 hrs 42 mins after mean time of sunrise. By 10h30 83% of all parasitoids had emerged.

In the incubator, mean time of emergence was 2 hrs 26 mins ± 10.11 mins (x ± SE) after start of the light phase, ranging from 40 mins to 6 hrs (Fig.14). After 3 hrs 30 mins of light, 81% of parasitoids had emerged, and 88% after 4 hours.

The results from the incubator confirmed that emergence of adult _T. howardi_ only begins after first light. Mean time of emergence after first light was slightly less in the incubator than in the laboratory. This was probably because light in the incubator was instantaneously bright, whereas the laboratory was illuminated gradually by the rising sun.

Earlier research confirms this trend of emergence after first light (Cherian & Subramaniam 1940), which is a habit also observed in the related _Tetrastichus sesamiae_ Risbec, known to emerge mainly between 08h00 and 09h00 (Okeyo-Owuor _et al._ 1991). Males generally emerged several minutes before the females, also previously observed in _Tetrastichus_ sp. (Moutia & Courtois 1952).

EMERGENCE AND MORTALITY RATES

The aim of this experiment was to determine what proportion of

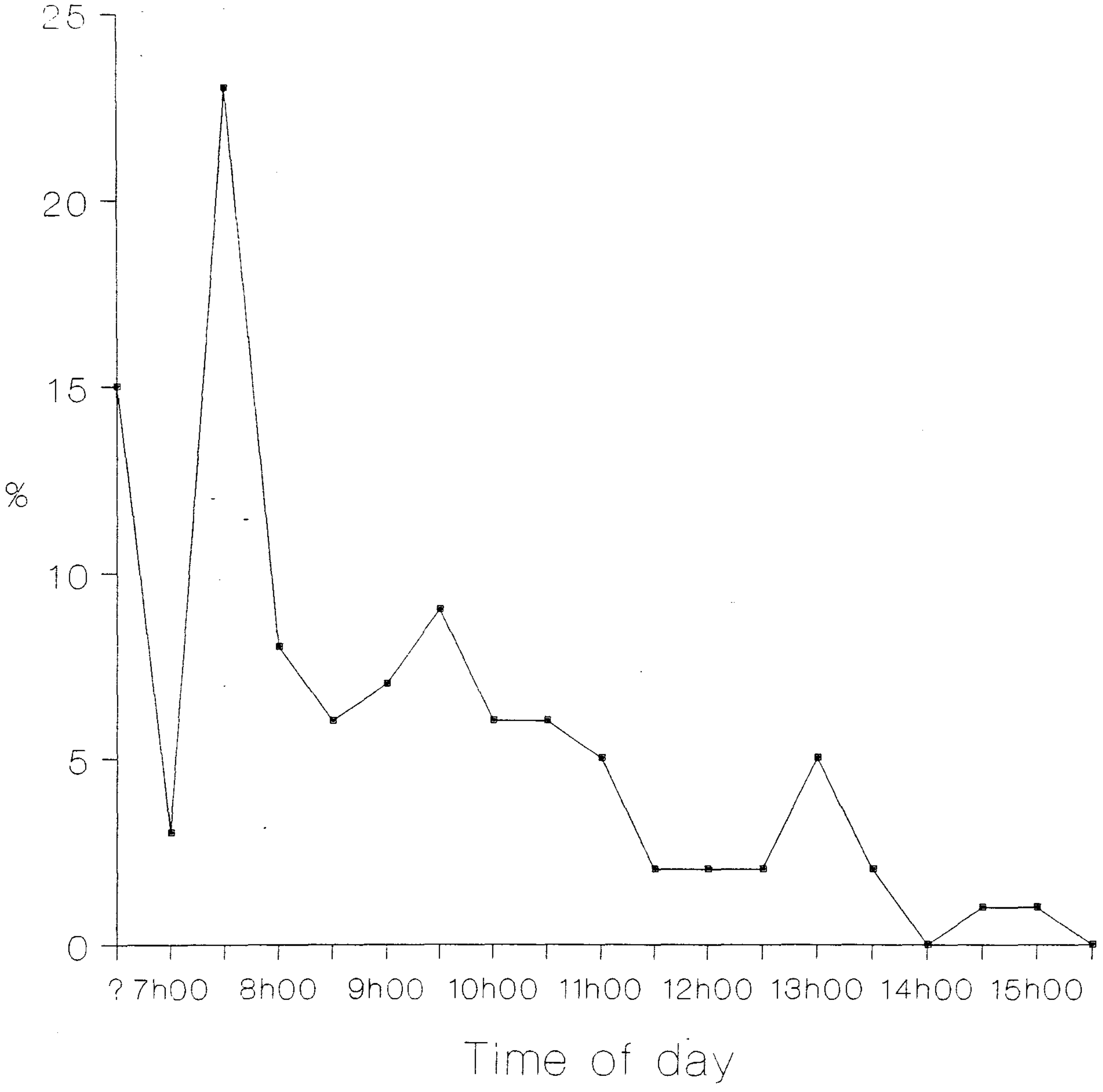

Fig.13 Times of emergence of *T.howardi* from *H.armigera* in the laboratory (? = 16h00 - 07h00).

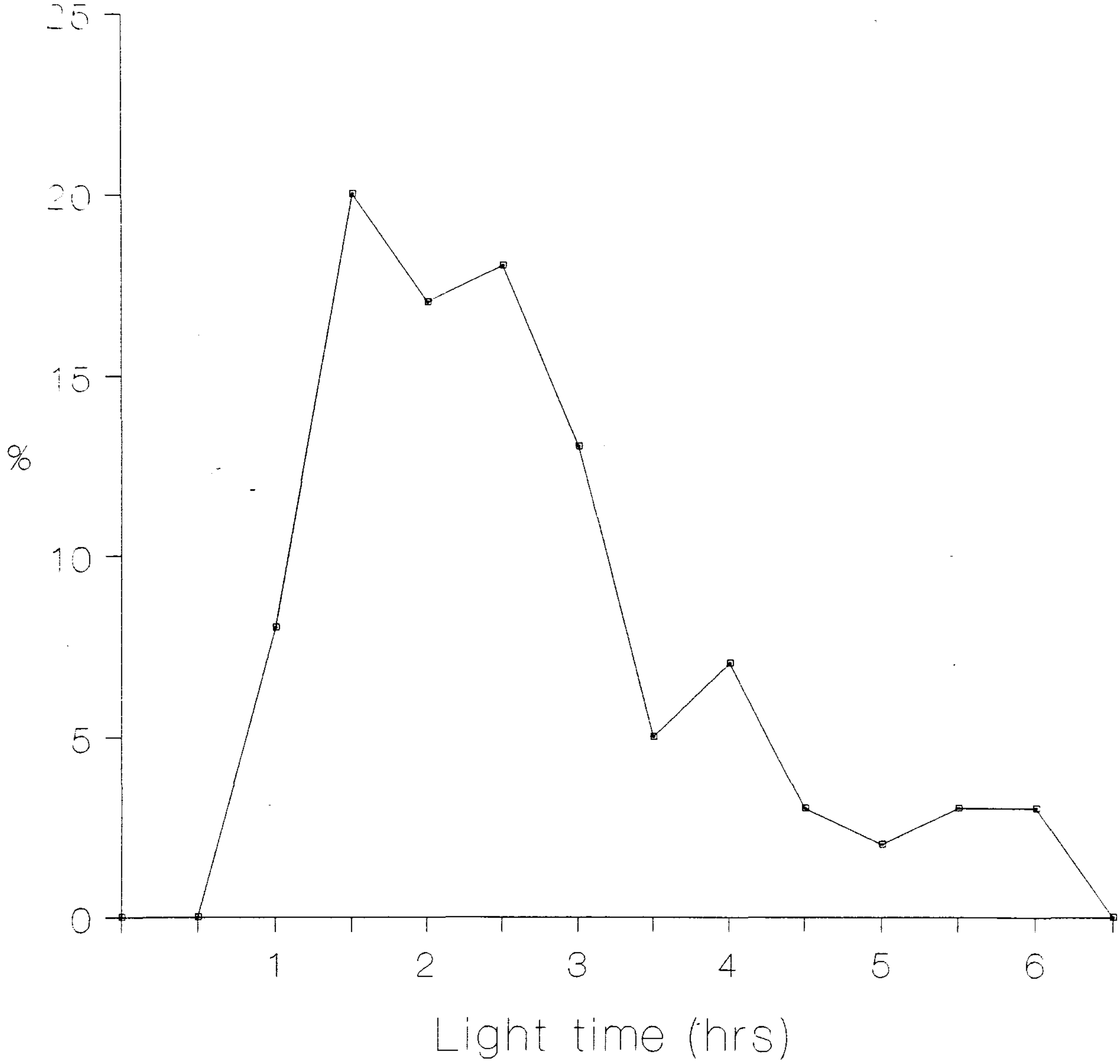

Fig.14 Times of emergence of T.howardi from H.armigera in incubator with L:D = 13:11.

parasitized hosts gave rise to T. howardi adults, and if this differed between different host species, and what were the comparative mortality rates of the hosts as a result of parasitoid attack.

Materials and Methods

Host pupae were presented individually to individual mated T. howardi females in glass vials. All females were about 2 days old. The parasitoids were observed and only parasitized hosts were taken. After oviposition, parasitoid females were removed from vials and the pupae were kept to check if emergence of parasitoids would occur. If no emergence occurred, pupae were dissected to determine if any development of parasitoids had taken place. This was repeated several times with each of 4 hosts: C. partellus, H. armigera, E. saccharina and P. laxa, using 15 to 20 hosts each time.

The experiments were repeated with all 4 host pupae, this time presenting pupae to large numbers of T. howardi in the wooden cages. In total, 705 host pupae were used.

Chi-square tests were applied to the results to determine if there were any significant differences of proportion of parasitized pupae giving rise to T. howardi adults and of overall mortality rates, between different host species and between singly parasitized and superparasitized hosts.

Results and Discussion

When parasitized by individual parasitoids there was a higher emergence rate from pupae than when they were superparasitized (Fig.15)(Table 6), however according to the Chi-square test, this was only significant for *H. armigera* (P = 0.002). A Chi-square test comparing mortality rates of hosts showed no significance because there were a few counts of less than 3. The differences in mortality rates were however not as great as the differences in emergence rates (Table 6).

When individually parasitized, emergence rate was significantly higher from *C. partellus* pupae than from *P. laxa* (x^2 = 114.47, P = 0.002), higher from *H. armigera* than from *E. saccharina* (x^2 = 22.60, P = 0.002) and from *P. laxa* (x^2 = 209.67, P = 0.002), and higher from *E. saccharina* than from *P. laxa* (x^2 = 13.32, P = 0.002).

When superparasitized, emergence rate was significantly higher from *C. partellus* pupae than from *P. laxa* (x^2 = 78.54, P = 0.002), higher from *H. armigera* than from *P. laxa* (x^2 = 162.52, P = 0.002), and higher from *E. saccharina* than from *P. laxa* (x^2 = 38.66, P = 0.002) (Fig.15).

Hosts' suitability may vary for a parasitoid (Doutt 1959). *T. sesamiae*, like *T. howardi*, has a wide host range. However, emergence rate of *T. sesamiae* from parasitized pupae differs from host to host, even amongst stem boring Lepidoptera (Okeyo-Owuor *et al.* 1991). For *N. vitripennis* percentages of emergence also differed according to the host species (Ohgushi 1960).

