CHAPTER 4

BIOLOGY OF *STETHORUS VAGANS*

4.1 ABSTRACT

Aspects of the biology of *Stethorus vagans* were studied at constant and fluctuating temperatures. The constant temperatures used were 12, 15, 20, 25 and 30°C and temperature varied from 12.7-32.1°C, with a photoperiod L:D 16:8 hours and at varying relative humidity (45-80 %). Larvae and adults were regularly fed with all stages of two-spotted mite, *T. urticae*, in modified petri dishes.

Newly laid eggs were translucent white, turning pale yellow after 4-5 hours. The mean egg dimensions were 0.36 x 0.19 mm. Eggs laid by unmated females did not hatch or show any signs of development. After egg hatching the newly emerged larva was white in colour, but soon become pale creamy-white. There were four larval instars, which were differentiated from each other by the presence of shed exuviae and differences in head capsule size. The pre-pupa, not a distinct stage in the life cycle but a quiescent period at the end of the 4th larval instar, lasting for a several hours. Pupae were oval, flattened and black-brown with fine hair-like setae on their dorsal side and a mean length and width of 1.06 x 0.74 mm. The adults were oval, convex and black with small yellow setae on their dorsal side.

The sex ratio was consistently 1:1. Mating was observed at all experimental temperatures (ie. 12-30°C). The mean duration of copulation decreased from 157.2 to 51.0 minutes as temperatures increased from 12°C to 30°C. There were also large variations in the duration of pre-oviposition, oviposition and post-oviposition periods over these temperatures. The pre-

oviposition period ranged from 12.5± 0.5 days at 12°C to 1.1 ± 0.4 days at 30°C and the oviposition period varied from 100.2 ± 7.5 to 18.3 ± 2.5 days at 12 and 30°C respectively. Mean fecundity was highest at 25°C with a lifespan oviposition of 189.7 ± 20.6 eggs /female and a mean oviposition rate during the reproductive period of 6.6 ± 0.3 eggs/female/day. Egg hatchability was also higher at 25°C compared with other constant and fluctuating temperatures. The post-oviposition period was 13.1 ± 0.6 and 1.3 ± 0.3 days at 12 and 30°C respectively. There was no significant difference between the longevity of males and females, with means of 125.6 ± 11.0 and 125.7 ± 7.6 days respectively at 12°C and 16.3 ± 4.1 and 17.9 ± 2.2 days at 30°C. The mean duration from commencement of egg laying to death of adults was 190.9 ± 10.0 days at 12°C and 27.1 ± 2.0 days at 30°C, while the mean generation time (egg to egg) was 77.8 ± 1.7, 15.5 ± 0.8 and 10.3 ± 0.4 days at 12, 25 and 30°C respectively.

4.2 INTRODUCTION

Biological control requires choice of appropriate natural enemies, which in turn requires knowledge of rearing, release, specific life history and behavioural characteristics of the biological control agent concerned (Albuquerque *et. al.* 1994).

Biological studies have been conducted overseas on several *Stethorus* species, including one Australian species, *S. nigripes* (Richardson 1977). In general the mean generation time for *Stethorus* spp. may be two weeks under favourable conditions, which is slightly longer than the development time required for most plant feeding mites (Moreton *et. al.* 1969; Helle & Sabelis 1985b). However their oviposition rate is higher and their oviposition period is longer than their prey if food is abundant (Moreton *et. al.* 1969; Jeppson 1975; Pavlova 1975; Singh & Ray 1977). Houston (1980), Gordon & Andreson (1979) and Britton & Lee (1972) described the holotypes of adults, pupae, and last instar larvae of the Australian species of *Stethorus*, viz *S. vagans*, *S. nigripes*, *S. fenestralis*, *S. obscuripensis* and *S. histrio*. However they did not attempt to describe their life histories. A review of the literature reveals no information on the biology and ecology of *S. vagans*, except for some scattered references to its presence in Australia (Bower & Thwaite 1995). Therefore the present studies were undertaken to elucidate information on aspects of its biology. This was considered essential in assessing its potential rate of population increase and for predicting of the number of generations that may occur within a year. The monitoring, collection and biological studies were all carried out at the Centre for Horticulture& Plant Sciences, University of Western Sydney, Hawkesbury, Richmond, NSW.

4.3 MATERIALS AND METHODS

4.3.1 Cultural Colony

Cultures of *S. vagans* were established in the laboratory from field collected adults on potted French bean plants at the Centre for Horticulture and Plant Sciences, University of Western Sydney Hawkesbury, Richmond, NSW (Chapter 2). Cultures were maintained under controlled conditions of 25 ± 2°C, RH 46-75% and a photoperiod L:D 16:8 hours in a constant temperature cabinet. Beetles were fed all stages of two-spotted mite, *T. urticae*, on French bean leaves (Chapter 2).

