CHAPTER 5

FEEDING BEHAVIOUR OF THE LADYBIRD *S. VAGANS*

5.1 ABSTRACT

Adult longevity and fertility of *S. vagans* were recorded on a range of alternative hosts in the laboratory, via a series of no choice tests. None of the alternative hosts were able to maintain fertility in female *S. vagans*, except broad mites, *Polyphagotarsonemus latus*. Although some eggs were laid when *S. vagans* were supplied with pollen & water, they did not hatch. The most effective food source for maintaining adult longevity was twospotted mite, *T. urticae* (38 ± 6.0 days), followed by broad mite, *Polyphagotarsonemus latus* (26.1 ± 0.9), rust mite, *Aucolops lycopersi* (23.47 ± 1.3), pollen & water (21.1 ± 2.7), and honey & water (18.94 ± 2.3). There was no significant difference between male and female longevity on the same host.

Time partitioning and prey preference of both adults and 4th instar larvae of *S. vagans* were studied in the laboratory. The behaviour of the predators was examined under different feeding regimes ie. newly emerged, satiated, and starved for 24 & 48 hours. The parameters assessed were the proportion of time spent by the predators searching, feeding, resting, walking and drinking water. All stages of the preferred host *T. urticae* were freely available during the experiment. The influence of starvation on predation and preference for prey stages were assessed. Newly emerged and fully fed predators spent most of their time resting and walking, while those starved for 24 & 48 hours spend significantly more time searching (28.7 & 27.9%) and feeding (46.7 & 54.9 %).

All stages of *S. vagans* preferred eggs of *T. urticae* to nymphal and adult stages, irrespective of their previous feeding regimes. More than 80% of the prey of newly emerged adults, satiated adults and newly emerged 4th instar larvae consisted of mite eggs, while they comprised more than 70% of the diet of 24 hour starved adults and 4th instar larvae. Adults and 4th instar larvae starved for 48 hours consumed less than 70% of their prey as eggs.

The rate of consumption by both adults and immature stages of *S. vagans* was observed at various densities of prey eggs and adult female mites. The total number of mite eggs consumed by 1st, 2nd, 3rd, and 4th larval instars were 27.93 ± 1.1, 50.12 ±1.0, 71.64 ± 1.5, and 152.36 ± 1.6 respectively. This compared with the numbers consumed by adult males, pre-ovipositing females, ovipositing females and post-ovipositing females, of 63.53 ± 0.4, 94.25 ± 0.5, 142.69 ± 0.53 and 57.1 ± 0.6 eggs per day respectively.

All motile stages of *S. vagans* responded positively to prey density and showed a type–II functional response. Their numerical response was measured by the successful completion of immature stages as well as the reproductive response of resultant adult females. To assess reproductive response, both gross and net oviposition rates were recorded at all prey densities. The gross fecundity was higher than net fecundity at low prey densities, because the adult predators were cannibalistic, eating their eggs because of lack of suitable prey. However both gross and net fecundity increased linearly until they reached a plateau at higher densities.

5.2 INTRODUCTION

Predation is an important component of ecological aspects because through predators the flow of energy continues throughout a community. It also regulates the populations on which they feed and maintains the fitness of these prey populations (Price 1997). Predation is common among ladybirds, and this aspect of their behaviour has received considerable attention, because of the economic importance of many of their prey (Majerus 1994; Price 1997).

Ladybirds are usually highly voracious and often have access to a wide range of prey. Records of prey taken in nature or accepted in the laboratory are numerous, and indicate a broad range of possible animal and plant sources eaten by adults, and to lesser extent larvae. Although the utilisation of alternative food may not be important for the reproductive potential of coccinellids, it appears to be essential for their survival during periods when their natural food sources are unavailable, scarce or building up (Helle & Sabelis 1985b; Majerus 1994).

An effective biological control agent is mainly selected on the basis of its functional and numerical responses to its prey. Functional response is the change in the number of prey eaten per unit time by each predator in relation to changes in prey density (Solomon 1949), while the numerical response is defined as the change in the predator numbers due to prey populations (Crawley 1975). Functional response curves were first derived by Solomon (1949) and then modified by Holling (1959, 1965 and 1966). He described four curves for different predator responses to their prey:

i) A linear rise to a plateau for crustacean predators (Type I).

ii) A curvilinear rise to a plateau for predatory insects (Type II).

iii) A s-shape curve rising to a plateau for vertebrate predators (Type III).

iv) A dome-shape response created by the disturbance of predators by prey activity at high prey density (Type IV).

The manner by which predators search, select, handle and consume prey, as well as their functional and numerical responses to prey densities are major parameters determining the success of biological control agents. These aspects have been reported in ladybirds by several authors, eg. the numerical response of *S. punctum picipes* to *T. urticae* populations in strawberries in British Colombia, Canada (Raworth 1990) and the functional response of *Chilocorus kuwanae* to the scale insect *Unaspis yanonensis* (Yang *et al.* 1997).

The studies described in this Chapter were undertaken to better understand the relationship of the coccinellid *S. vagans* to its prey in terms of its host location, alternative hosts, time and resource partitioning, rate of prey consumption and functional & numerical responses.

5.3 MATERIALS AND METHODS

5.3.1 Culture of *S. vagans*

Large numbers of *S. vagans* pupae were collected from the field on gerbera (*Gerbera jamesonii*) leaves which were naturally infested with *T. urticae*. These field collected pupae were cultured in plastic boxes 30 x 12 x 6 cm in which a hole 22 x 7 cm was cut in the lid and covered with 70 μ nylon mesh. All adults were identified to species after emergence and *S. vagans* were cultured in separate boxes. These culture were maintained in a controlled temperature cabinet at 25°C, under photoperiod L:D 16: 8. All collections and experimental work were conducted at the Centre for Horticulture and Plant Sciences, University of Western Sydney, Richmond campus (Chapter 2).

5.3.2 Alternative hosts

Two hundred newly emerged beetles were randomly selected from the stock colony and paired in 20 replicates on each of the potential food sources in modified petri dishes (Chapter 2). Rate of predation by paired adult *S. vagans* was measured in the laboratory at 25 ± 2°C on the different food hosts. Other parameters assessed were mating, fecundity, egg hatchability and adult longevity.

Treatments were:

1. Starved, no food
2. Water only
3. Honey and water
4. Pollen and water
5. Citrus aphids, *Toxoptera citricidus* (Hemiptera: Aphididae) (all stages)
6. White fly, *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae) (eggs only)

7. Predatory mites, *Phytoseiulus persimilis* (Acarina: Phytoseiidae) (eggs only)

8. Rust mites, *Aucolops lycopersi* (Acarina: Eriophyidae) (all stages)

9. Broad mites, *Polyphagotarsonemus latus* (Acarina: Tarsonemidae) (all stages)

10. Twospotted mite *T. urticae* (Acarina: Tetranychidae) (all stages) (Control)

The above diets were exposed to adult *S. vagans* either inside or on the nylon screen of the modified petri dishes. The base was covered with dry filter paper to absorb any excrement of hosts or predators, thereby preventing contamination. The number and quantity of each food type was consistent in each treatment, except for Treatment 1 (starved). In each dish a fresh bean leaf disc not infested with *T. urticae* was supplied daily to the pair for oviposition.

In Treatment 2, water was supplied constantly through a cotton roll placed on the mesh screen, and was renewed daily. In Treatment 3, honey diluted with water in a ratio of 3: 1 (v/v) was provided in the same manner as water in Treatment 2. These cotton rolls were renewed every second day; to prevent rolls from becoming dry and also to avoid any risk of contamination. In Treatment 3, mixed pollen obtained from honeybees, *Apis mellifera*,, was supplied in a small plastic lid (3mm diam.) placed on the dry filter paper, while water-soaked cotton rolls were placed over the nylon screen covering the dishes as inTreatment 2. Cotton rolls were renewed daily, while pollen was replaced every two days, because it developed fungal growth if kept longer. The number of aphids, white fly eggs, predatory mite eggs, rust mites, broad mites and twospotted mites were counted carefully on the host leaf arenas before the predators were confined. After a 24 hour feeding period the pairs of *S. vagans* were transferred to new containers and the remaining number of each host counted and recorded. Mating, fecundity and longevity of *S. vagans* were determined by observing all dishes 12

hourly. The number of eggs laid was counted and the period between adult emergence and death was calculated as longevity.