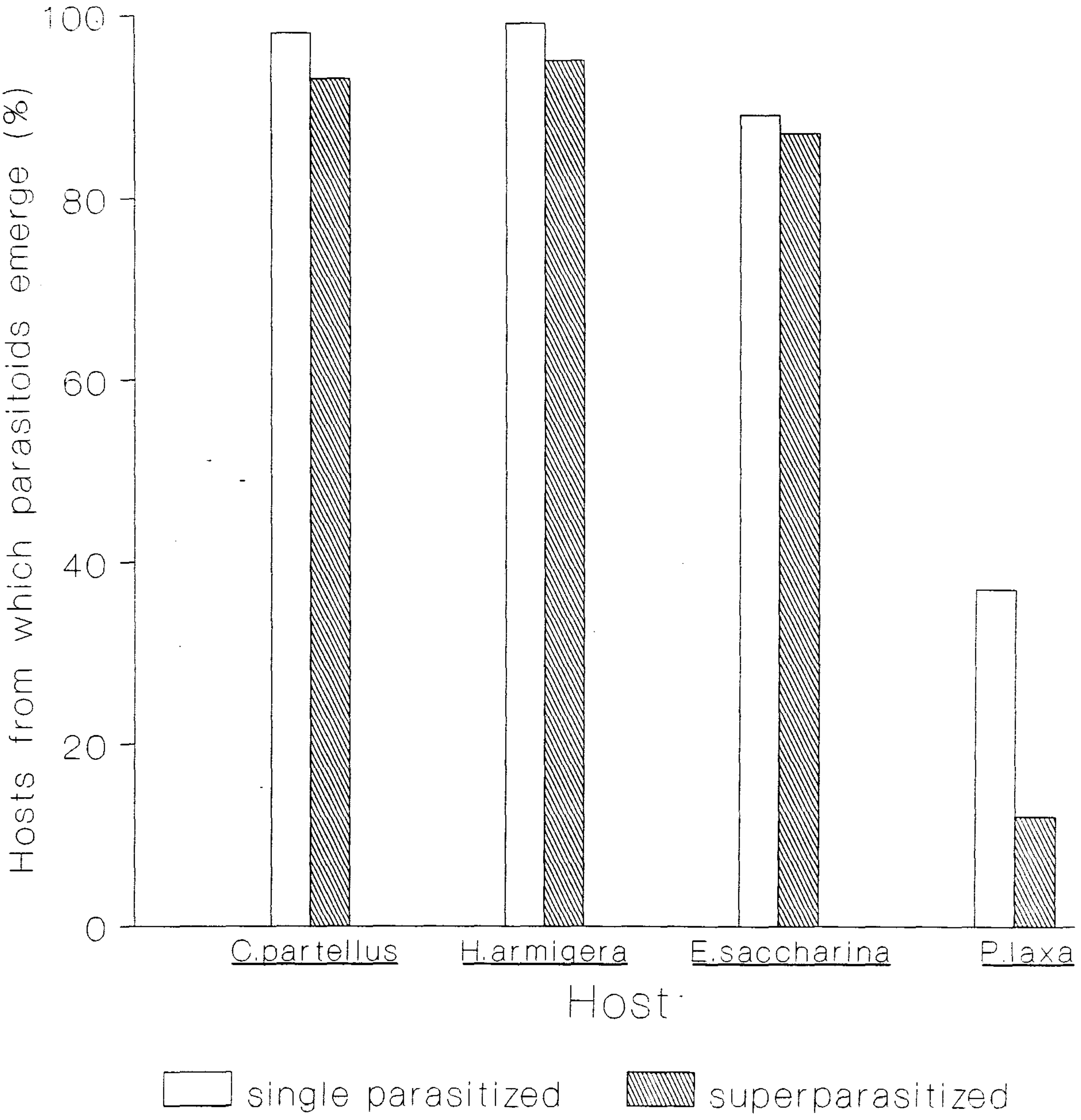

Fig.15 Percentage of T.howardi parasitized host pupae giving rise to parasitoids, and the difference between singly and superparasitized pupae.

Table 6. Emergence rates of *T. howardi* from singly parasitized and superparasitized hosts, development without emergence, and total mortality of hosts.

Parasitization	Host	n	% Mortality	% Hosts, parasitoids emerging from	% Unemerged larvae	adults
single	*C.p.*	81	100	97.53	0	0
	H.a.	102	100	99.02	0	0
	E.s.	21	90.48	80.95	4.76	0
	P.l.	65	87.69	32.31	9.23	18.46
super	*C.p.*	93	93.55	87.10	2.15	0
	H.a.	255	96.86	91.76	0.39	0.78
	E.s.	34	91.18	79.41	5.88	0
	P.l.	54	92.59	11.11	14.81	40.74

C.p. = *C. partellus*, *H.a.* = *H. armigera*, *E.s.* = *E. saccharina*, *P.l.* = *P. laxa*.

Mortality rates of hosts always exceeded *T. howardi* emergence rates in these experiments, but only significantly so for *P. laxa*, both in the singly parasitized (t = -6.27, P = 0.003) and superparasitized (t = -53.89, P = 0.0000007) pupae. The dipteran puparia were obviously far less suitable hosts for *T. howardi* than the lepidopteran pupae. The large difference between the mortality rate of, and parasitoid emergence rate from the puparia was due to two reasons: the inability of *T. howardi* to complete development, probably because of physiological unsuitability of the host (Ohgushi 1960); the inability of fully developed adults to emerge. *Disochaeta* sp., also a tetrastichine, as a hyperparasite of dipteran puparia, was unable to make an emergence hole in the puparium wall and consequently died (Clausen 1940).

EMERGENCE NUMBERS AND SEX RATIO

In earlier work, the fertility of T. howardi females was found to be 101 progeny with 92% females when reared on H. armigera (Kfir et al. 1993). The aim of this experiment was to determine numbers and sex ratio of T. howardi emerging from different hosts, when parasitised by individual parasitoid females and when superparasitized.

Materials and Methods

C. partellus, H. armigera, and E. saccharina pupae, and P. laxa puparia were presented individually to individual mated T. howardi females in vials. The host pupae were left with the parasitoids for about 24 hours. The parasitoids were then removed, and the pupae retained in the vials until emergence of parasitoids. After the emerged parasitoids had died they were counted and sexed for each host pupa.

A similar experiment was conducted with the same hosts. This time host pupae were presented to T. howardi in cages and so were superparasitized.

The number of parasitoids emerging from each host species, according to whether the pupa had been singly parasitized or superparasitized, was recorded. Before the means were compared the data was subjected to Taylor's Power Law to determine the distribution pattern and to calculate the relevant transformation. Data was transformed and ANOVAs were performed on it, followed by an LSD multiple range test.

A Chi-square test was used to determine if there were any significant differences in sex ratios (expressed as percentage females but using total numbers for the Chi-square) of the emergent parasitoids from the different host species, and between those hosts singly parasitized and superparasitized.

A regression analysis was applied to the data to determine if there was any significant correlation between total number of emergent parasitoids and the sex ratio.

Results and Discussion

The results are presented in Table 7. Pooling data (x) for singly parasitized pupae and for super-parasitized pupae separately, Taylor's Power Law revealed a clumped distribution for both (Θ = 69,90° and 52,92° respectively). Consequently a log x transformation was applied to the data (Elliott 1983). An ANOVA revealed a significant difference in the mean numbers of parasitoids emerging from the singly parasitized hosts (F-ratio = 8.95; $P < 0.0001$), and the super-parasitized hosts (F-ratio = 34.38; $P < 0.00001$). LSD multiple range tests showed that the emergence number from singly parasitized _P_. _laxa_ pupae was significantly different from the other hosts, which were not significantly different from one another. Numbers emerging from superparasitized _P_. _laxa_ puparia were significantly different for _P_. _laxa_ from the other 3 hosts, and from

E. *saccharina* also significantly different from the other 3 hosts. Numbers from *C*. *partellus* and *H*. *armigera* were not significantly different from each other.

Table 7. Numbers and sex ratios of *T*. *howardi* emerging from various parasitized and superparasitized hosts.

Parasit-ization	Host	n	x parasites emerg/host	SE	Signif. diffs. in x	% Females	Signif. diffs. in % F
single	*C*.*p*.	21	55.48	6.48	a*	94.37	a*
	H.*a*.	20	55.55	8.32	a	94.69	a
	E.*s*.	10	55.60	8.66	a	94.06	a
	P.*l*.	8	15.25	2.30	b	91.80	a
super	*C*.*p*.	12	342.00	23.01	c	89.42	b
	H.*a*.	30	392.27	41.16	c	81.73	c
	E.*s*.	9	124.22	33.01	d	75.76	d
	P.*l*.	15	37.13	7.78	e	75.22	d

C.*p*. = *C*. *partellus*, *H*.*a*. = *H*. *armigera*, *E*.*s*. = *E*. *saccharina*, *P*.*l*. = *P*. *laxa*.

* Values followed by the same letter are not significantly different ($P < 0.0001$, LSD multiple range test for x; $P < 0.05$, Chi-square test for % Females).

A Chi-square test revealed that there was a significant difference in sex ratios ($x^2 = 466.38$; $P < 0.00001$). There was no significant difference in sex ratio between parasitoids emerging from different singly parasitized host species (Table 7). Several of the differences in sex ratio observed for parasitoids emerging from the various superparasitized host species were significantly different, as well as the differences between singly and superparasitized hosts (Table 7).

The lower numbers of _T_. _howardi_ emerging from _P_. _laxa_ was due to the comparatively smaller size of the pupae. The sex ratio of emergent parasitoids being significantly lower from _P_. _laxa_ than from the other three hosts, confirmed that it was a less suitable host. Consequently the results from _P_. _laxa_ were excluded from the regression analysis in which percentage of females was correlated with total number. This revealed a strongly significant correlation ($r = 0.05$; $F1,100 = 34.15$; $P < 0.00001$) (Fig.16).

The number of individuals that develop in a single pupa varies according to the size of the pupa (Moutia & Courtois 1952; Okeyo-Owuor _et al._ 1991). This has been found in certain parasitoids, such as the eulophid _E_. _kuwanae_, to be a result of fewer eggs being oviposited on smaller hosts (Uematsu 1981). If this is true in _T_. _howardi_, it is probably only to a limited extent, as _T_. _howardi_ is highly gregarious and superparasitization often occurs in the laboratory. It is more likely that the lesser number of adult parasites emerging in smaller pupae is due to deficiency of food material for the developing larvae (Rudriah & Sastry 1959).

The sex ratio shows a marked preponderance of females, which is characteristic of the tetrastichine subfamily (Clausen 1940). Amongst hymenopteran parasites, there are many cases where mostly females emerge from large host individuals and mostly males from small hosts (Doutt 1959). Uematsu (1981) believes that parasitoids may have the ability to modify the sex ratio of the progeny according

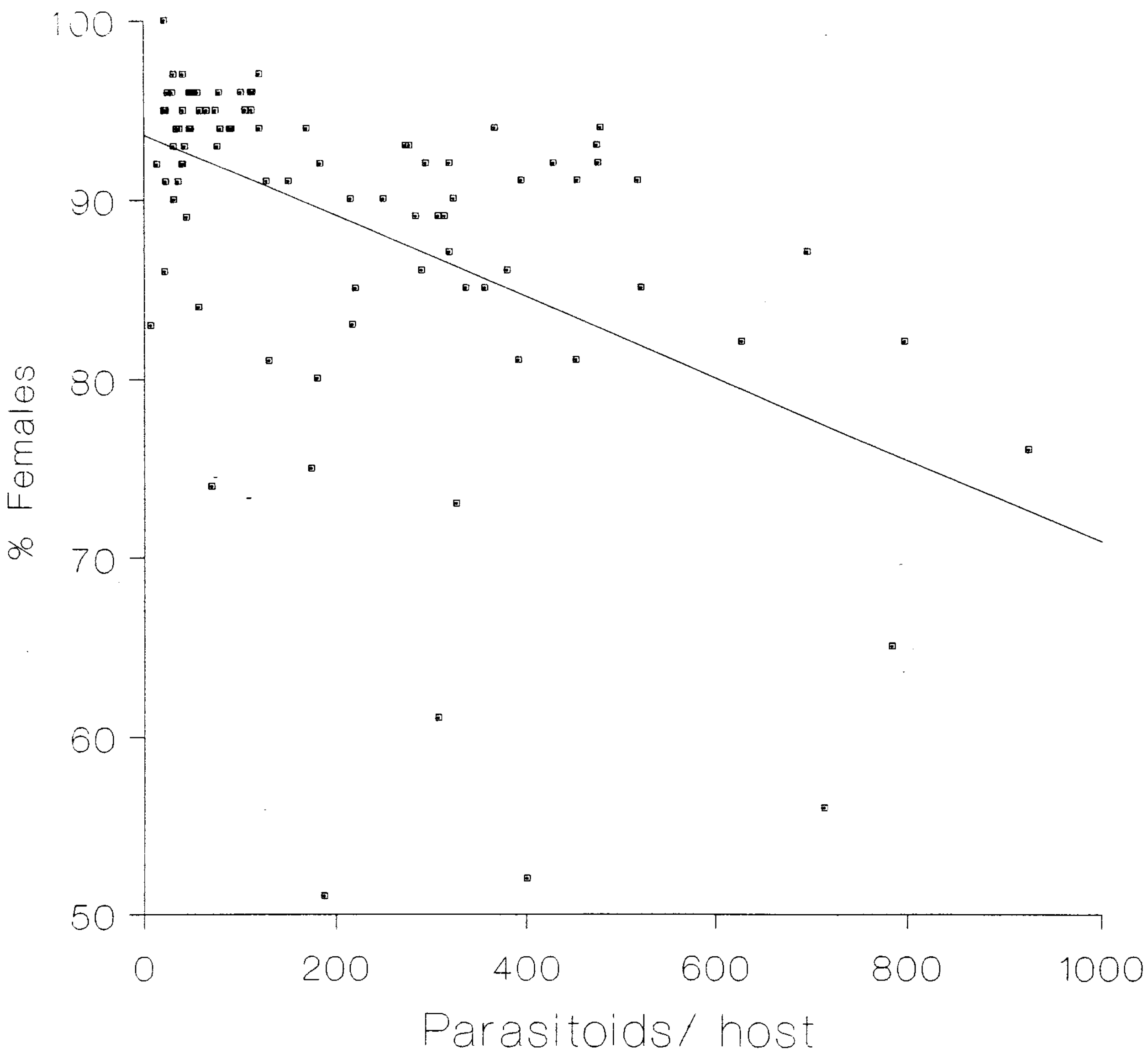

Fig.16 Relationship between number of *T.howardi* emerging per host pupa, and percentage females emerging ($y = 93.82 - 0.02x$; $r = 0.05$; $P < 0.00001$).

to the host size. This would allow more efficient utilization of host resources, as males are smaller than females and therefore require less energy to mature.