4.3.2 Biological Studies

4.3.2 1 Life cycle

All life cycle observations were made on first generation offspring from *S. vagans* adults collected in the field. The parental pairs were randomly selected from the stock colony. Each pair (male & female) was placed on a 2.5-cm diameter leaf disc infested with all stages of *T. urticae*. These leaf discs were maintained on water-saturated foam in modified sealable petri dishes (Chapter 2). Five pairs were randomly allocated to various constant (12, 15, 20, 25, and 30°C) and fluctuating (12.7-32.1°C) temperatures in the laboratory. Eggs deposited by pairs at each temperature were collected 12 hourly. Each egg was carefully isolated by cutting the section of leaf containing the egg and placing it individually on a 4.7-cm diameter filter paper (Whatman: Catalogue number 1820 047), in a new 5-cm diameter petri dish. Eggs collected from each temperature were exposed to the same temperature as previously, and replicated 50 times. After egg hatching, the larval instars were supplied daily with all stages of *T. urticae* and were retained in the same petri dish until adult emergence. The presence of shed exuviae as well as changes in head capsule size were used to differentiate between larval instars. Measurements of the head capsule as well as dimensions of life stages of *S. vagans*

were made using a stereo-zoom microscope (compound microscope, BM series, Olympus Optical Co. Japan) fitted with an ocular micrometer (at 400 times magnification). The duration of immature stages was assessed at 12 hourly intervals as described in Chapter 2.

After adult emergence, sexes were identified and paired on the day of emergence. All pairs were placed on mite infested bean leaf discs (2.5 cm diameter), and kept at the same temperature at which they had been previously raised to study their reproductive biology. Reproduction was evaluated by observing each pair 12 hourly until the commencement of oviposition. The number of eggs laid by each female was counted daily until its death. Eggs were removed and placed at the same temperature in separate petri dishes, where the number of newly hatched larvae was recorded daily until no eggs hatched for several days. Egg hatchability (%) was calculated as well as pre-oviposition, oviposition and post-oviposition periods and adult longevity.

4.3.2.2 Sex ratio and mating behaviour

The sex ratio of *S. vagans* was investigated because of its importance in population dynamics. Sex ratio was determined for each constant and fluctuating temperature. Twelve hourly observations were made of the petri dishes containing pupae. Any newly emerged adults observed were transferred singly into empty sealable petri dishes to identify their sex by observing them with a binocular microscope. Identification was based on size (males are slightly smaller than females) as well as the presence of a cleavage in the 10th abdominal segment of males (Britton & Lee 1972).

Mating behaviour during pre-oviposition, oviposition and post-oviposition periods was observed for each temperature regime in which the corresponding immature stages had been

previously raised. Adults were paired after emergence on two-spotted mite infested leaf discs and the frequency and duration of copulation was noted at frequent (3 hourly) intervals. Once copulation was noted commencing pairs were observed constantly to determine the duration of copulation.

4.3.2.3 Effect of male and female ratio on oviposition and fertility

A separate experiment was also conducted to assess the effect of mating and the male: female ratio on oviposition and fertility, as well as on frequency and duration of copulation. Virgin male and female cultures were produced by isolating newly emerged adults which were raised singly from eggs maintained at a constant temperature of 25 ± 2°C. The following treatments were established using the same modified petri dishes used for assessment of immature stage development.

(i) T1: 10 newly emerged unmated females confined singly.

(ii) T2: 10 newly emerged females mated once and then confined singly.

(iii) T3: 10 newly emerged females and males confined in pairs (control).

(iv) T4: 10 newly emerged females each confined with 2 males.

All adults were paired on mite infested leaf discs (Chapter 2). Each day the number of eggs laid were recorded and the leaf discs were transferred to new dry filter papers in petri dishes. The number of eggs laid per day and their viability were compared between treatments. Conditions during the experimental period were 25°C ± 2°C, photoperiod 16L: 8D hours and relative humidity 46-80%. Observations were continued until adult death.

Data were collected for the following parameters where appropriate:

-Duration of copulation

-Frequency of copulation

-Behaviour of the two males during copulation

-Oviposition and fecundity

-Egg hatching

4.3.3 Diapause Studies

4.3.3.1 Laboratory experiment

To investigate the possibility of diapause in any stage of *S. vagans* a separate experiment was conducted at a range of temperatures and daylengths in the laboratory as described below.