5.3.3 Time partitioning behaviour

We examined time partitioning behaviour in both adults and 4th instar larvae. Newly emerged beetles were randomly selected from the foundation colony to observe their time partitioning behaviour. Their behaviour was examined individually and compared between satiated and starved for (24 & 48 hours) as well as newly emerged (less than 24 hours) adults. Satiated and newly emerged individuals were tested immediately after selection, while the remainder were maintained in a culture fed with excess *T. urticae* for a week. Each adult treatment was replicated 30 times (15 males and 15 females). Each trial comprised of one individual adult on a 2.5 cm diameter leaf disc infested with all stages of *T. urticae* placed on moist foam in a 5 cm diameter modified petri dish.

To observe time partitioning behaviour of newly emerged, satiated, and starved (24 & 48 hours) 4th instar larvae, 150 newly laid eggs of *S. vagans* were exposed at 25°C ± 2 in a controlled temperature cabinet. The newly emerged larvae were fed excess all stages of *T. urticae* until the 4th instar. Newly emerged 4th instar larvae were immediately exposed to the prey arena as they shed their 3rd larval instar exoskeleton. For satiated 4th instars they were fully fed for one day before assessing their time partitioning behaviour, while other larvae were starved for 24 & 48 hours; however they were fully fed for one day prior to starvation. Each treatment was replicated 30 times. In each trial prey were supplied with all stages of *T. urticae* on 4.7 cm diameter dry filter papers by brushing infested French bean leaves (Chapter 2). Both adults and larvae of *S. vagans* were observed individually for a two hour period

under a binocular microscope (20 x magnification) and tested only once. Four stopwatches were used (one for each behaviour category) to record the following behaviours:

(i) Searching: defined as slow forward movement

(ii) Feeding: defined as a successful capturing, manipulating and consuming prey. It was considered to be terminated when the predator discarded the exoskeleton.

(iii) Resting: the residual time during which the beetle was not actively searching or feeding, but located in one place.

(iv) Other activities eg. walking (rapid movement, not searching & drinking water): In this category beetles were also observed with other activities such as rubbing their elytra on the edge of leaves or foam.

In these experiments the time spent in each behaviour was recorded as well as the number and stage of each mite prey consumed.

5.3.4 Prey consumption

Newly emerged *S. vagans* adults were randomly selected soon after emergence from the stock colony to investigate the rate of prey consumption for adult males and for pre-ovipositing, ovipositing and post-ovipositing females. Twenty individuals from each of the above groups were exposed to a *T. urticae* density of 200 eggs/arena. For the pre-oviposition category females were selected prior to mating, while for oviposition and post-oviposition they were chosen at the peak of the oviposition period and several days after ceasing egg laying respectively.

The prey consumption rate of immature stages of *S. vagans* was also observed. Approximately 45 newly laid eggs of *S. vagans* were incubated at 25 ± 2°C in a controlled temperature cabinet (as previously described in Chapter 2). The newly emerged 1st instar larvae were confined with the same mite egg density used for adult stages. All larval instars were observed at the same prey density and followed until they moulted to the subsequent instar or died.

The prey consumption experiments were conducted in modified sealable petri dishes (Chapter 2) with a 4.7 diameter dry filter paper (Whatman Catalogue Number 1870 047) on their base onto which the mite eggs were placed. The total internal surface area was 32.48 cm^2 (diameter = 8 cm, height = 0.3). Mite eggs were obtained by brushing infested French bean leaves onto sheets of paper using a mite-brushing machine (as described in Chapter 2). The sheets were retained for approximately 20 minutes to enable the motile mite stages to move off; any remaining motile stage were removed by an aspirator. Each predatory stage of *S. vagans* was provided with 200 mite eggs, which were transferred to the filter paper with a fine camel hair brush. The predators were transferred to a new container at the end of each 24 hour period and the number of mite eggs remaining in each dish was recorded.

5.3.5 Functional and Numerical Response

The functional and numerical responses of *S. vagans* to various densities of *T. urticae* were conducted in the laboratory at 25 ± 2°C and photoperiod L:D 16: 8. Initially adult predators were randomly selected from the culture colony and paired on infested bean leaf discs in modified petri dishes. *S. vagans* eggs obtained from these leaf discs were kept at the same temperature for incubation. Fifteen newly hatched larvae were confined singly at nine prey density levels until they died or pupated. Mite eggs were supplied at eight levels of

abundance from 2 to 200, while no mite eggs were supplied for the starvation control (Chapter 2). The level of prey densities selected for these experiments were based on the number of *T. urticae* observed in field and greenhouse populations. The number of mite eggs at different levels of mite infestation on young leaves of French bean plants (ie developing populations) was estimated by randomly selecting approximately 20 leaves infested with different levels of twospotted mite from the glasshouse culture and cutting 2.5 cm diameter discs from them. Mite egg numbers on these discs were counted using a stereomicroscope. It was noted that young leaves showing marked symptoms of mite feeding (i.e very heavy populations) contained > 40-50 mite eggs/ disc, while heavy, moderate, light and very light infestations had approximately 30-40, 20-30, 10-20, and < 10 mite eggs/ disc respectively. The number of mite eggs consumed and the development of each *S. vagans* individual were recorded 12 hourly. The predators were transferred to new dishes containing the appropriate number of mite eggs every 24 hours. Any larval instar that survived for some time but was unable to moult or pupate and eventually died was excluded from the data.

A separate series of investigations were run for ovipositing female *S. vagans* at eight levels of *T. urticae* eggs (from 0 to 200) similarly to that described in Section 5.3.4. Ten predators were confined individually at each density in modified petri dishes and observed 8 hourly for five consecutive days. Every 24 hours the predators were transferred to new dishes containing the same commencing number of prey. To establish adult *S. vagans* functional response to adult *T. urticae*, 20 ovipositing *S. vagans* females were confined individually in modified petri dishes at 5, 10, 20 and 30 adult female mites on 2.5-cm leaf discs. Leaf discs were cut from mite-free French bean plants, which were infested with the appropriate number of adult *T. urticae* prior to exposure to *S. vagans* females. These discs were observed 12 hourly, and

the predators were transferred to a new arena after 24 hours, with the number of prey remaining counted and recorded. This experiment was run for 5 consecutive days.

The numerical response of adult females *S. vagans* was also studied at various prey densities with *T. urticae* eggs. Newly emerged females were kept with males and fed for one week. The female predators were then exposed individually in modified petri dishes to 0, 10, 25, 50, 75, 100, 125, 150 and 200 mite eggs per day. Predators in the control treatment were provided with all stages of twospotted mite. All predators were observed at 12 hourly intervals and any prey eggs consumed were recorded as well as predators eggs oviposited. The beetles were examined twice daily, because at low prey levels they were observed to consume their own eggs. They were transferred to new level of prey with relevant number of eggs every24 hours. Eggs oviposited were recorded at 12 hourly intervals and the investigations were conducted over a five day period.

5.4.4 Statistical Analysis

Analysis of variance (ANOVA) was used to determine whether there was any significance difference in *S. vagans* longevity and fecundity associated with different alternative hosts. Where significant differences occurred means were separated by using Least Significant Difference (LSD). ANOVA was also used to identify significant differences between mean predation rates and handling time as well as to determine significance differences in the rate of prey consumption between the motile stages of *S. vagans*. In all cases the statistical package CoStat (CoHort Software P.O.19272, Minneapolis, MN 55419, USA) was used. Graphs were drawn using Origin 4.1 (Software for Technical Graphics and Data Analysis for Windows).

5.5 RESULTS

5.5.1 Alternative Hosts

The effect of alternative hosts on adult *S. vagans* survival, mating frequency, fecundity, and egg hatchability is summarised in Table 5.1. Mating was not recorded in any host treatment except for broad mites and twospotted mites. No oviposition was therefore recorded on any alternative hosts. The mean mating frequency recorded with twospotted mite was 16 times more than for broad mite, while fecundity was 9 times higher with the same host. Egg hatchability and daily egg production was also greater on the primary host, *T. urticae*. Broad mite was the only alternative prey treatment in which viable eggs were laid. A few eggs were laid in the pollen & water treatment, but they did not hatch and were considered to be non-viable. There were also significant differences in adult *S. vagans* longevity associated with different alternative hosts. It was highest in the broad mite treatment and lowest in the starved and rust mite treatments, while it significantly increased in pollen & water, honey & water, predatory mite, water, white-fly and aphid treatments, respectively (Fig 5.1).