INFLUENCE OF FEMALE SIZE ON FERTILITY AND SEX RATIO

Materials and Methods

Female parasitoids were separated into large and small females. Each group consisted of 40 and 50 individuals respectively. The mass of each group was measured on a digital scale, sensitive to 10^{-4} g (four decimal places of a gram), and divided by 40 and 50 respectively, to get the mean mass of individual per group. It was not possible to measure the mass of individual parasitoids as the digital scale was not sensitive enough. The mated females were then each presented with an H. armigera pupa for 24 hours. The pupae were then retained individually in marked vials until emergence of parasitoids. Parasitoids were counted and sexed. Mean number of individuals emerging, was then calculated for each group. Means were compared using a t-test on transformed data. Sex ratio of the two groups was compared with each other using a Chi-square test.

In a second experiment, female T. howardi of varying sizes, were placed individually with H. armigera pupae for 24 hours. Pupae were then removed and placed in marked vials. Females' body lengths and head widths were measured using a dissecting microscope with calibrated eye piece. When T. howardi emerged from parasitised pupae, they were counted and sexed. Numbers and percent females emerged were then each correlated with both body length and head width of parent female in turn, using regression analyses.

Results and Discussion

In the first experiment T. howardi emerged from only 17 pupae from each group (Table 8). Fertility proved to be significantly greater for the larger females (mean mass = 5.625×10^{-4}g) than for the smaller females (mean mass = 5×10^{-5}g). This was indicated by a t-test ($t = 7.22$; $P < 0.05$) performed on the transformed data. There was also a significant difference in the sex ratios (Table 8) of the two groups ($x^2 = 5.12$; $P = 0.02$).

Table 8. Fertility and sex ratio of large and small T. howardi females.

Female Size	n	Fertility	SE	% Females
Large	17	77.00	5.08	95.58
Small	17	32.71	2.93	92.17

In the second experiment parasitoids emerged from 20 of the pupae. Significant correlations were found between numbers emerging and body length of parent female ($r = 0.07$; $P = 0.0003$) (Fig.17), numbers emerging and head width of parent female ($r = 0.07$; $P = 0.001$) (Fig.18), percentage of females emerging and body length of parent female ($r = 0.06$; $P = 0.01$) (Fig.19), and percentage of females emerging and head width of parent female ($r = 0.06$; $P = 0.007$)

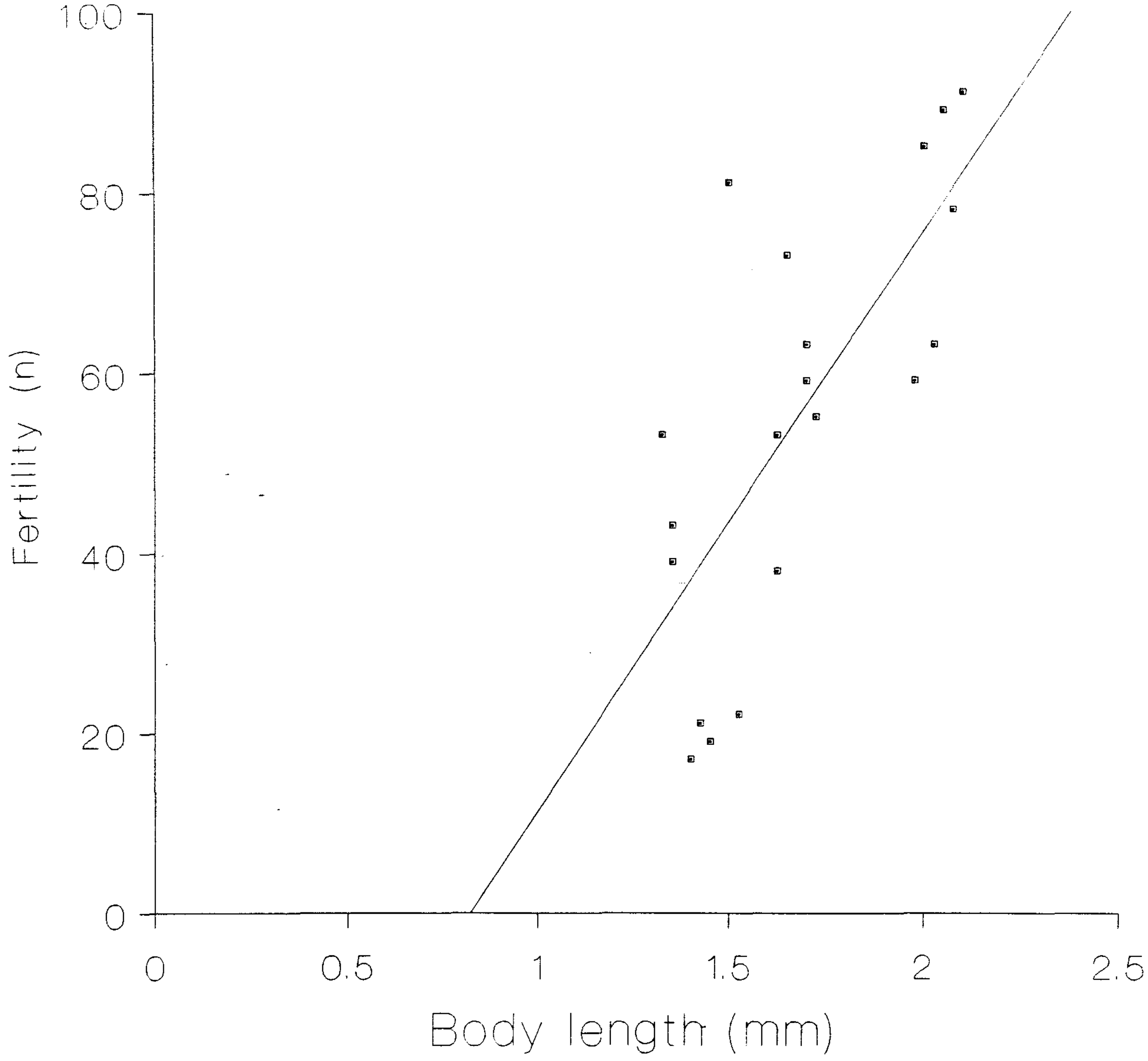

Fig.17 Relationship between body length of female T.howardi and fertility (y = -50.77 + 62.94x); r = 0.07; P = 0.0003).

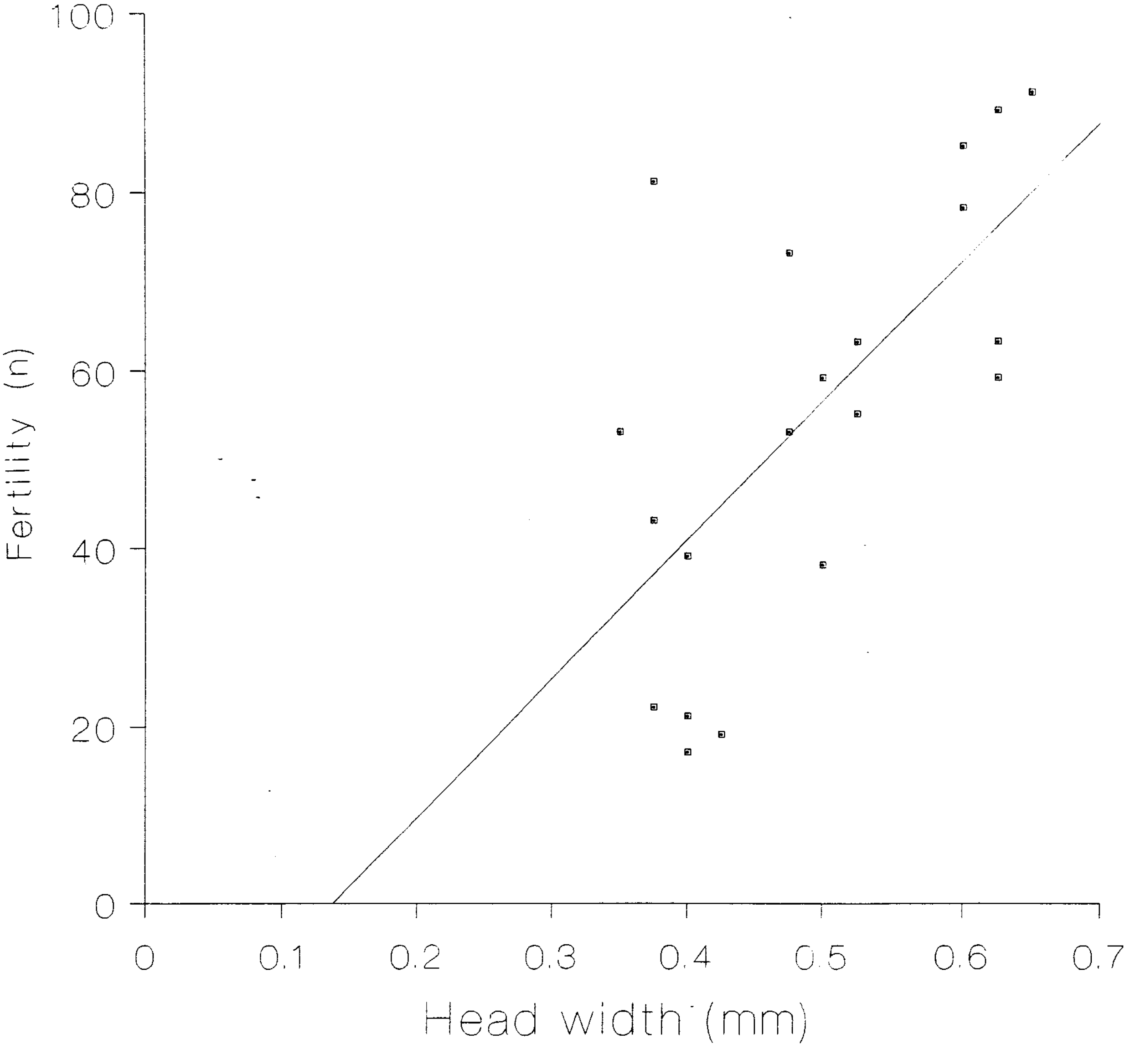

Fig.18 Relationship between head width of female T.howardi and fertility ($y = -21.48 + 155.80x$; $r = 0.066$; $P = 0.001$).