	Light: Dark	Temperature
1. Short day and low temperature	6: 18	11 ± 0.5°C
2. Short day and high temperature	6: 18	32 ± 0.5°C
3. Long day and low temperature	18: 6	11 ± 0.5°C
4. Long day and high temperature	18: 6	32 ± 0.5°C

To commence this study virgin males and females were raised at a constant temperature of 25 ± 2°C as previously described. Newly emerged adults were randomly selected and confined in pairs on 2.5 cm leaf discs infested with all stages of *T. urticae*. Twenty replicates were randomly allocated to each of the four treatments. These pairs of adults were placed in their allocated treatment for mating and oviposition. Eggs deposited in each treatment were placed singly on dry filter paper in separate modified petri dishes (Chapter 2) and placed back in the same treatment. Development was followed by examining each petri dish every 12 hours from egg incubation until adult death.

4.3.3.2 Field collection

Adult *Stethorus* were field collected over a three-year period from potted bean plants for a stock colony (Chapter 2). However from March 1996 to February 1998 all leaves collected from the field were thoroughly examined under a stereo microscope for different stages of *S. vagans*. The numbers of each stage were recorded, to assess whether diapause occurred at any stage in the field.

4.3.4 Statistical Analysis

4.3.4.1 Life cycle

The variability of duration of all life cycle stages at all temperatures was assumed to be normally distributed; thus an analysis of variance (ANOVA) of the data was undertaken using the statistical software package CoStat (CoHort Software P.O.Box 19272, Minneapolis, MN 55419, USA).

4.3.4.2 Sex ratio

Sex ratios were analysed to test the null hypothesis that female: male = 1:1. For this purpose, the χ^2 goodness of fit test was applied using the statistical function of Excel 5 (Microsoft Office).

4.3.4.3 Effects of male: female ratio on oviposition and fertility

The effect of male to female ratios on fecundity and egg viability (ie. female fertility) was also analysed with ANOVA from CoStat CoStat (CoHort Software P.O.Box 19272, Minneapolis, MN 55419, USA).

4.3.4.4 Diapause

The oviposition and fertility rates of all *S. vagans* females were calculated by analysis of variance (ANOVA) using CoStat.

4.4 RESULTS

4.4.1 Description of Life Cycle

4.4.1.1 Egg

Newly laid eggs were translucent white, turning pale yellow after 4-5 hours. They were smooth, elongate and rounded at both ends. The mean size of the eggs was 0.36 x 0.19 mm (Table 4.1). They were laid horizontally on the lower surface of the leaves along the midrib and lateral veins in an exposed position. They were commonly deposited singly, but occasionally in pairs among large mite colonies. Fertilised eggs appeared granular in the first two days, while two red eyespots developed one-day before hatching. The eggs became transparent on the day of hatching and the developing embryo could clearly be seen through the chorion of the egg. Unfertilised eggs were slightly smaller and did not show any colour changes as they gradually shrivelled and died.

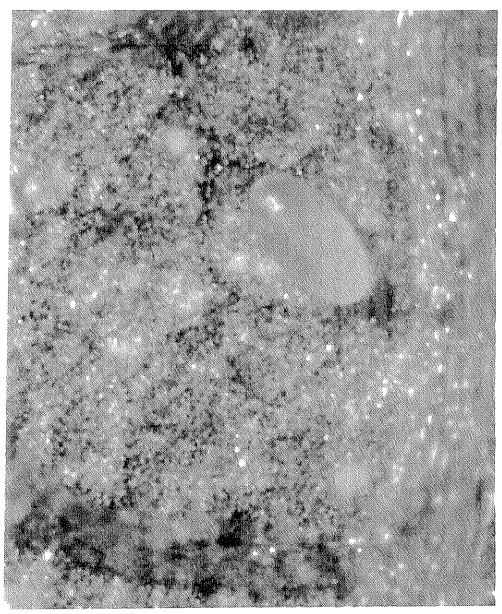

Fig. 4.1 Egg of *S. vagans*

4.4.1.2 Larva

Larvae moulted three times with instars closely resembling each other: the presence of a shed exoskeleton and head capsule size was used to differentiate between instars (Table 4.1). Prior to moulting, larvae stopped moving and fixed themselves by the 10^{th} abdominal segment to a surface and remained inactive for 3-5 hours. Ecdysis began at the head and continued along the back throughout the abdomen. The shed exoskeleton remained fixed the substrate and the larva emerged from the exoskeleton of the former instar.