Table 5.1 Mean mating, fecundity, egg hatchability and adult longevity in starved adult *S. vagans* with different food sources.

	n	Mating	Fecundity	%Hatch	Egg/♀/day	Adult Longevity	
		Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Mean ± S.E.	Female Mean ± S.E.	Male Mean ± S.E.
Starved	20	0.0	0.0	0.0	0.0	4.9 ± 0.3^{a}	3.7 ± 0.4^{a}
Water	20	0.0	0.0	0.0	0.0	9.0 ± 0.7^{b}	7.6 ± 0.5^{b}
Honey & Water	20	0.0	0.0	0.0	0.0	18.9 ± 2.3^{c}	17.7 ± 2.3^{c}
Pollen & water	20	0.0	4	0.0	0.4	21.1 ± 2.7^{cd}	18.2 ± 3.0^{c}
Aphid	20	0.0	0.0	0.0	0.0	5.0 ± 0.5^{a}	3.5 ± 0.1^{a}
Whitefly (egg)	20	0.0	0.0	0.0	0.0	5.9 ± 0.6^{ab}	4.0 ± 0.4^{a}
Predatory Mite	20	0.0	0.0	0.0	0.0	7.2 ± 0.3^{ab}	6.7 ± 0.2^{ab}
Rust Mite	20	0.0	0.0	0.0	0.0	23.5 ± 1.3^{de}	22.7 ± 0.9^{d}
Broad Mite	20	1 ± 0.0	10.1 ± 1.0	67.0 ± 2.6	2.98 ± 0.7	26.1 ± 0.9^{e}	25.7 ± 0.9^{e}
Twospotted	20	16	90.7 ± 4.3	81.6 ± 8.1	6.23 ± 6.4	38.0 ± 6.0^{f}	37.5 ± 4.4^{f}

* Numbers followed by similar letters are not significantly different at $p \leq 0.05\%$

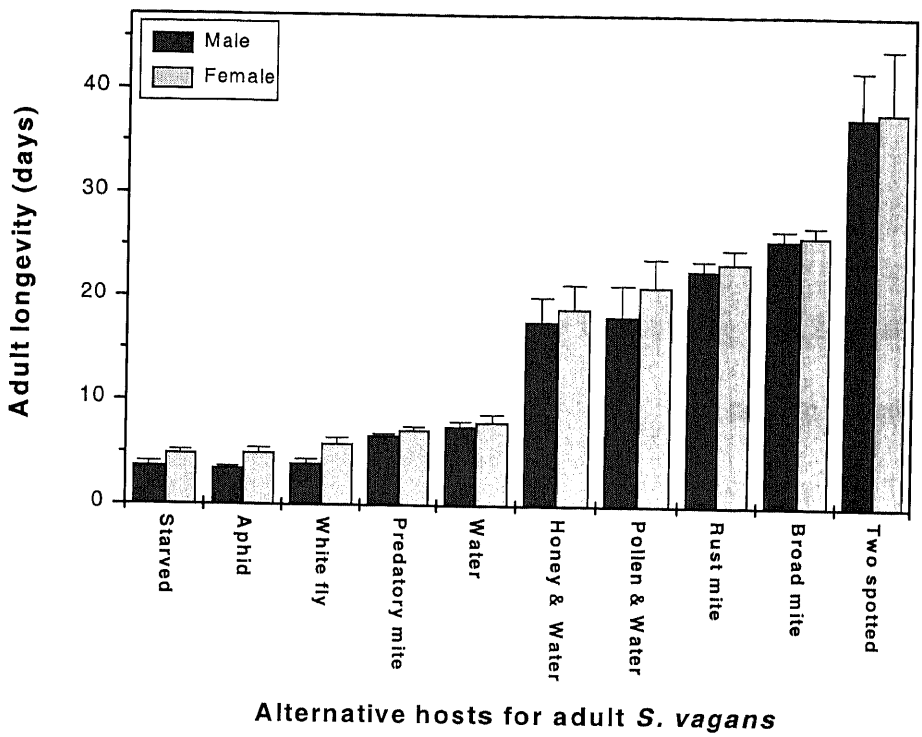

Fig 5.1 Mean adult longevity of *S vagans* with different hosts.

5.5.2 Time Partitioning

The results for time partitioning behaviour are presented in Table 5.2. Satiated adult *S. vagans* spend most of their time resting (60.3 ± 5.3%), followed by walking (30.5 ± 5.6%), feeding (4.97 ± 0.7%) and searching (4.23 ± 0.8%). Adults starved for 24 & 48 hours spent a higher proportion of their time feeding (i.e. 46.7 ± 8.6% & 54.9 ± 6.5% respectively). This was followed by searching, resting, and walking with proportions of 28.9 ± 7.7 & 27.9 ± 6.4, 13.4 ± 1.7 & 9.5 ± 1.5, and 10.4 ± 1.7 & 3.9 ± 0.6% respectively. They were also recorded drinking water, although for short periods (0.6 ± 0.4% & 3.9 ± 1.2 % at 24 & 48 hours starved respectively) (Fig 5.2).

Adults tested within 24 hours of emergence spent most of their time either resting (44.4 ± 5.2%) or walking (40.7 ± 5.3%), and very little time searching (7.6 ± 0.6%) or feeding (7.3 ± 0.6%). There was no significant difference between behaviour of male and female adults in any category, whether satiated, starved or newly emerged.

The mean time spent by satiated 4th instar larvae in searching was 2.9 ± 0.8 %, feeding 2.8 ± 0.6 %, resting 65.4 ± 6.5 % and walking 28.9 ± 5.8 %. This pattern differed greatly in larvae starved for 24 hours, which spent significantly more time searching (30.5 ± 6.3) and feeding (47.7 ± 5.8) than resting (18.9 ± 2.2) and walking (2.9 ± 1.3 %). This pattern was even more pronounced in the 48 hour starved larvae with results recorded in the above categories recorded as 33.5 ± 4.2, 56.5 ± 3.8, 10.0 ± 1.7 % and 0.0% respectively. Newly emerged 4th instars spent more time resting (41.1 ± 5.9 %) with only 8.7 ± 0.9 % spent on searching, 9.3 ± 0.6 % for feeding, 40.9 ± 6.0% and 0.0% for walking (Table 5.2).

Both adults and 4th instar larvae consumed more eggs > nymphs > adults of *T. urticae* irrespective of whether they were fully fed, starved or newly emerged (Table 5.3). Satiated adult *S. vagans* ate more eggs (82.0 ± 5.1%) than nymphs (11.0 ± 4.3%) and adult (6.8 ± 2.7%) prey. Predation on eggs was lower in adult predators starved for 24 and 48 hours, however numerical consumption of eggs was still higher than of nymphal or adult mites. Newly emerged 4th instar larvae also preferred eggs to all other mite stages and consumed the highest percentage of eggs (83.0 ± 4.8%) compared with fully fed (70.0 ± 1.2%) and starved larvae (73.3 ± 4.4 & 69.6 ± 4.3% for 24 & 48 hours starved respectively). However the total consumption of nymphs and adult *T. urticae* decreased in satiated larvae compared with newly emerged larvae.

Table 5.2 Percentage time spent by *S. vagans* in various activities when provided *T. urticae* as prey.

Stages	n	Searching	Feeding	Resting	Walking	Drinking Water
		Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE	Mean ± SE
Satiated adult	30	4.2 ± 0.8^{a}	5.0 ± 0.7^{a}	60.3 ± 5.3^{c}	30.5 ± 5.6^{bc}	0.00^{a}
Starved adult (24hour)	30	28.9 ± 7.7^{cd}	46.7 ± 8.6^{b}	13.4 ± 1.7^{a}	10.4 ± 1.7^{a}	0.6 ± 0.4^{a}
Starved adult (48hour)	30	27.9 ± 6.4^{cd}	54.9 ± 6.5^{b}	9.5 ± 1.5^{a}	3.9 ± 0.6^{a}	3.9 ± 1.2^{b}
Newly emerged adult	30	7.6 ± 0.6^{ab}	7.3 ± 0.6^{a}	44.4 ± 5.2^{b}	40.7 ± 5.3^{c}	0.0
Satiated 4th instar	30	2.9 ± 0.8^{a}	2.8 ± 0.6^{a}	65.4 ± 6.5^{c}	28.9 ± 5.8^{b}	0.0^{a}
Starved 4th instar (24h)	30	30.5 ± 6.3^{cd}	47.7 ± 5.8^{b}	18.9 ± 2.2^{a}	2.9 ± 1.3^{a}	0.0^{a}
Starved 4th instar (48h)	30	33.5 ± 4.2^{e}	56.5 ± 3.8^{b}	10.0 ± 1.7^{a}	0.0^{a}	0.0^{a}
Newly emerged 4th instar	30	8.7 ± 0.9^{ab}	9.26 ± 0.6^{a}	40.94 ± 6^{b}	41.1 ± 5.9^{c}	0.0^{a}

* Numbers in same columns followed similar letters are not significantly different at $p \leq 0.05\%$

Table 5.3 Mean number and percentage of stages of *T. urticae* consumed by *S. vagans* adults and 4th instar larvae.