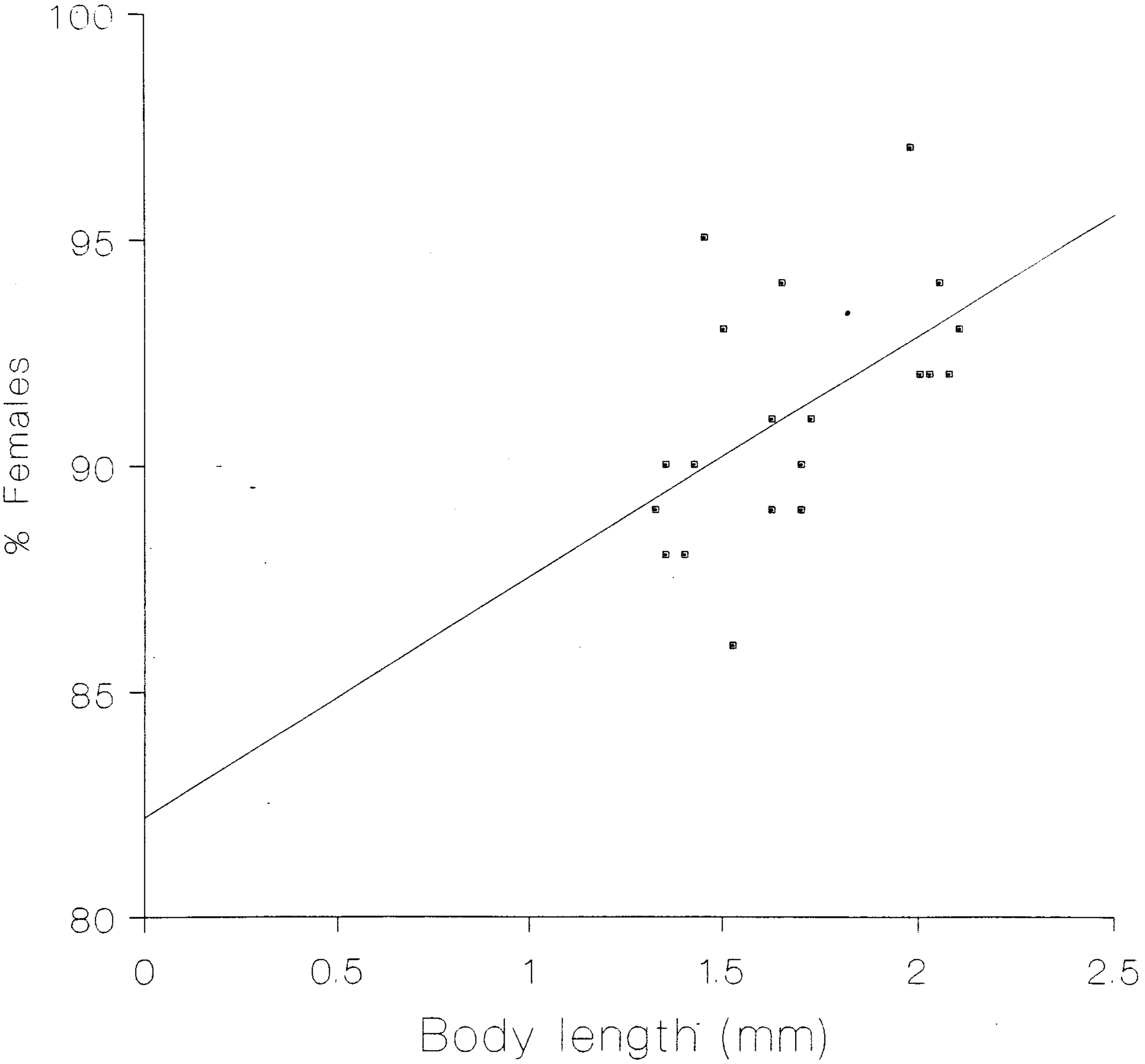

Fig.19 Relationship between body length of female T.howardi and sex ratio (y = 83.76 + 15.15x; r = 0.056; P = 0.01).

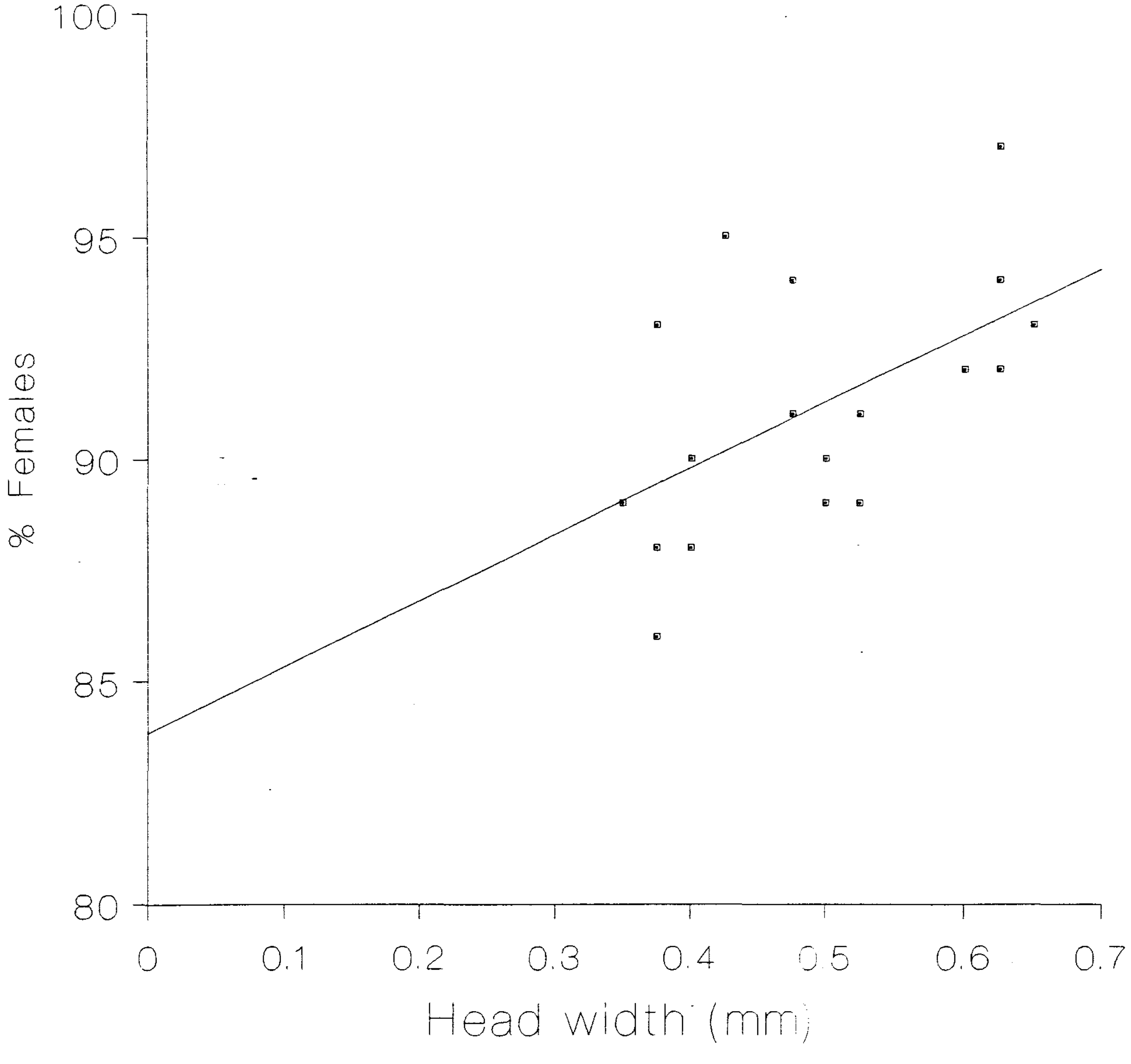

Fig.20 Relationship between head width of female *T.howardi* and sex ratio ($y = 83.76 + 15.15x$; $r = 0.058$; $P = 0.007$).

(Fig.20).

In Tetrastichus incertus Ratzeburg, fecundity and clutch size were highly correlated with the weight of the female adult (Pitcairn & Gutierrez 1992). Pitcairn and Gutierrez (1992) also concluded that the fitness of T. incertus females is increased by being large. If male eggs are a smaller reproductive investment than female eggs, then this could explain the difference observed in sex ratio.

INFLUENCE OF HOST AGE ON FERTILITY AND SEX RATIO

Materials and Methods

C. partellus pupae of different ages, 1 - 5 days old, were presented in numbers of 14 to 20 each, to T. howardi in cages. They were removed the following day, having been exposed to parasitization for ± 17 hours. The pupae were kept singly in sealed and marked vials. Numbers and sex ratios of individuals emerging were recorded.

A similar experiment was conducted using H. armigera pupae of 1 - 4 days old.

Another similar experiment with H. armigera was conducted, this time exposing the pupae to individual T. howardi females for 24 hours.

To determine if there was any significant difference in mean numbers emerging from hosts of different ages, data (after being transformed if necessary) were subjected to ANOVA tests followed by LSD multiple range tests. Sex ratios were compared with Chi-square tests to check for significant differences.

Results and Discussion

Number of T. howardi emerging from both hosts, showed an overall decrease from 1 day old pupae to the oldest pupae (Table 9). From C. partellus an ANOVA showed significant differences between days (F-ratio = 4.07; P = 0.005) (Table 9). This was less significant for H. armigera (F-ratio = 1.90; P = 0.1).

The parasitoid sex-ratios from the C. partellus pupae were not taken into consideration as they were completely abnormal, probably due to gross superparasitization. However, a Chi-square test did show a significant decrease in percentage females emerging from H. armigera pupae according to increased age (x^2 = 6.98; P = 0.008) (Table 9).

There were no significant differences in numbers (F-ratio = 0.38; P = 0.76) and sex ratio (x^2 = 0.29; P = 0.96) of parasitoids emerging from singly parasitized H. armigera (Table 9).

Table 9. Numbers and sex ratio of *T. howardi* emerging from superparasitized *C. partellus* pupae, and superparasitized and singly parasitized *H. armigera* pupae.

Parasitization	Host	Pupal Age (days)	n	x	SE	Signif. diffs. in x	% Fem.	Signif. diffs. in % F
Super	*C.p.*	1	20	235.30	19.47	a*	–	–
		2	20	223.35	27.00	a	–	–
		3	14	144.36	15.22	b	–	–
		4	14	146.50	18.16	b	–	–
		5	19	144.95	17.07	b	–	–
	H.a.	1	20	468.70	58.89	c	84.45	a*
		2	19	490.95	38.53	d	80.56	b
		3	20	330.20	40.65	c e	79.52	b
		4	16	291.62	32.20	e	75.45	c
Single		1	8	31.50	5.65	f	92.50	d
		2	7	28.00	6.96	f	94.10	d
		3	7	34.00	5.67	f	93.30	d
		4	6	33.83	7.73	f	93.30	d

C.p. = *C. partellus*, *H.a.* = *H. armigera*.

* Values followed by the same letter are not significantly different ($P < 0.05$; LSD multiple range test for x, and Chi-square test for % Fem.).

Mean number of adults developing per host was found to decrease with increasing age of host for both *T. sokolowskii* (Mushtaque 1990) and *T. brevistigma* (Hammerski *et al.* 1990). The reason for this was not known but females could possibly lay fewer eggs in older pupae, or the parasitoid survival could be lower in older pupae (Hammerski *et al.* 1990). Mortality of *Eurytoma* sp. (Hymenoptera: Eurytomidae) increases with age of host, as percentage of females produced decreases (Tagawa & Fukushima 1993). These facts suggest that older hosts are less suitable for *Eurytoma* sp. than are younger ones, even though there was no significant decrease in hosts parasitized. Different stages of host are known to attract oviposition of different gender eggs in the solitary aphelinid parasitoid, *Coccophagus atratus* Compere (Donaldson & Walter 1991). The difference in sex ratio and numbers emerging in *T. howardi* indicate a decreasing suitability of the hosts with increasing age. This is not a factor when the host is parasitized by only a single female and number of eggs laid is well below what the host can support.

CHAPTER 7

LONGEVITY OF ADULT PARASITOIDS

Materials and Methods

T. howardi were separated into 4 groups for comparison. These were:

1st comparison:

1. mated females,
2. mated males,
3. virgin females,
4. virgin males;

2nd comparison:

5. females kept separately,
6. males kept separately,
7. females kept with males,
8. males kept with females;

3rd comparison:

9. females with food and water,
10. males with food and water,
11. females with only water,
12. males with only water,
13. females without food and water,
14. males without food and water;

4th comparison:

15. females with water and hosts,
16. males with water and hosts,
17. females without hosts (with water),
18. males without hosts (with water).

Categories 1 and 5, and 2 and 6 were the same insects, as were categories 7 and 9, 8 and 10, 11 and 17, and 12 and 18. In all experiments males and females were kept together, except categories 1,2,3 and 4 (5 and 6), giving a total of 8 groups. All groups of insects, unless otherwise stated, were fed on honey and water, were not presented with hosts, and were kept in small perspex cages (250 x 160 x 150 mm) ventilated with fine gauze netting. Each category consisted of 8 - 104 insects.