Four larval instars were recorded. The newly emerged larvae were white, but soon become pale creamy-white. The pink coloured contents of the alimentary canal (turning dark after feeding) were visible through the larval body. All instars possessed numerous dark brown setae over the tergites and pleurites with dark brown pigmentation at the bases of the dorsal setae. The measurements of body length, width and head capsule are presented in Table 4.1. The analysis of variance shows that the mean body length, width and head width of each instar different significantly ($p \leq 0.05$).

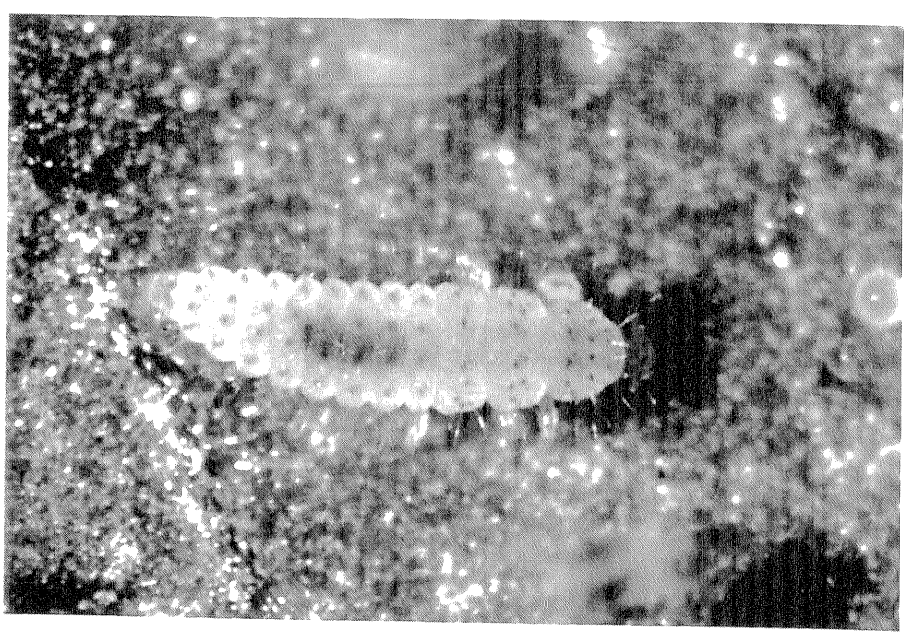

Fig. 4.2 Larva of *S. vagans*

4.4.1.3 Pre-pupa

When the final instar stopped feeding, it attached itself by the anal cremaster to the substrate. The larva shrank and gradually hunched up its back. This marked the prepupal stage, which was cream in colour. This stage was not a distinct stage in the life cycle, but a quiescent period at the end of 4th instar, lasting for only a few hours.

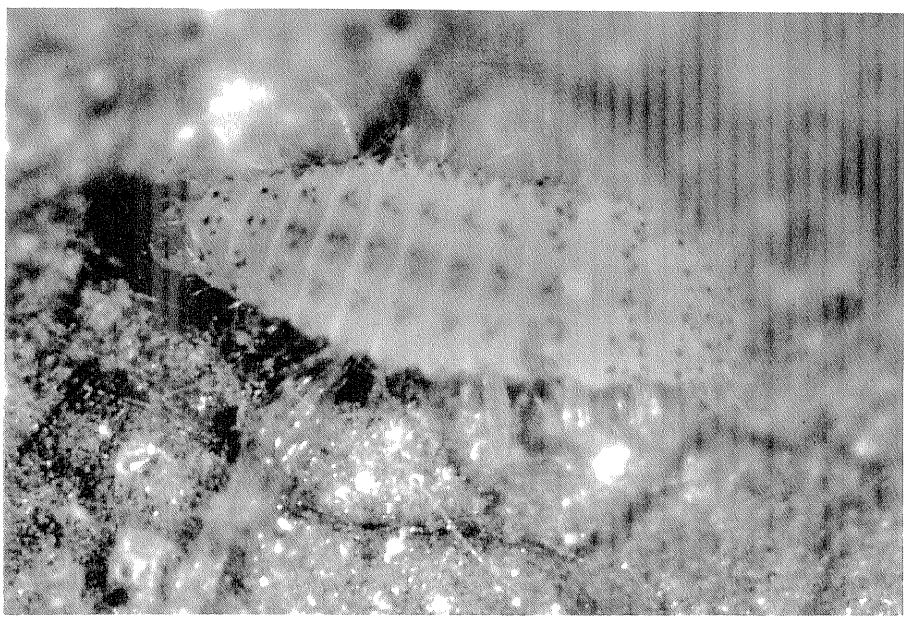

Fig. 4.3 Pre-pupal stage of *S. vagans*

4.4.1.4 Pupa

The pupa was oval, flattened, and subtruncate anteriorly and tapered posteriorly. It was creamy in colour for the first few hours then became uniform black-brown. The body was covered with fine hair-like setae and the abdominal segments, wing pads and legs of the adult were very prominent. In the field, pupae were found in different locations but most frequently

on the underside of the leaves. In the laboratory they were found on leaf discs as well as on the inner surface of petri dishes. The mean length and width at widest point of the pupa was 1.06 x 0.74 mm respectively (Table 4.1).