Stages	Feeding							
	Egg		Nymph		Adult		Total	
	No. eggs	% eggs	No. nymph	% nymph	No. adults	% adults	Mean No	% Total
Satiated adults	5.4 ± 0.7	82.0 ± 5.1	1.2 ± 0.1	11.3 ± 4.3	1.0 ± 0.0	6.8 ± 2.7	6.4 ± 0.6	100
Starved adults (24hour)	12.5 ± 1.5	77.6 ± 5.3	2.6 ± 0.4	17.8 ± 3.1	1.4 ± 0.2	4.6 ± 1.8	15.8 ± 1.3	100
Starved adults (48hour)	7.4 ± 5.8	54.7 ± 2.6	3.0 ± 2.0	25.5 ± 14.6	1.9 ± 1.2	19.8 ± 17.8	12.1 ± 6.5	100
Newly emerged adults	6.4 ± 0.6	89.4 ± 3.2	0.6 ± 0.2	8.6 ± 3.2	0.2 ± 0.1	3.7 ± 2.5	7.1 ± 0.5	100
Satiated 4th instars	3.8 ± 0.7	70.1 ± 1.2	1 ± 0.0	6.9 ± 2.9	1.0 ± 0.0	3.1 ± 2.1	4.4 ± 0.9	100
Starved 4th instars (24h)	25.8 ± 2.6	73.3 ± 4.4	6.5 ± 1.0	19.4 ± 3.2	2.5 ± 0.5	7.3 ± 1.4	34.8 ± 2.0	100
Starved 4th instars (48h)	31.4 ± 3.0	69.6 ± 4.3	10.8 ± 1.7	24.9 ± 4.1	2.1 ± 0.3	4.8 ± 0.7	44.6 ± 2.4	100
Newly emerged 4th instar	6.7 ± 0.6	83.6 ± 4.8	0.8 ± 0.2	12.4 ± 3.4	0.2 ± 0.1	4.0 ± 2.7	6.9 ± 0.9	100

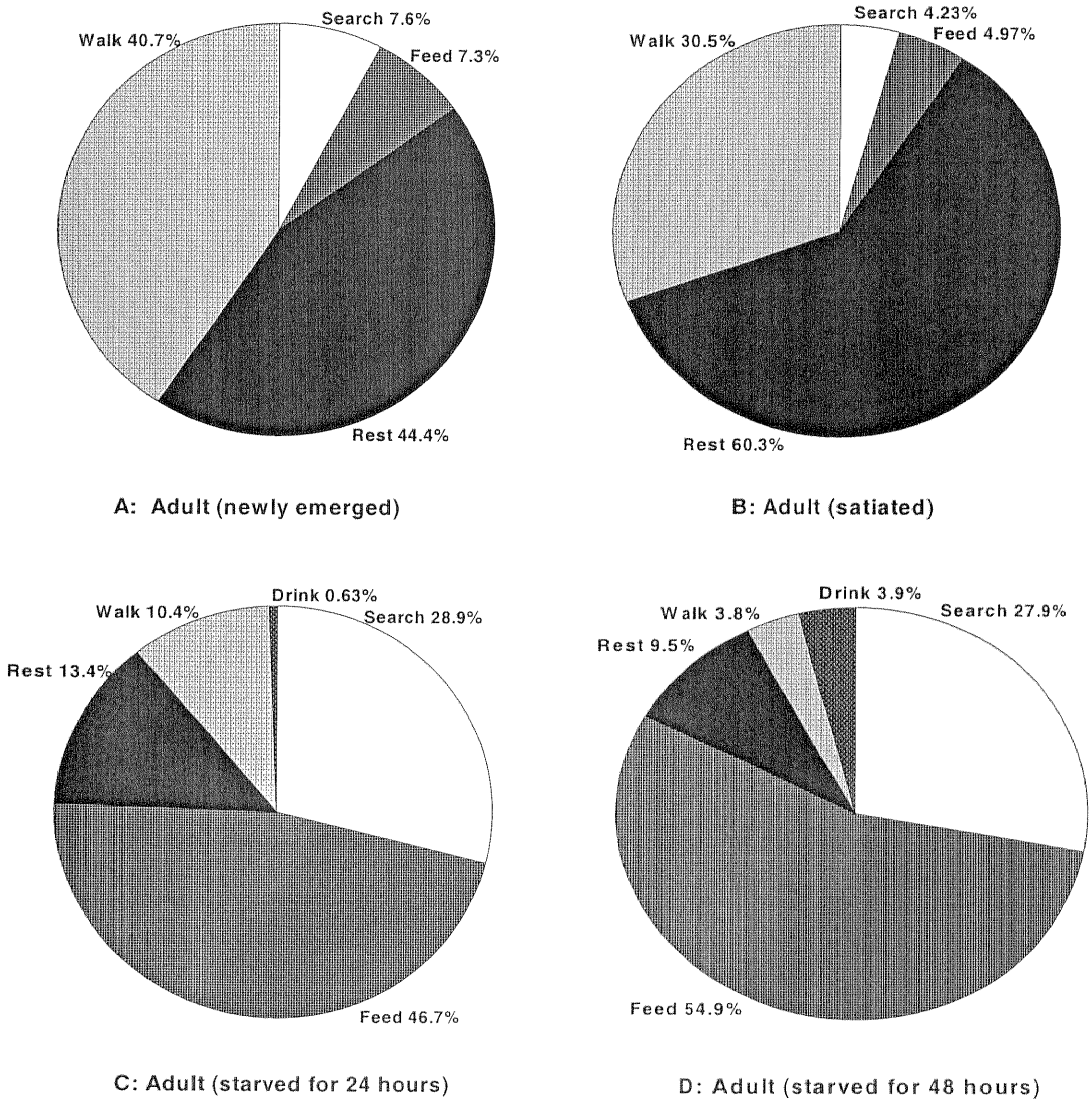

Fig 5.2 Proportion of time spent by adult *S. vagans* in different activities when provided with *T.urticae* as prey.

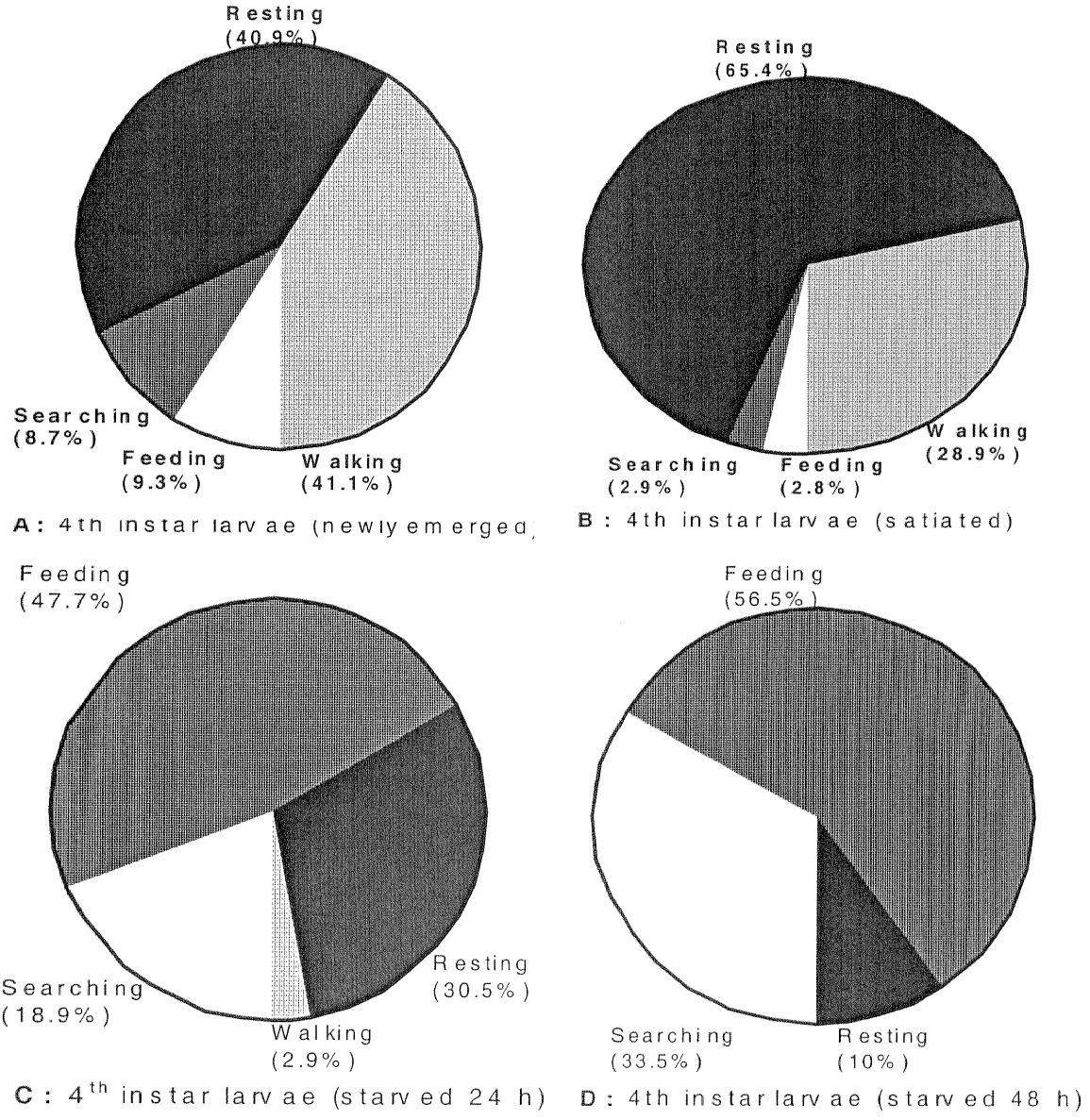

Fig 5.3 Proportion of time spent by fourth instar larvae of *S. vagans* in different activities when provided with *T.urticae* as prey.