Time of emergence of parasitoids was recorded and soon after they were placed in the cages. Cages were checked twice daily and all dead individuals removed and recorded, until no surviving parasitoids remained.

The data was analyzed using the GLM with the Poisson Distribution. To make the desired comparisons between longevities, means were compared using multiple comparison Bonferroni LSD tests.

Results and Discussion

In all cases, there were significant differences in mean longevity between males and females, under the same conditions (Table 10). Longevity ranged from a mean of 5.43 days for females and 3.09 days for males, to a mean of 52.50 days for females and 28.56 for males, depending on conditions (Table 10).This was significantly longer than was observed in earlier work (Cherian & Subramaniam 1940; Rudriah & Sastry 1959; Kfir et al. 1993).

Table 10. Longevity of _T_. _howardi_ under different conditions.

Category	Sex	n	Longevity (days)	SE	Signif. diffs. in Longevity
ISH	F	83	49.61	1.39	a*
	M	24	27.42	1.04	b
VSH	F	80	52.50	1.30	a
	M	63	24.05	0.94	b
ITH	F	25	47.36	2.60	a
	M	10	26.10	3.70	b
ITN	F	28	5.43	0.12	d
	M	42	3.09	0.10	e
ITW	F	47	10.42	0.35	c
	M	8	5.75	0.37	d
ITP	F	104	27.96	0.30	b
	M	12	25.67	0.38	b

I = mated, V = virgin, S = sexes separate, T = sexes together, H = honey and water, N = no honey or water, W = water only, P = pupae (hosts) and water.

* Values followed by the same letter are not significantly different ($P < 0.05$; multiple comparison LSD Bonferroni test).

All significant differences in mean longevity were shown up by the Bonferroni LSD tests (Table 10). There was no significant difference in the longevity of mated and virgin parasitoids ($t = 2.68$; $P < 0.05$) (Fig.21). The same test showed no significant difference in longevity between sexes kept separately and males and females kept together ($t = 2.68$; $P = 0.05$) (Fig.22), despite earlier findings to the contrary (Kfir *et al.* 1993). In the third comparison, *T. howardi* with honey and water lived significantly longer than those with only water, which in turn lived longer than those with no food or water ($t = 2.99$; $P < 0.05$) (Fig.23). These findings correlate with the general trend found in the genus (Ooi 1988; Mushtaque 1990; Okeyo-Owuor *et al.* 1991). Okeyo-Owuor *et al.* (1991) found that longevity was dependent on the quality and concentration of the food provided. Parasitoids presented with hosts and water lived significantly longer than those without hosts ($t = 2.68$; $P < 0.05$) (Fig.24). In *Dicondylus indianus* Olmi (Hymenoptera: Dryinidae) supplied with hosts, longevity was shortest at the lowest densities, but it exceeded the longevity of wasps deprived of hosts (Sharagard *et al.* 1991). However, *D. indianus* is a host feeder. *T. howardi*, unlike *T. flavigaster* (Moran *et al.* 1969), was never observed host feeding.

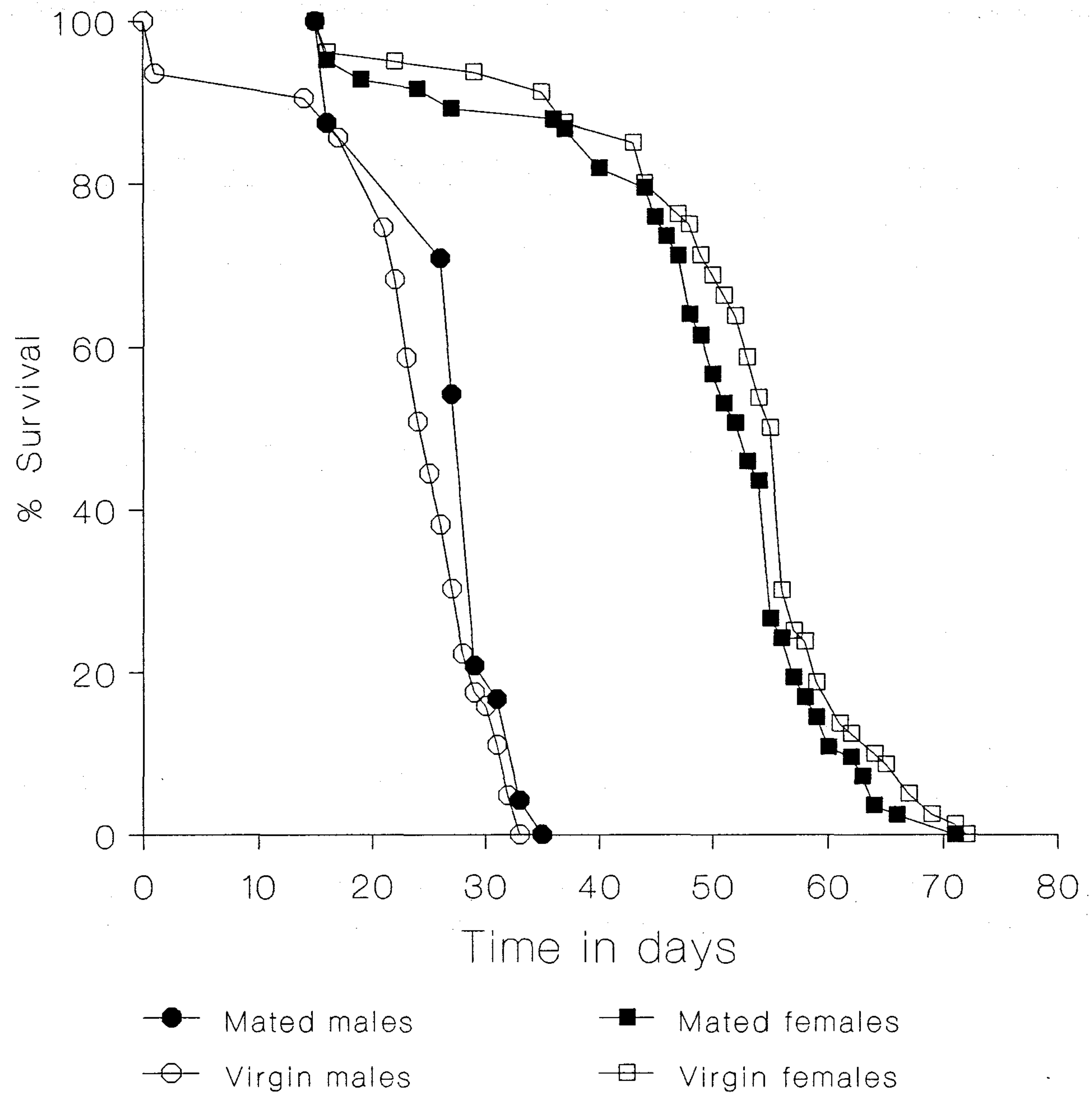

Fig.21 Longevity of T.howardi: mated versus virgin males and females.

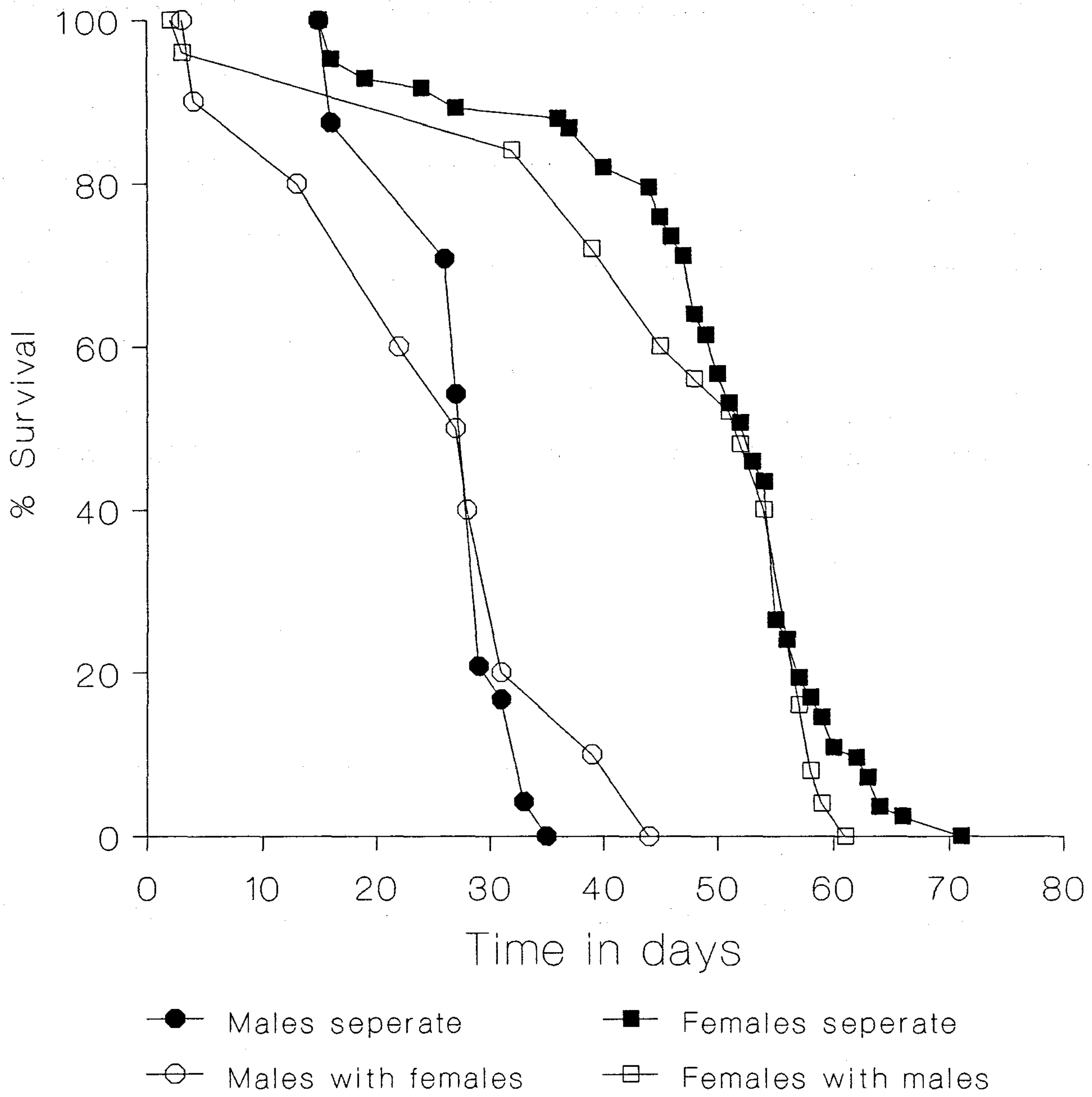

Fig.22 Longevity of _T.howardi_: Males and females kept seperately versus males and females kept together.

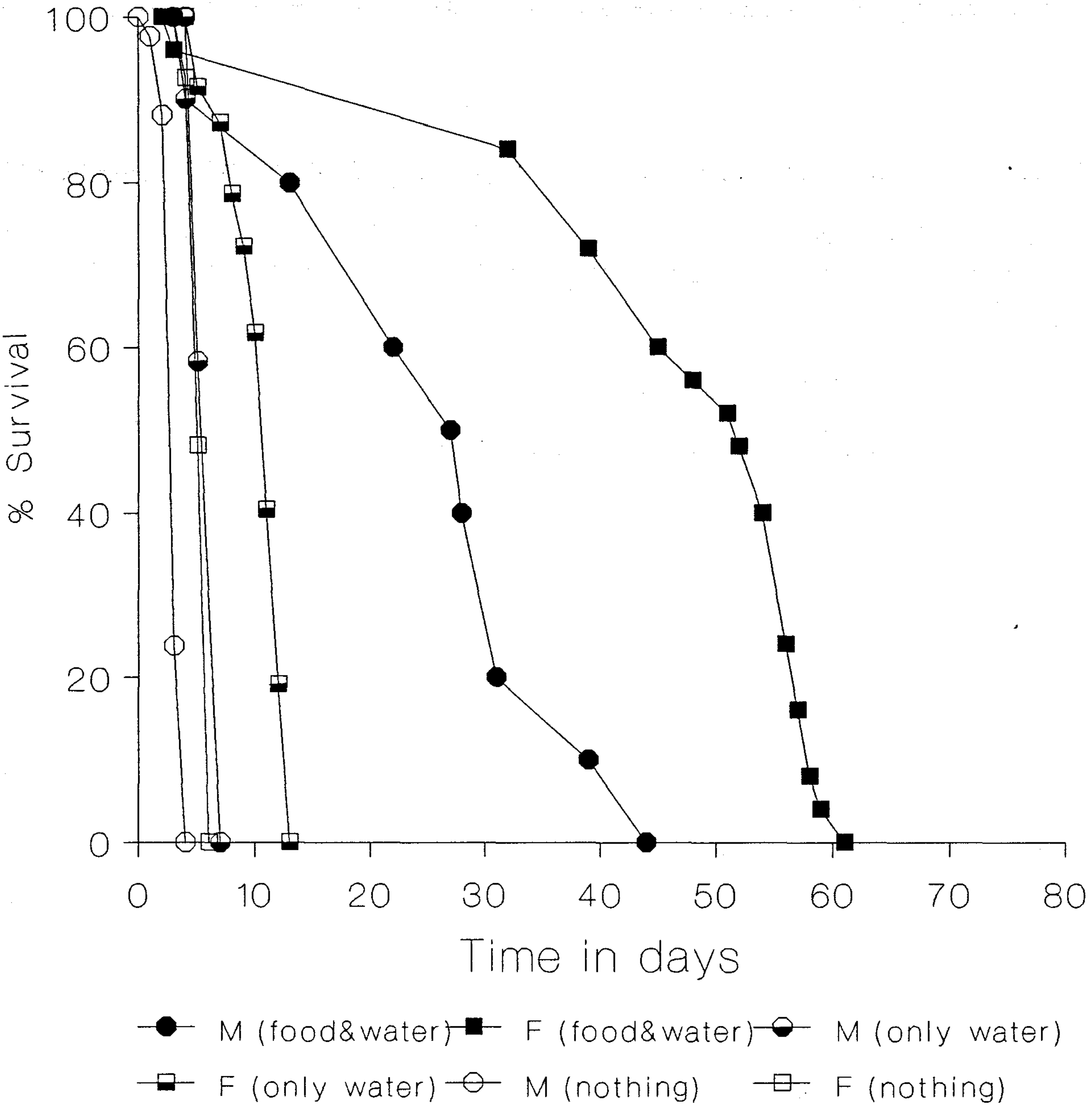

Fig.23 Longevity of T.howardi: influence of presence and absence of food and/or water.

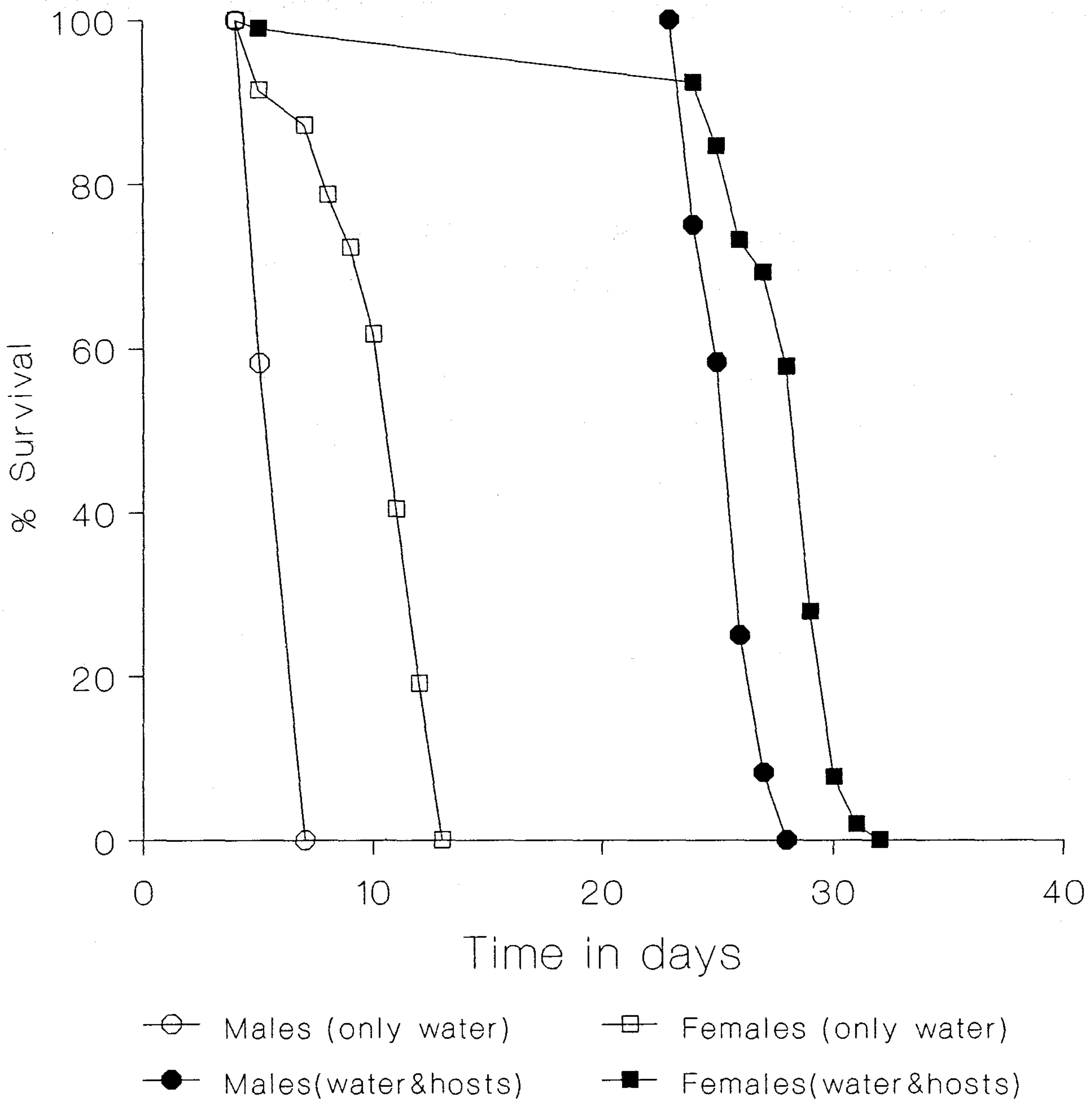

Fig.24 Longevity of T.howardi: influence of hosts.

CHAPTER 8

RELEASE AND RECOVERY

Materials and Methods

T. howardi was released in the 1993 growing season in a sorghum field of about 5000m^2 at the government experimental farm near Brits, Transvaal (25°38'S, 27°47'E, elevation 1100m) South Africa. Sorghum had been planted at the start of the rainy season in October 1992. As soon as plants were about 400 mm tall they were randomly sampled in groups of 25 a week. The plants were removed and dissected in the laboratory to record all borer infestation. At the first appearance of borer pupae in January 1993, a gauze tent was erected in the middle of the field. The tent covered about 250 plants. C. partellus infestation of the plants in the tent was augmented by artificial infestation of the plants with first instar larvae from the laboratory culture. 190 of the plants in the tent were inoculated, each with 2 larvae.

Simultaneously, weekly releases of T. howardi were made both in the tent and in the open field for 21 consecutive weeks until no more C. partellus pupae were forming. Parasitoids were released from parasitised H. armigera pupae. Taking into account that ± 390 parasitoids develop per host, it was calculated that a total of 174000 T. howardi individuals were released in the field at an average of around 8600 a week. A total of approximately 53 000 parasitoids were released in the tent at an average of around 2600 a week.

Weekly, 25 plants from the open field and 8 - 10 plants from the tent were collected at random and dissected. Pupae found were kept singly in marked vials for emergence.

In the same area a total of around 17 600 individuals were released against P. xylostella in cabbages, over a period of 6 weeks at an average of 2900 T. howardi per week.

In the Delmas area of the Transvaal Highveld (25°09'S, 28°41'E, elevation 1600m) around 78 000 T. howardi were released in a sorghum field, over 13 weeks, at an average of about 6000 per week.

Results

From the tent at Brits one T. howardi parasitised pupa was recovered, in April. The pupa was unfortunately broken during dissection of the sorghum stalk and so the larvae, identified as T. howardi did not develop to adulthood. One recovery was also made from the field at Brits in March. This included 38 females and 2 males.

Several recoveries of T. howardi were made from P. xylostella pupae collected from the cabbages in an independent study.

No recoveries were made from the sorghum at Delmas.

Discussion

Before release of T. howardi, the possible advantages and disadvantages of such an action were carefully assessed. Two important questions arose: should generalist parasitoids be released as natural enemies in biological control programmes, and should facultative hyperparasitoids be released?

It has often been suggested that for biological control of pests, the host specificity of the natural enemies is not critical (Cock 1986). However, there has recently been a swing away from this by some researchers (Howarth 1991; Lockwood 1993). Importantly, most recorded incidents of generalist natural enemies switching hosts have been island cases, particularly Hawaii (Gagne 1972; Howarth 1983), which are ecologically completely different from mainlands.

Throughout its distribution T. howardi has been recorded as a parasitoid of Lepidoptera and mainly stem borers (Ayyar 1927; Cherian & Subramaniam 1940; Rudriah & Sastry 1959; Boucek 1988). In fact, there is only one recorded case of T. howardi being recovered from anything other than stem borers, and that was on diamondback moth, P. xylostella in Malaysia (Ooi & Kelderman 1979; Ooi 1979). The polyphagous nature of T. howardi was shown only under laboratory conditions by Cherian & Subramaniam (1940) and Rudriah & Sastry (1959) who reared it respectively from 15 and 17 different lepidopteran hosts. Kfir et. al. (1993) reared the parasitoid from 11 lepidopteran, 1 coleopteran, 4 hymenopteran and 2 dipteran hosts.

There appear to be 4 distinct and consecutive steps whereby a parasitoid-host relationship is successfully initiated, and through their operation the natural host list of a parasite becomes restricted (Flanders 1953; Doutt 1959; Vinson 1976). These are: host habitat finding, host finding, host acceptance, and host suitability. T. howardi is adapted in its host searching strategy to penetrate tunnels excavated by stem borers, and is therefore adapted to the typical habitats of stem borers. As the habitat and host searching strategy are highly specialized, the parasitoid will therefore initially seek such an environment.

Because hyperparasitism has traditionally been viewed as detrimental to biological control, it has been policy of biological control projects to exclude hyperparasites during the introduction of natural enemies (Flanders 1943; Doutt & DeBach 1964; Laing & Hamai 1976; McDonald & Kok 1991). However, there is some indication that the negative influence of hyperparasitoids on biological control might have been overemphasized (LaSalle 1993), as real evidence for this is lacking (Luck _et al._ 1981; Nealis 1983). In fact, it has been suggested that under certain conditions the presence of a hyperparasitoid might actually improve a biological control system by changing it from one which displayed periodic pest outbreaks to one of continuous subeconomic pest population level (Luck _et al._ 1981; LaSalle 1993). Nevertheless, it has been concurred that as their impact on biological control is still uncertain, the conservative policy of excluding all exotic obligate hyperparasitoids should continue (Bennett 1981; Luck et. al. 1981; Kfir _et al._ 1993).

_T._ _howardi_ is a facultative hyperparasitoid. The question of importing and releasing such a natural enemy is a different issue. Facultative hyperparasitoids which preferentially act as hyperparasitoids should not be used in biological control (Bennett 1981: Cock 1986; Sullivan 1987). However, May & Hassell (1981) showed that a system with a facultative hyperparasitoid, which does not distinguish between parasitized and unparasitized hosts, had the same population dynamics as a system with two parasitoids which attack the same stage of the host. If one accepts that multiple introductions are favourable, there is no reason not to introduce such a facultative hyperparasitoid.

Some cases of successful pest control by a facultative hyperparasitoid have been documented, including a few by Luck _et al._

(1981). Oomyzus sokolowskii Kurdj. (Hymenoptera: Eulophidae) has been introduced into several countries for control of P. xylostella on cole crops (Fitton & Walker 1992). It attacks both the diamondback moth and its braconid parasite Cotesia plutellae Kurdj. O. sokolowskii achieved levels of parasitism up to 68 - 100% in Barbados, and together with C. plutellae satisfactorily suppressed P. xylostella in the Cape Verde Islands (Cock 1985).

Ehler (1979) demonstrated that the total parasitism on a cecidomyiid gall-forming midge, Rhopalomyia californica Felt, in California, was consistently higher when the facultative secondary parasitoid, Zatropis capitis Burks (Hymenoptera: Pteromalidae), was present compared to when primary parasitoids acted alone. The study showed that as the number of parasitoid species increased, the efficacy of the individual species diminished while the combined efficacy of the species increased.