5.5.3 Rate of prey consumption

The rates of consumption by immature and mature stages of *S. vagans* when provided surplus *T. urticae* eggs are given in Table 5.4. The 4th instar larva was the most voracious stage followed by 3rd, 2nd and 1st larval instars. The mean number of mite eggs consumed by 1st, 2nd, 3rd, and 4th instar larvae was 27.0 ± 1.1, 50.0 ± 1.0, and 71.0 ± 1.5 and 152.4 ± 1.7 per larva in a 24 hour period, respectively. Ovipositing adult females consumed more than did pre-ovipositing and post-ovipositing females (viz. mean 142.7 ±0.5, 94.3 ± 0.5 and 57.1 ± 0.6 eggs per day respectively), while males consumed 63.5 ± 0.4 eggs per day (Table 5.4).

Table 5.4 Mean number of *T. urticae* eggs consumed by various stages of *S. vagans*.

Predator Stages	Number observed n	Mite eggs consumed /day /individual Mean ± S.E.
Larva		
1st instar larva	30	27.9 ± 1.1[a]
2nd instar larva	25	50.1 ± 1.0[b]
3rd instar larva	25	71.6 ± 1.5[e]
4th instar larva	25	152.4 ± 1.7[h]
Adult		
Male	20	63.5 ± 0.4[d]
Female		
Pre-oviposition	20	94.3 ± 0.5[f]
Oviposition	20	142.7 ± 0.5[g]
Post-oviposition	20	57.1 ± 0.6[c]

Numbers in same columns followed similar letters are not significantly different at $p \leq 0.05\%$

5.5.4 Functional Response

The functional response and development of immature stages of *S. vagans* were studied at nine prey density levels. Newly emerged larvae failed to survive more than one day without food; therefore "zero prey density" was not included in the data analysis.

The results of the functional response of all larval instars of *S. vagans* are presented in Table 5.5. The response of the 1st, 2nd and 3rd instars increased linearly from low to medium prey densities and then plateaued for higher densities (i.e 150 & 200 mite egg /density), while for 4th instars it increased linearly until it reached a plateau at the highest density (i.e 200 mite eggs /density) (Fig. 5.3). There was no significant difference in the response of adult *S. vagans* to mite eggs at low prey densities, because in the 10 and 25 mite egg treatments (per petri dish for 24 hours), they were able to be consumed within the first 8 hours of the feeding period. In the 50 mite egg treatment adult *S. vagans* were able to completely consume all prey within 16 hours and in the 100 egg treatment most eggs were consumed within 24 hours. The consumption response increased linearly at low prey densities until it plateaued at higher prey densities (Table 5.6, Fig 5.4). A similar response was observed when adult *S. vagans* were fed on adult mite prey. The number of adult *T. urticae* consumed by adult female *S. vagans* increased linearly from low (5 mites/day) prey density until it reached a plateau at higher prey densities (10-30 mites/day) (Fig.5.5). The mean number of mites consumed by a *S. vagans* female at the various prey densities tested was 4.5 ± 0.1, 7.9 ± 0.3, 8.6 ± 0.3 and 8.2 ± 0.3 per day respectively (Table 5.7).

Development of immature stages of *S. vagans* was also observed at all eight prey densities. The larval instars consumed all mite eggs at lower prey densities within the 24 hour

investigation period. The minimum number of prey eggs required for normal development of each instar is shown in Table 5.8. The greatest number of prey was required for completion of the 4th larval instar followed by the 3rd instar. There were differences in the relative response of 2nd instar larvae, depending on prey density. In general, more prey were consumed at high prey densities to complete each *S. vagans* instar. For 1st instar larvae this difference was almost 5 fold (ie. from prey density of 2 to 200 mite eggs), for 2nd instars 4 fold (from 2 to 200 mite eggs) and for 3rd and 4th instars 2 fold (from 10 to 200 mite eggs).

No 2nd instar larva completed development at prey levels < 5 eggs /day and no 3rd or 4th instar larvae completed their development at < 10 mite eggs /day.

Table 5.5 Functional response of immature stages of *S. vagans* to prey density (number of *T. urticae* eggs consumed per day).

Stages	Number of predatory days	Prey density (mite eggs /day)							
		2	5	10	25	50	100	150	200
1st instar		2.0 ±0.0	4.8 ± 0.1	9.9 ± 0.1	20.6 ± 0.4	19.0 ± 1.3	23 ± 0.7	24.1 ± 0.7	24.4 ± 0.8
	N*	93	49	38	31	31	35	35	34
2nd instar		2.0 ±0.0	5.0 ± 0.1	9.8 ± 0.1	21.6 ± 0.5	37.2 ± 1.6	40.1 ± 0.2	42.28 ± 2.1	44.9 ± 1.8
	N*	3	51	31	22	21	23	23	21
3rd instar		-	5.0 ± 0.0	9.9 ± 0.0	25.0 ± 0.0	46.7 ± 0.7	54.5 ± 3.5	62.9 ± 2.6	67.0 ± 2.6
	N*	-	16	73	28	31	22	25	22
4th instar*		-	-	10.0 ± 0.0	25.0 ± 0.0	49.5 ± 2.0	91.8 ± 1.1	138.0 ± 2.3	146.0±2.7
	N*			125	64	46	44	39	37

N*= Number of predator-days (replicates 15 X days) for each density level

Table 5.6 Functional response of adult *S. vagans* to *T. urticae* egg density at 25 ±2°C.

Prey density (Mite egg/day)	Number of prey (mite eggs)consumed			Proportion
	8 hour*	16 hour*	24 hour*	
10	9.8 ± 0.0	9.9 ± 0.1	10.0 ± 0.0	1
25	24.9 ± 0.1	25.0 ± 0.0	25.0 ± 0.0	1
50	41.0 ± 1.0	50.0 ± 0.0	50.0 ± 0.0	1
75	43.0 ± 0.9	72.3 ± 0.6	75.0 ± 0.0	1
100	43.98 ± 4.6	83.7 ± 0.6	93.9 ± 0.8	0.94
125	53.5 ± 0.7	97.2 ± 0.7	105.9 ± 0.6	0.85
150	41.0 ± 0.8	102.0 ± 0.9	111.6 ± 1.3	0.74
200	52.2 ± 1.3	112.8 ± 1.2	134.7 ± 1.5	0.67

*Each reading is the mean of 50 predatory days (i.e. 10 replicates x 5 days)

Table 5.7 Functional response of adult *S. vagans* to adult *T. urticae* density at 25 ± 2°C.

Prey density (adult mite /day)	Number of observations n	Total (mites eaten /day)	Mean (mites eaten /day/*S. vagans*)
5	20	90	4.5 ± 0.1
10	20	158	7.9 ± 0.3
20	20	171	8.6 ± 0.3
30	20	164	8.2 ± 0.3

Table 5.8 Total number of eggs consumed by each immature stage of *S. vagans* at various prey densities and total number of eggs required to complete development at 25°C.