Despite warnings against the introductions of facultative hyperparasitoids (Mertins & Coppel 1973; Weseloh et al. 1979), it would be wise to evaluate separately for each candidate species depending on the availability of conventional natural enemies and the seriousness of the insect pest problem (Sullivan 1987).

The case for release of T. howardi is greatly strengthened by three factors: T. howardi is cardinally a primary parasitoid and in fact never recorded as a hyperparasitoid in nature; the chief indigenous natural enemy of stem borers, C. sesamiae (Kfir 1990), is not seriously attacked by T. howardi; recent attempts to establish seven exotic parasitoids against stem borers failed (Skoroszewski & Van Hamburg 1988; Kfir 1992).

Plans to release T. howardi in the West Indies were halted when its hyperparasitism on tachinid puparia was discovered in the laboratory (Bennett 1965). As tachinids play only a negligible role in natural control of maize and sorghum pests in the Highveld region of the Transvaal (Kfir _et al._ 1993), this does not apply here.

A short life cycle, high fecundity and longevity, preponderance of females over males, and amenability to easy multiplication in the laboratory are other points in favour of release of _T. howardi_ as a biocontrol agent (Cherian & Subramaniam 1940; Rudriah & Sastry 1959). Some drawbacks are its polyphagous nature, its inability to breed on young larval stages which are destructive to crops, and eggs laid in clusters in the host.

CHAPTER 9

GENERAL DISCUSSION

The purpose of these studies was to augment the natural enemy complex of lepidopterous stem borers in cereal crops and to improve their natural control. In addition, the aim was to study the biology of T. howardi, and to determine whether it should be released. If released, its establishment and spread were to be monitored, as well as the effect on the biological control of the stem borers.

C. partellus and B. fusca can cause severe yield losses. Therefore, controlling the borers to a level below economic threshold is essential. Chemical control is not only extremely expensive (Van Hamburg 1987) but often impractical due to the elusive life style of the borer larvae. This is why the biological control option is being explored.

Introductions of natural enemies have been made against stem borers since 1914, there being 215 records of releases world wide (Greathead 1990). Establishments of natural enemies have been few, and control of pests even rarer. Using New World tachinids and C. flavipes, 2 cases of partial control (out of 130 introductions) of the American sugarcane borer, Diatraea saccharalis F., have been achieved, these being in Barbados and on some Caribbean islands (Betbeder-Matibet 1989; Greathead 1990). Another biological control success was the introduction of C. flavipes against Chilo sacchariphagus Bojer in Madagascar (Mohyuddin et al. 1981). It is notable that all successes in this field occur on islands.

In Africa, attempts to introduce natural enemies of graminaceous stem borers have met with little success during the last 30 years.

In Kenya, Uganda, and Tanzania, several species of Trichogrammatidae, Braconidae, Ichneumonidae, and Tachinidae have been imported and released but none have been recovered (Ingram 1983). In the Old World in spite of massive effort to introduce New World tachinids, which breed well on *Chilo* spp. and other Old World stem borers in insectaries, none have become established (Greathead 1990). In view of the largely negative results of past classical biocontrol introductions against stem borers the prospects do not appear very promising. However, apparent possibilities should be investigated and unless it has been shown that natural enemies are physiologically incapable of colonizing, introductions are worth attempting (Greathead 1990).

Using eulophids against stem borers was probably first attempted in 1950 in South India (Rudriah & Sastry 1959). *T. howardi* was released against sugarcane borers, especially *S. nivella*, but no recoveries were made. *T. israeli* was exported to Trinidad in 1959 for control of pyralid sugarcane borers (Bennett 1965). Unfortunately, the eulophid also readily attacked tachinid puparia in the laboratory, themselves parasitic on the sugarcane borers, and release was halted. The same reasoning was applied to the importation of *T. howardi* to Trinidad in 1963, and release never occurred (Bennett 1965). From 1965 to the 1970s, 3 species of Eulophidae, *Pediobius furvus* Gahan, *Trichospilus diatraeae* Cherian & Margabandhu, and *T. israeli* were released into Reunion Island to combat *C. sacchariphagus* and *Sesamia calamistis* Hampson (Lepidoptera: Noctuidae), mainly on sugarcane. Very few recoveries were made, and these were only of *T. diatraeae* on *S. calamistis*.

Stem borers are difficult targets for biocontrol. Nevertheless, prospective natural enemies should continue to be sought. T. howardi, from laboratory biological studies and knowledge of its field behaviour, showed substantial promise. T. howardi was easy to mass rear in the laboratory, producing great numbers in short periods of time and a strong preponderance of females. Despite proving facultatively hyperparasitic under artificial conditions, T. howardi has never been observed as such in its natural environment. The main indigenous parasitoid of C. partellus and B. fusca, C. sesamiae is not significantly attacked by the eulophid. Neither is T. howardi's ability to develop on tachinids of any concern, as the flies play a negligible role in natural control of pests of maize and sorghum in the Transvaal Highveld (Kfir et al. 1993). The chances of establishing exotic tachinids are also very slim. T. howardi was highly polyphagous in the laboratory but in the field has virtually always been found on stem borers. This is probably due to its highly specific habitat niche, and the preference which it demonstrated under experimental conditions, for phytophagous over parasitic insects.

T. howardi was released in this study for only one season. Great numbers were released but very few recoveries were made. This may partly be a reflection on the relatively small samples of sorghum plants which were taken from the release area. It is impossible after one season of release to determine whether establishment has or will take place. Because the stem borers diapause as mature larvae in the dry stalks of maize and sorghum plants during winter (Kfir 1990b; Kfir 1991), there are no host pupae present for the parasitoid. Therefore, for its survival, T. howardi would have to over-winter in an alternative host, and then parasitize the stem borers during the next growing season.

If _T. howardi_ should establish and spread it is not believed that it alone would be the solution to the stem borer problem on summer grain crops in South Africa. However, it is hoped, in accordance with the initial aim of the project, that _T. howardi_ will augment the existing natural enemy complex of the lepidopterous stem borers and contribute to the improvement of the natural control.

REFERENCES

ANDOW, D.A. & PROKRYM, D.R. 1990. Plant structural complexity and host-finding by a parasitoid. Oecologia 82: 162-165.

ARTHUR, A.P. 1962. Influence of host tree on abundance of Itoplectis conquisitor (Say) (Hymenoptera: Ichneumonidae), a polyphagous parasite of the European Pine Shoot Moth Rhyacionia buoliana (Schiff.) (Lepidoptera: Olethreutidae). Canadian Entomologist 94: 337-347.

AYYAR, T.V.R. 1927. The parasitic Hymenoptera of economic importance noted from South India. Bulletin of Entomological Research 18: 73-78.

BATE, R., VAN RENSBURG, G.D.J. & GILIOMEE, J.H. 1991. Distribution and occurrence of the stem-borer Chilo partellus (Swinhoe) (Lepidoptera: Pyralidae) on maize in South Africa. Journal of African Zoology 105: 11-15.

BENNETT, F.D. 1965. Tests with parasites of Asian graminaceous moth-borers on Diatraea and allied genera in Trinidad. Technical Bulletin, Commonwealth Institute of Biological Control 5: 101-116.

BENNETT, F.D. 1981. Hyperparasitism in the practice of biological control. In: ROSEN, D. (ed.) The Role of Hyperparasitism in Biological Control: A symposium. Division of Agricultural Sciences, University of California 4103: 43-49.

BETBEDER-MATIBET, M. 1989. Biological control of sorghum stem borers. International Workshop on Sorghum Stem Borers, 1987: International Crops Research Institute for the Semi-Arid Tropics: 89-93.

BOUCEK, Z. 1988. Australian Chalcidoidea (Hymenoptera). A biosystematic revision of genera of 14 families, with a reclassification of species. CAB International, U.K., 832pp.

CAMORS Jr., F.B. & PAYNE, T.L. 1972. Response of *Heydenia unica* (Hymenoptera: Pteromalidae) to *Dendroctonus frontalis* (Coleoptera: Scolytidae) pheromones and a host-tree terpene. Annals of the Entomological Society of America 65: 31-33.

CHERIAN, M.C. & SUBRAMANIAM, C.K. 1940. *Tetrastichus ayyari* Rohw. - a pupal parasite of some moth-borers in South India. Indian Journal of Entomology 2: 75-77.

CLAUSEN, C.P. 1940. Entomophagous Insects. McGraw-Hill Book Company, New York: 135-156.

COCK, M.J.W. 1985. A review of biological control of pests in the Commonwealth Caribbean and Bermuda up to 1982. Technical Communication 9. Commonwealth Institute of Biological Control, Commonwealth Agricultural Bureau, 218pp.

COCK, M.J.W. 1986. Requirements for biological control: an ecological perspective. Biocontrol News and Information 7: 7-16.

DOBSON, A.J. 1983. An Introduction to Statistical Modelling. Chapman & Hall, London.

DONALDSON, J.S. & WALTER, G.H. 1991. Host population structure affects field sex ratios of the heteronomous hyperparasitoid, *Coccophagus atratus*. Ecological Entomology 16: 35-44.

DOUTT, R.L. 1959. The biology of parasitic Hymenoptera. Annual Review of Entomology 4: 161-182.

DOUTT, R.L. & DeBACH, P. 1964. Some biological control concepts and questions. In: DeBACH, P. (ed.) Biological Control of Insect Pests and Weeds. Chapman & Hall, London: 118-142.

EHLER, L.E. 1979. Utility of facultative secondary parasites in biological control. Environmental Entomology 8: 829-832.

EISJACKERS, H.J.P. & VAN LENTEREN, J.C. 1970. Host choice and host discrimination in *Pseudocoila bochei*. Netherlands Journal of Zoology 20: 414.

ELLIOTT, J.M. 1983. Some Methods for the Statistical Analysis of Samples of Benthic Invertebrates, 2nd ed. Titus Wilson & Son Ltd., Kendal, 159pp.

ETIENNE, J. 1974. Introduction of Eulophidae into Reunion Island for the control of sugarcane borers. Proceedings of the International Society of Sugar Cane Technology 15: 387-392.

FITTON, M. & WALKER, A. 1992. Hymenopterous parasitoids associated with diamondback moth: the taxonomic dilemma. In: TALEKAR, N.S. (ed.) Diamondback Moth and Other Crucifer Pests, Proceedings of the Second International Workshop, Asian Vegetable and Development Centre. Shanhua, Taiwan: 225-232.

FLANDERS, S.E. 1943. Indirect hyperparasitism and observations on three species of indirect hyperparasites. Journal of Economic Entomology 36: 921-926.

FLANDERS, S.E. 1947. Elements of host discovery exemplified by parasitic hymenoptera. Ecology 28: 299-309.

FLANDERS, S.E. 1951. Mass culture of California red scale and its golden chalcid parasites. Hilgardia 21: 1-42.

FLANDERS, S.E. 1953. Variations in susceptibility of citrus-infesting coccids to parasitization. Journal of Economic Entomology 46: 266-269.

GAGNE, W.C. 1972. Notes and exhibitions: *Apotoforma* sp. Proceedings of the Hawaiian Entomological Society 21: 158-159.

GREATHEAD, D.J. 1990. Utilization of natural enemies of Chilo spp. for management in Africa. Insect Science and its Application 11: 749-755.

HAMMERSKI, M.R., HALL, R.W. & KEENEY, G.D. 1990. Laboratory biology and rearing of Tetrastichus brevistigma (Hymenoptera: Eulophidae), a larval-pupal parasitoid of the elm leaf beetle (Coleoptera: Chrysomelidae). Journal of Economic Entomology 83: 2196-2199.

HOWARTH, F.G. 1983. Classical biocontrol: Panacea or Pandora's box. Proceedings of the Hawaiian Entomological Society 24: 239-244.

HOWARTH, F.G. 1991. Environmental impacts of classical biological control. Annual Review of Entomology 36: 485-509.

INAYATULLAH, C. 1983. Host selection by Apanteles flavipes (Cameron) (Hymenoptera: Braconidae): Influence of host and host plant. Journal of Economic Entomology 76: 1086-1087.

INGRAM, W.R. 1983. Biological control of graminaceous stem borers and legume pod borers. Insect Science and its Application 4: 205-209.

JACKSON, D.J. 1937. Host selection in Pimpla examinator F. Proceedings of the Royal Entomological Society of London, Series A 12: 81-91.

KFIR, R. 1988. Hibernation by the lepidopteran stalk borers, Busseola fusca and Chilo partellus on grain sorghum. Entomologia Experimentalis et Applicata 48: 31-36.

KFIR, R. 1990a. Parasites of the spotted stalk borer, Chilo partellus (Lepidoptera: Pyralidae) in South Africa. Entomophaga 35: 403-410.

KFIR, R. 1990b. Prospects for cultural control of the stalk borers, Chilo partellus (Swinhoe) and Busseola fusca (Fuller), in summer grain crops in South Africa. Journal of the Entomological Society of Southern Africa 53: 41-47.

KFIR, R. 1991. Duration of diapause in the stem borers, Busseola fusca and Chilo partellus. Entomologia Experimentalis et Applicata 61: 265-270.

KFIR, R. 1992. Seasonal abundance of the stem borer, Chilo partellus (Lepidoptera: Pyralidae), and its parasites on summer grain crops. Journal of Economic Entomology 85: 518-529.

KFIR, R., GOUWS, J. & MOORE, S.D. 1993. Biology of Tetrastichus howardi (Olliff) (Hymenoptera: Eulophidae): A facultative hyperparasitoid of stem borers. Biocontrol Science and Technology 3: 149-159.

KFIR, R., VAN HAMBURG, H. & VAN VUUREN, R. 1989. Effect of stubble treatment on the post-diapause emergence of the grain sorghum stalk borer, Chilo partellus (Swinhoe) (Lepidoptera: Pyralidae). Crop Protection 8: 289-292.

KIDD, N.A.C. & JERVIS, M.A. 1991a. Host-feeding and oviposition strategies of parasitoids in relation to host stage. Researches of Population Ecology 33: 13-28.

KIDD, N.A.C. & JERVIS, M.A. 1991b. Host-feeding and oviposition by parasitoids in relation to host stage: consequences for parasitoid-host population dynamics. Research of Population Ecology 33: 87-99.

KIRSTEN, F. & KFIR, R. 1991. Rate of development, host instar preference and progeny distribution by Pauesia sp. (Hymenoptera: Aphidiidae), a parasitoid of Cinara cronortii Tissot & Pepper (Homoptera: Aphididae). Journal of the Entomological Society of Southern Africa 54: 75-80.

LAING, J.E. & HAMAI, J. 1976. Biological control of insect pests and weeds by imported parasites, predators and pathogens. In: HUFFAKER, C.B. & MESSENGER, P.S. (eds.) Theory and Practice of Biological Control. Academic Press, New York: 685-743.

LaSALLE, J. 1993. Parasitic Hymenoptera, biological control and biodiversity. In: LaSALLE, J. & GAULD, I.D. (eds.) Hymenoptera and Biodiversity. CAB International, U.K.: 197-215.

LOCKWOOD, J.A. 1993. Environmental issues involved in biological control of rangeland grasshoppers (Orthoptera: Acrididae) with exotic agents. Environmental Entomology 22: 503-518.

LUCK, R.F. MESSENGER, P.S. & BARBIERI, J.F. 1981. The influence of hyperparasitism on the performance of biological control agents. In: ROSEN, D. (ed.) The Role of Hyperparasitism in Biological Control: A Symposium. Division of Agricultural Sciences, University of California 4203: 34-42.

MALLY, C.W. 1920. The maize stalk borer, Busseola fusca Fuller. Bulletin of the Department of Agriculture, Union of South Africa no.3, 111pp.

MATTHEE, J.J. 1974. Pests of graminaceous crops in South Africa. Entomology Memoir, Department of Agricultural Technical Services, Republic of South Africa no.40, 24pp.

MAY, R.M. & HASSELL, M.P. 1981. The dynamics of multiparasitoid-host interactions. American Naturalist 117: 234-261.

McCULLAGH, P. & NELDER, J.A. 1985. Generalized Linear Models. Chapman & Hall, New York.

McDONALD, R.C. & KOK, L.T. 1991. Hyperparasites attacking Cotesia glomerata (L.) and Cotesia rubecula (Marshall) (Hymenoptera:

Braconidae) in Southwestern Virginia. Biological Control 1: 170-175.

MERTINS, J.W. & COPPEL, H.C. 1973. Adaptation of native and exotic hyperparasites to the introduced primary sawfly parasite, Exenterus amictorius (Panzer) in Wisconsin. Entomophaga 18: 19-23.

MOHYUDDIN, A.I. 1990. Biological control of Chilo spp. in maize, sorghum and millet. Insect Science and its Application 11: 721-732.

MOHYUDDIN, A.I. & GREATHEAD, D.J. 1970. An annotated list of parasites of graminaceous stem borers in East Africa, with a discussion on their potential in biological control. Entomophaga 15: 241-247.

MOHYUDDIN, A.I., INAYATULLAH, C. & KING, E.G. 1981. Host selection and strain occurrence in Apanteles flavipes (Cameron) (Hymenoptera: Braconidae) and its bearing on biological control of graminaceous stem-borers (Lepidoptera: Pyralidae). Bulletin of Entomological Research 71: 575-581.

MORAN, V.C., BROTHERS, D.J. & CASE, J.J. 1969. Observations on the biology of Tetrastichus flavigaster Brothers & Moran (Hymenoptera: Eulophidae), parasitic on psyllid nymphs (Homoptera). Transactions of the Royal Entomological Society of London 121: 41-58.

MOUTIA, L.A. & COURTOIS, C.M. 1952. Parasites of the moth-borers of sugar-cane in Mauritius. Bulletin of Entomological Research 43: 325-359.

MUSHTAQUE, M. 1990. Some studies on Tetrastichus sokolowskii Kurd. (Eulophidae: Hymenoptera), a parasitoid of diamondback moth in

Pakistan. Pakistan Journal of Zoology 22: 37-43.

NADARAJAN, L. & JAYARAJ, S. 1975. Influence of various hosts on the development and reproduction of the pupal parasite, *Tetrastichus israeli* M.and K. (Eulophidae: Hymenoptera). Current Science 44: 458-460.

NEALIS, V.G. 1983. *Tetrastichus galactopus* (Hym.: Eulophidae), a hyperparasite of *Apanteles rubecula* and *Apanteles glomeratus* (Hym.: Braconidae) in North America. Journal of the Entomological Society of British Columbia 80: 25-28.

OHGUSHI, R.I. 1960. Studies on the host selection by *Nasonia vitripennis* parasitic on house fly pupae. Physiological Ecology (Japan) 9: 11-31.

OKEYO-OWUOR, J.B., OLOO, G.W. & AGWARO, P.O. 1991. Bionomics of *Tetrastichus sesamiae* (Hymenoptera: Eulophidae), a pupal endo-parasitoid of *Maruca testulalis* (Lepidoptera: Pyralidae). Entomophaga 36: 417-423.

OOI, P.A.C. 1988. Laboratory studies of *Tetrastichus sokolowskii*. Entomophaga 33: 145-152.

OOI, P.A.C. & KELDERMAN, W. 1979a. A parasite of the diamond-back moth in Cameron Highlands, Malaysia. Malaysian Agricultural Journal 51: 187-190.

OOI, P.A.C. & KELDERMAN, W. 1979b. The natural enemies of *Plutella xylostella* (L.) in Cameron Highlands, Malaysia. Malaysian Agricultural Journal 52: 77-84.

PITCAIRN, M.J. & GUTIERREZ, A.P. 1992. Influence of adult size and age on the fecundity and longevity of *Tetrastichus incertus* (Hymenoptera: Eulophidae). Annals of the Entomological Society of America 85: 53-57.

RAO, V.P. 1965. Natural enemies of rice stem borers and allied species in various parts of the world and possibilities of their use in biological control of rice stem borers in Asia. Technical bulletin, Commonwealth Institute of Biological Control 6:1-68.

READ, D.P., FEENY, P.P. & ROOT, R.B. 1970. Habitat selection by the aphid parasite *Diaeretiella rapae* (Hymenoptera: Braconidae) and hyperparasite *Charips brassicae* (Hymenoptera: Cynipidae). Canadian Entomologist 102: 1567-1578.

RUDRIAH, M.P. & SASTRY, K.S.S. 1959. Studies on the biology of *Tetrastichus ayyari* Rohwer, with attempts to utilise it in the control of sugar cane borers. Indian Journal of Entomology 20: 189-198.

SAHRAGARD, A., JERVIS, M.A. & KIDD, N.A.C. 1991. Influence of host availability on rates of oviposition and host-feeding, and on longevity in *Dicondylus indianus* Olmi (Hym., Dryinidae), a parasitoid of the rice brown planthopper, *Nilaparvata lugens* Stål (Hem., Delphacidae). Journal of Applied Entomology 112: 153-162.

SAMSON-BOSHUIZEN, M., VAN LENTEREN, J.C. & BAKKER, K. 1974. Success of parasitization of *Pseudocoila bochei* Weld (Hum., Cynip.): a matter of experience. Netherlands Journal of Zoology 24: 67-85.

SKOROSZEWSKI, R.W. & VAN HAMBURG, H. 1987. The release of *Apanteles flavipes* (Cameron) (Hymenoptera: Braconidae) against stalk-borers of maize and grain-sorghum in South Africa. Journal of the Entomological Society of Southern Africa 50: 249-255.

STREAMS, F.A., SHAHJAHAN, M. & LeMASURIER, H.G. 1968. Influence of plants on the parasitization of the tarnished plant bug by

Leiophron pallipes. Journal of Economic Entomology 61: 996-999.

SULLIVAN, D.J. 1987. Insect Hyperparasitism. Annual Review of Entomology 32: 49-70.

TAGAWA, J. & FUKUSHIMA, H. 1993. Effects of host age and cocoon position on attack rate by the hyperparasitoid, Eurytoma sp. (Hym.: Eurytomidae), on cocoons of the parasitoid, Cotesia (= Apanteles) glomerata (Hym.: Braconidae). Entomophaga 38: 69-77.

UEMATSU, H. 1981. The ovipositional behaviour in Euplectrus kuwanae Crawford (Hymenoptera: Eulophidae), a parasitoid of Argyrogramma albostriata (Bremer et Grey) (Lepidoptera: Noctuidae). Applied Entomology and Zoology 16: 443-450.

VAN HAMBURG, H. 1979. The grain-sorghum stalk-borer, Chilo partellus (Swinhoe) (Lepidoptera: Pyralidae): seasonal changes in adult populations in grain sorghum in the Transvaal. Journal of the Entomological Society of Southern Africa 42: 1-9.

VAN HAMBURG, H. 1987. A biological control approach to pest management in grain crops with special reference to the control of stalk borers. Technical Communication, Department of Agriculture and Water Supply, Republic of South Africa 212: 52-55.

VAN LENTEREN, J.C. 1976. The development of host discrimination and the prevention of superparasitism in the parasite Pseudocoila bochei Weld (Hym.: Cynipidae). Netherlands Journal of Zoology 26: 1-83.

VAN RENSBURG, G.D.J. & BATE, R. 1987. Preliminary studies on the relative abundance and distribution of the stalk borers Busseola fusca and Chilo partellus. Technical Communication, Department of Agriculture and Water Supply, Republic of South Africa 212:

49-52.

VAN RENSBURG, J.B.J., WALTERS, M.C. & GILIOMEE, J.H. 1987. Ecology of the maize stalk borer, *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae). *Bulletin of Entomological Research* 77: 255-269.

VAN RENSBURG, J.B.J., WALTERS, M.C. & GILIOMEE, J.H. 1988. Mortality in natural populations of the maize stalk borer *Busseola fusca* (Fuller) (Lepidoptera: Noctuidae) in South Africa. *Phytophylactica* 20: 17-19.

VAN RENSBURG, N.J. & VAN HAMBURG, H. 1975. Grain sorghum pests: an integrated control approach. *Proceedings of the 1st Congress of the Entomological Society of Southern Africa*: 151-162.

VINSON, S.B. 1976. Host selection by insect parasitoids. *Annual Review of Entomology* 21: 109-133.

VINSON, S.B. & PIPER, G.L. 1986. Source and characterization of host recognition kairomones of *Tetrastichus hagenowii*, a parasitoid of cockroach eggs. *Physiological Entomology* 11: 459-468.

WESELOH, R.M., WALLNER, W.E. & HOY, M.A. 1979. Possible deleterious effects of releasing *Anastatus kashmirensis*, a facultative hyperparasite of the gypsy moth. *Environmental Entomology* 8: 174-177.

WILLIAMS, T. 1991. Host selection and sex ratio in a heteronomous hyperparasitoid. *Ecological Entomology* 16: 377-386.