Stages	Number of observations	Prey density (mite eggs /day)							
		2	5	10	25	50	100	150	200
1st instar		12.7 ± 0.3	15.4 ± 0.2	23.3 ± 1.1	35.1 ± 1.5	41.4 ± 1.5	55.8 ± 1.9	61.8 ± 1.99	64 ± 2.7
	N	15	15	15	15	15	15	15	15
2nd instar		3	16.3 ± 0.3	21.0 ± 1.0	29.2 ± 1.4	43.6 ± 2.1	50.8 ± 3.6	55.5 ± 3.8	66.6 ± 3.7
	N		10	15	15	15	15	15	15
3rd instar				49.6 ± 0.2	40.3 ± 1.4	92.4 ± 4.1	66.2 ± 5	90.4 ± 3.9	94.0 ± 6.0
	N			11	15	15	15	15	15
4th instar				142.0 ± 1.7	150.0 ± 1.9	107.8 ± 5	209.7 ± 5	281.0 ± 9.5	272.0 ± 9.6
	N			11	15	15	15	15	15

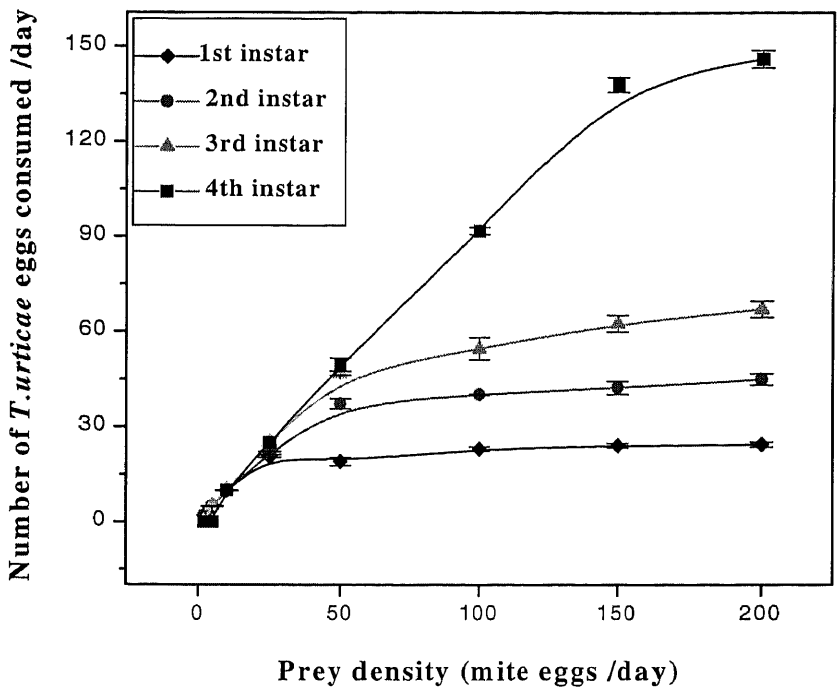

Fig. 5.4 Functional response of immature stages of *S. vagans* to *T. urticae* egg density.

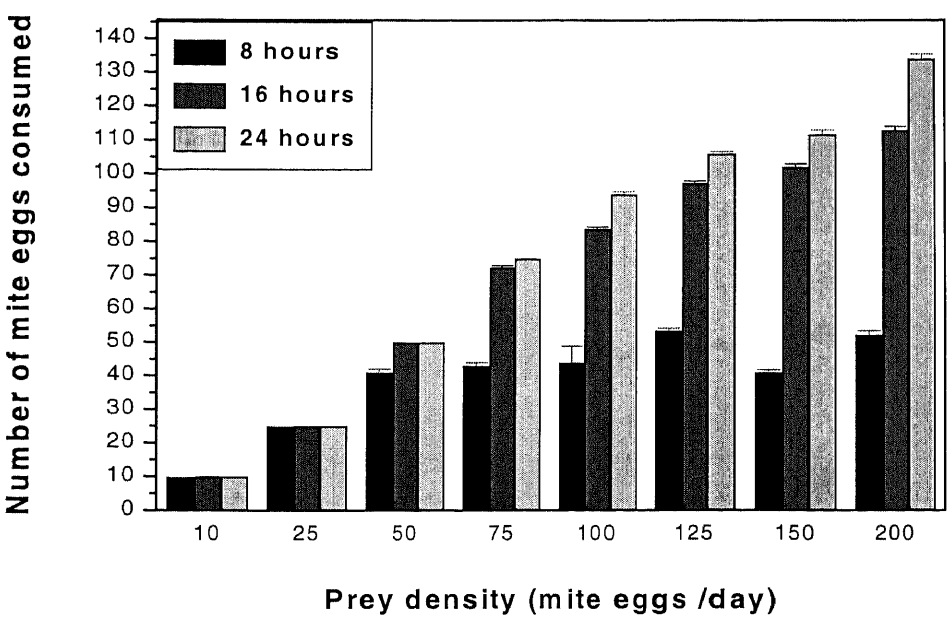

Fig. 5.5 Functional response of adult *S. vagans* to *T. urticae* egg density.

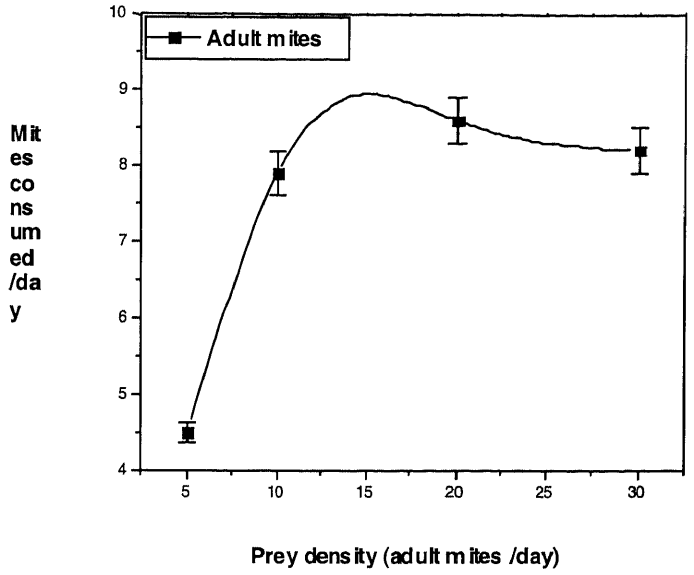

Fig 5.6 Functional response of adult *S. vagans* to adult *T. urticae*.

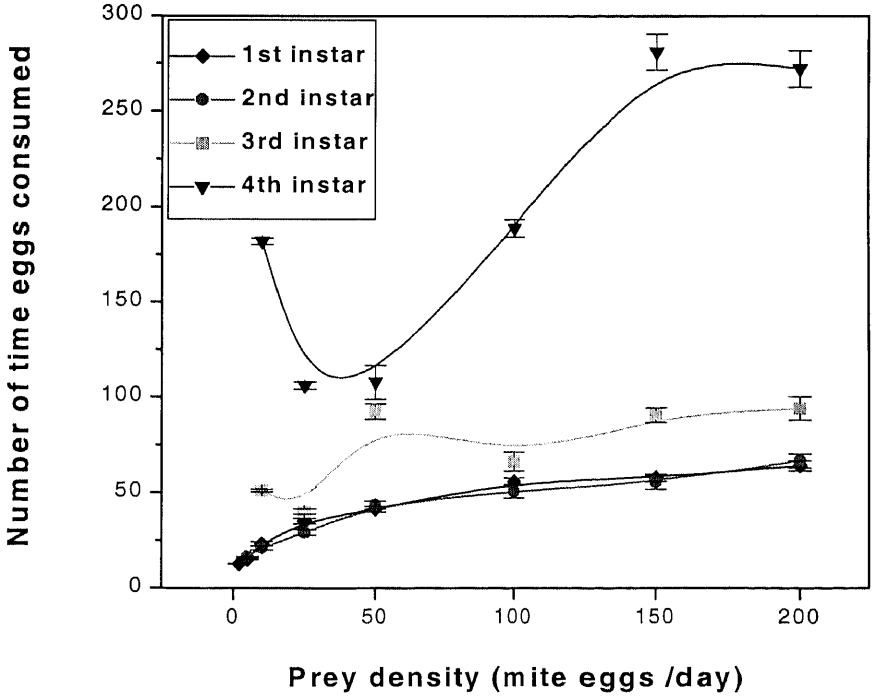

Fig. 5.7 Number of prey required by immature stages of *S. vagans* for development.

5.5.5 Numerical response

The developmental time and survival of preimaginal stages of *S. vagans* were both strongly influenced by prey density. The relative development time for all immature stages combined (ie. 1st instar emergence to pupation) was 17.9 days at low prey density (25 mite eggs /day). There was no significant difference in rate of development at higher prey densities. However survival rate was significantly higher at prey densities of 100 eggs/day or above (Table 5.9, Fig 5.7).

The minimum number of prey required for survival of 1st, 2nd, 3rd and 4th larval instar survival were 10, 25, 100 and 100 eggs /day respectively. Without prey no development occurred and the 1st instar larvae could not survive more than one day, while only 15% of 1st instar larvae completed this stage at the lowest prey density (2 mite eggs /day), but died soon after moulting. At low prey levels (i.e. 5 mite-eggs per day) 75% of the 2nd instar larvae completed this stage but could not survive to the 3rd instar. Only 3 out of 16 larvae completed the 4th instar at 10 mite eggs /day, with a mean development period of 18 days. The minimum number of prey needed for 90 % survival of 1st, 2nd, 3rd and 4th instars were 2-5, 7.5, 17.5 and 37.5 mites per day respectively (Table 5.10). Maximum survival (100%) of immature stages was recorded only at prey densities of ≥ 100 eggs /day, while none reaching the pupal stage that had consumed less then 25 mite eggs /day.

The numerical response of adult female *S. vagans* was also examined using the parameters of survival and reproduction in response to changes in prey density (Table 5.11). Adults could not survive after the 5th day of starvation, but they could survive for short periods (11 days) at low prey density (i.e. 10 mite eggs /day). Adult *S. vagans* responded to prey density. Both mean gross and mean net fecundity for adult females was low at low prey densities, but

increased linearly with increasing prey density, plateauing at high densities (i.e. 200 mite eggs /day) (Table 5.11). Gross fecundity was higher than net fecundity at lower prey densities, because the predators ate their own eggs. However the net fecundity increased consistently with prey number until it equalled gross fecundity at the highest prey density. It required 100 ± 0.0 mite eggs at low prey density (10 mite eggs/ day) and 18.8 ± 0.3 mite eggs at high prey density (200 mite eggs /day) to produce one *S. vagans* egg (Table 5.12).

Table 5.9 Number of days required for immature stages of *S. vagans* to complete development at various *T. urticae* densities at 25 ± 2°C.

Stages	Number of individuals commencing each stage	Prey density (mite eggs /day)							
		2	5	10	25	50	100	150	200
1st instar		6.2 ± 0.1	3.3 ± 0.6	2.5 ± 0.1	2.1 ± 0.9	2.1 ± 0.1	2.3 ± 0.7	2.3 ± 0.1	2.2 ± 0.1
	N	15	15	15	15	15	15	15	15
2nd instar			3.4 ± 0.8	2.1 ± 0.9	1.5 ± 0.8	1.4 ± 0.1	1.6 ± 0.3	1.5 ± 0.1	1.4 ± 0.1
	N	3	10	15	15	15	15	15	15
3rd instar				4.9 ± 0.7	1.8 ± 0.6	2.0 ± 0.1	1.5 ± 0.1	1.6 ± 0.9	1.5 ± 0.1
	N			11	15	15	15	15	15
4th instar				15.0 ± 0.5	4.3 ± 0.8	3.1 ± 0.1	2.9 ± 0.6	2.6 ± 0.1	2.5 ± 0.1
	N				13	14	15	15	15
Pupa					4.3 ± 0.1	3.9 ± 0.1	3.6 ± 0.1	3.5 ± 0.7	3.5 ± 0.1
	N				12	13	15	15	15
Total									

Table 5.10 Numerical response of immature stages of *S. vagans* to *T. urticae* densities and their survival to the next stage at 25 ± 2°C.

Predator stages	Prey density (mite eggs /day)							
	2	5	10	25	50	100	150	200
1st instar larva	15	100	100	100	100	100	100	100
2nd instar larva	0	75	100	100	100	100	100	100
3rd instar larva	0	0	80	100	100	100	100	100
4th instar larva	0	0	15	90	95	100	100	100
Pupa	0	0	0	85	90	100	100	100

Table 5.11 Numerical response and conversion of prey into progeny by female *S. vagans* at different prey (*T. urticae*) densities.

Prey density (mite eggs/day)	Mean mite eggs consumed/• /day	Eggs laid/• /day (gross fecundity)	Eggs surviving/• /day (net fecundity)	Mite eggs consumed /*S. vagans* egg laid	Mite eggs consumed /S. vagans surviving egg
10	10.0 ± 0.0	0.2 ± 0.0	0.1 ± 0.0	50.0 ± 0.0	100.0 ± 0.0
25	25.0 ± 0.0	0.6 ± 0.1	0.2 ± 0.0	43.0 ± 3.7	125.0 ± 0.0
50	50.0 ± 0.0	2.2 ± 0.1	0.8 ± 0.1	23.3 ± 1.0	67.4 ± 6.1
75	75.0 ± 0.0	3.3 ± 0.1	2.1 ± 0.2	23.3 ± 0.8	40.1 ± 3.8
100	93.9 ± 0.7	5.8 ± 0.1	4.8 ± 0.1	16.1 ± 0.3	19.7 ± 0.4
125	121.8 ± 0.4	6.7 ± 0.2	5.7 ± 0.1	18.3 ± 0.5	21.5 ± 0.6
150	127.7 ± 0.9	7.0 ± 0.1	6.8 ± 0.1	18.3 ± 0.2	18.8 ± 0.3
200	139.0 ± 0.5	7.5 ± 0.1	7.5 ± 0.1	18.8 ± 0.3	18.8 ± 0.3

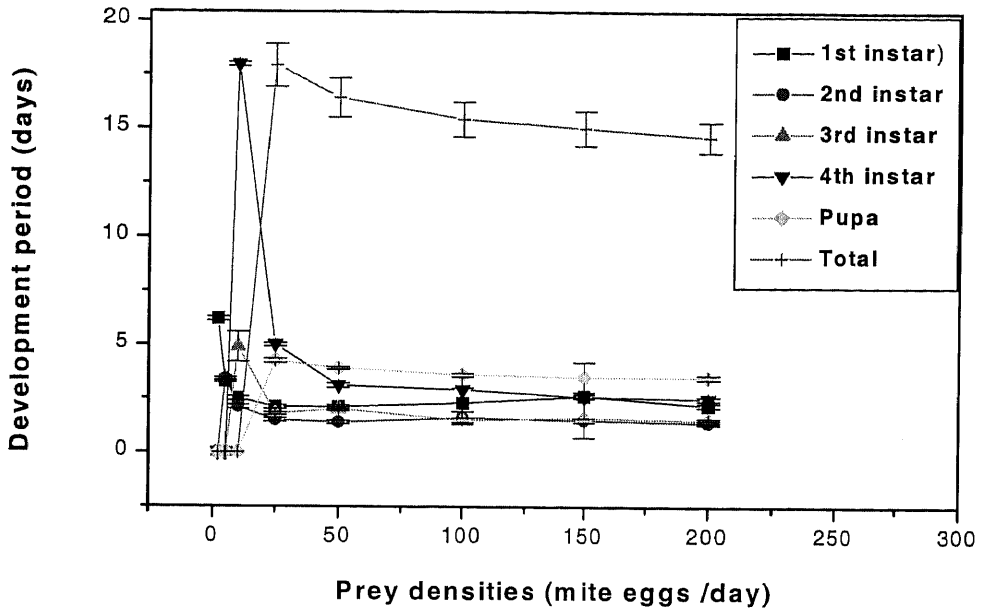

Fig 5.8 Number of days required for immature stages of *S. vagans* to complete their development at various prey densities.

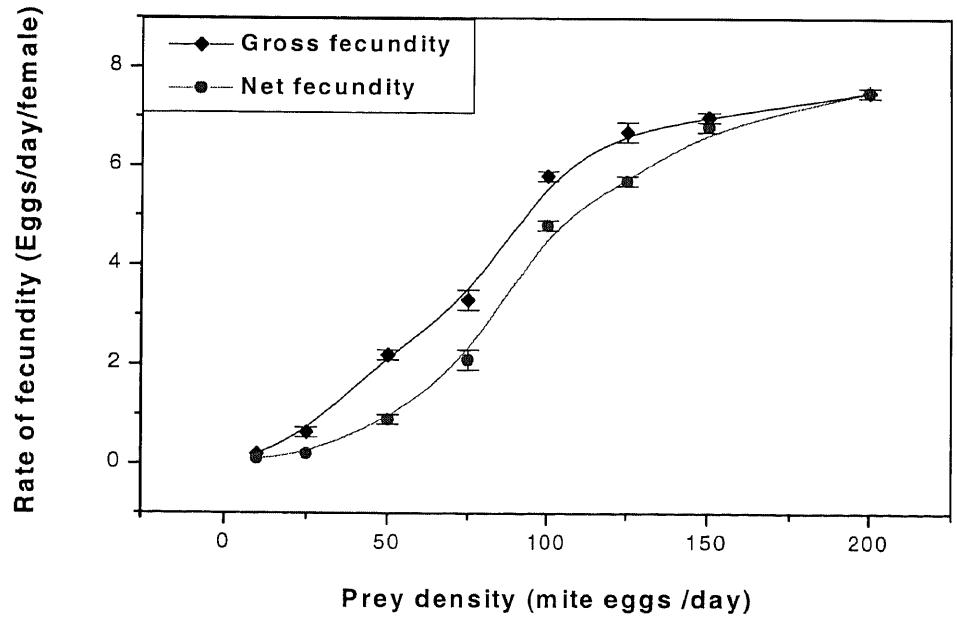

Fig 5.9 Numerical response of adult female *S. vagans* to prey densities.

5.6 DISCUSSION

5.6.1 Alternative prey to *T. urticae*

No mating or oviposition was recorded for *S. vagans* on any host, except for pollen & water, broad mite and twospotted mite (control). The mean number of eggs laid per female when reared on pollen & water and broad mites was very low (4.0 ± 0.4 and 10.1 ± 1.0, respectively) when compared with *T. urticae* (90.7 ± 4.3). Eggs laid in the pollen & water treatment did not hatch, whereas egg hatchability in the broad mite treatment was 67 %. This was, however, significantly less than that recorded in the *T. urticae* treatment (81.6%) (Table 5.1). Adult longevity increased compared with the starved control, with the provision of alternative hosts such as water, honey & water, aphids and white fly eggs but no reproduction occurred on these hosts. Adult longevity in relation to host was *T. urticae* > broad mites > rust mites > pollen & waters > honey & water > water > *P. persimilis* eggs > white flies > aphids > starved (control). However no significant difference in longevity was recorded between adults fed on aphids or white flies. There was no significant difference recorded between male and female longevity on any of the above diets.

Our results with *S. vagans* are similar to reports of authors working with other *Stethorus* spp. such as Putman (1955a) and Kehat (1967) who concluded that *S. punctillum* fed on aphids, phytoseiid mites and scale insects but these hosts were not sufficient for development or oviposition, and Kamiya (1966) and Hoy *et al.* (1979) who reported that *S. japonus* fed on plant resins, sweet foliar secretions, and honey & water, which increased longevity but did not result in copulation or reproduction.

In this study broad mite was found to be the only effective alternative host for *S. vagans*, not only increasing adult longevity, but also producing fertile eggs. While the other alternative hosts did not support reproduction, all significantly increased adult longevity. Therefore these alternative prey may assist in sustaining *S. vagans* in localities where its primary host is in low in numbers or diapausing.

It appears from the results that *T. urticae* is an important food source for *S. vagans*, not only for adult survival but also for successful reproduction. A puzzling aspect is that *S. vagans* is native to Australia, but *T. urticae* and broad mites are introduced species; therefore it remains unclear that what was the original host(s) of *S. vagans* before the introduction of these species. Other mite species native to Australia such as tydeids, oribatids, stigmatideids and tetranychids, such as *T. ludeni* may have been primary hosts of *S. vagans* before the introduction of *T. urticae* to Australia. None of these species were included in these investigations.

5.6.2 Time partitioning and stage preference

The results in Table 5.2 show that adult *S. vagans* spent more time searching and feeding when starved for 24 or 48 hours than when satiated or newly emerged. Adults starved for 48 hours spent more time drinking water than when starved for only 24 hours. Less drinking also occurred in 48 hour starved adults once they had a successfully prey capture and ingested haemolymph. However they also attacked a greater proportion of adult mites under these conditions, in which case each individual provided more food than did individual prey eggs or nymphs. Beetles starved for 48 hours occasionally regurgitated the mite haemolymph back into the prey's body for a considerable time after commencing feeding.

Larvae of *S. vagans* responded similarly to adults with respect to time partitioning. Fourth instar larvae starved for 24 & 48 hours spent more time searching and feeding than did fully fed and newly emerged larvae. The proportion of time spent searching and feeding by larvae starved for 24 hours was greater than for the larvae starved for 48 hours (Table 5.2). The reason may be that 48 hour starved larvae attacked more motile stages than those starved for 24 hours, which may have provided more haemolymph for nourishment than did eggs.

All stages of *S. vagans* had a strong preference for mite eggs when given a choice of all mite stages (Table 5.3). Starved predators consumed more motile stages of *T. urticae* than did satiated ones, but this was still a lower proportion than eggs. Satiated and newly emerged predators were more selective and preferred eggs to other mite stages which they encountered during searching. Houck (1991) recorded similar results for satiated adult *S. punctum*, which consumed 92.7% eggs, 3.6% nymphs and 3% adult *T. urticae* respectively, while for 24 hour starved adults these figures were 88.4, 3.6, 7.5% respectively. Therefore its appears that *Stethorus* spp. have a strong preference for eggs when satiated, but this preference changes (ie. they become less selective) when they are starved.

The process of feeding consists of piercing prey, sucking haemolymph and its regurgitation back into the body of the captured prey. It also includes the predator chewing and consuming body. In a number of encounters motile mites were obsessive being attacked and even damaged, but still were able to escape from their predators. This was more common when the predators were satiated or newly emerged.

5.6.3 Rate of consumption

The rate of prey consumption for all motile stages of *S. vagans* was similar to those recorded for relative stages of *S. nigripes* (Richardson 1977). However the consumption rate was less than that reported for some other species of *Stethorus*, namely *S. punctillum* and *S. punctum* (Putman 1955a; Hull *et al.* 1977b). This is possibly because *S. vagans* is smaller than these other species.

5.6.4 Functional response

The functional response of all motile stages of *S. vagans* to *T. urticae* increased curvilinearly to a plateau at high prey densities. This type of curve is characteristic of a type-II functional response (Holling 1965), which has been demonstrated in a number of insect and other invertebrate predators to their prey. Richardson (1977) recorded a similar curve for *S. nigripes* with the same host, *T. urticae*. Munyaneza & Obrycki (1997) also reported a type-II functional response for the coccinellid *Colemoegilla maculata* feeding on Colorado potato beetle *Leptinotarsa decemlineata* (Coleoptera: Chrysomelidae).

An interesting functional response has been reported in *S. bifidus* and its prey, *T. lintearius,* in which an increasing proportion of mites were killed as mite density increased, because of decreased mean feeding time and reduced extraction of the body contents. This behaviour was not observed in *S. vagans.*

5.6.5 Numerical response

The mean developmental time for *S. vagans* immature stages was significantly shorter at higher prey densities i.e. 14.7 days at a prey density of 25 mite eggs per day, but only 11.1 days at 200 mite eggs /day. The developmental rate recorded in the control treatment was

13.7 ± 0.2 days at 25 ± 2°C, which is longer than that recorded at higher densities of mite eggs. This may be because more motile stages of prey were present in the control treatment and therefore may be less nutritious than mite eggs or it may be because it is more difficult to locate and capturing motile stages. Another reason is that motile mites may interfere with *Stethorus* activities such as searching or eating, although this was not observed. Our results are very similar to those reported by Richardson (1977) for *S. nigripes*, another Australian species.

The numerical response of larval instars of *S. vagans* was also strongly correlated with mite egg densities (Table 5.8, Fig. 5.6). For 95% survival immature *S. vagans* required 75 mite eggs per day compared with 70 mite eggs /day for *S. nigripes* (Richardson 1977). Both larvae and adults of *S. vagans* had a type-II response at higher prey densities, which has been recorded for a number of insect predators including *S. nigripes* (Richardson 1977).

The reproductive response of adult *S. vagans* females was positively correlated with mite egg densities. The gross and net fecundity increased linearly with mite density and plateaued at high prey density. A similar response was reported for *S. nigripes* (Richardson 1977). The net fecundity rate was lower than gross fecundity because adults ate their own eggs at lower prey densities. Cannibalism ceased at higher prey densities. Cannibalism is a common phenomenon in a number of insect predators especially ladybirds, at low prey densities. Richardson (1977) for example observed the same type of behaviour in *S. nigripes* and Majerus (1994) reported that ladybirds *Adalia bipunctata* and *F. hebraea* fed on their own eggs or early instar larvae if prey was scarce. The cannibalism of *S. vagans* at lower prey densities has important ecological significance. First it provides enough energy and water to

enable predators to survive when prey is scarce. Second it likely to reduce inter-specific competition in the population and hence prevents population collapse.

We was calculated that at lower prey densities *S. vagans* required 25-35 mite eggs to produced an egg, which declined to 16 to 20 mite eggs at higher prey densities. The efficiency in converting prey to predator progeny was correlated with prey density as sigmoid curve (Fig.5.9). These results were supported by Richardson (1977) who reported that *S. nigripes* required 30-40 *T. urticae* eggs to produced one egg.

This is likely to make *S. vagans* an effective predator at all prey densities, as while the number of mite eggs consumed at high prey density is lower and their rate of oviposited under these condition is higher.