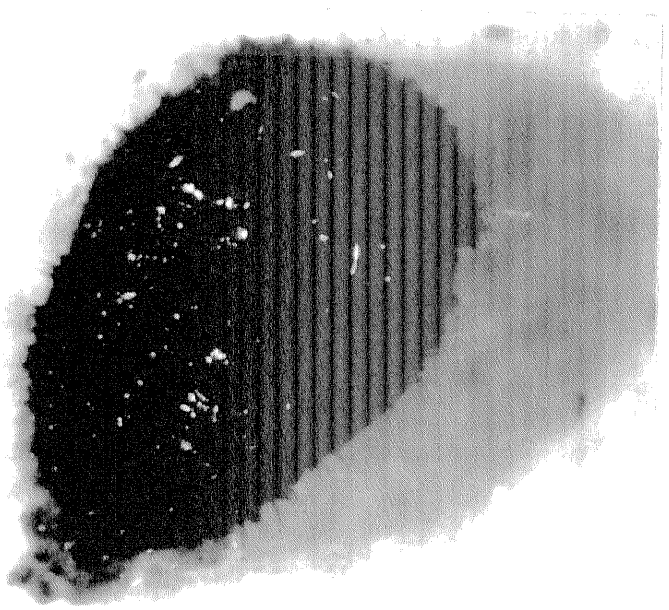

Fig. 4.4 Pupal stage of *S. vagans*

4.4.1.5 Adult

Adults emerged from the pupal skin, by splitting it transversely and longitudinally on the ecdysial sutures in the thoracic region. The beetles took several minutes to emerge completely. Once free they sat beside their pupal cases, where they remained for approximately an hour. The newly emerged beetles were light yellow in colour for first the few minutes then slowly changed through orange to black. The pronotum, however was

completely black at eclosion. Adults were convex and oval, being wide in the middle and narrow at both ends. Males were smaller than females and were differentiated from the females by a cleavage of their 10^{th} abdominal sternite. The mean length and width of males and females were 1.04 x 0.75 mm and 1.15 x 0.79 mm respectively. The mean adult longevity was 125.7 ± 7.6, 72.6 ± 3.3, 41.9 ± 3.3, 26.6 ± 2.3, and 17.9 ± 2.2 days at 12, 15, 20, 25, and 30°C respectively, and 30.0 ± 1.6 days at the fluctuating temperatures (12.7-32.1°C). The total life span from commencement of oviposition to adult death ranged from 190.9 ± 10.0 to 27.1 ± 2.0 days at constant temperatures of 12 and 30°C respectively, and 27.1 ± 2.0 days at fluctuating temperatures (Table 4.2).

Table 4.1 Measurements (mm) of all stages of *S. vagans*

Stages		n	Length		Width		Head capsule	
			Range	Mean	Range	Mean	Range	Mean
Egg		10	0.33-0.40	0.36	0.18-0.20	0.19	-	-
1st instar		10	0.50-0.75	0.64	0.10-0.13	0.12	0.10-0.11	0.106[a]
2nd instar		10	0.85-0.98	0.91	0.15-0.18	0.16	0.13-0.14	0.131[b]
3rd instar		10	1.08-1.25	1.14	0.20-0.28	0.22	0.15-0.16	0.156[c]
4th instar		10	1.5-2.08	1.84	0.38-0.45	0.41	0.18-0.19	0.181[d]
Pupa		10	1.0-1.25	1.06	0.63-0.80	0.74	-	-
Adult	Male	10	0.98-1.08	1.04	0.70-0.80	0.75	-	-
	Female	10	1.10-1.20	1.15	0.78-0.80	0.79	-	-

*Numbers in same columns followed by different letters are significantly different at $p \leq 0.05\%$

Table 4.2 Mean duration ± SE (days) (of *S. vagans* life stages at different constant and fluctuating temperatures.

Stage		Constant Temperature					Fluctuating Temperature
		12°C	15°C	20°C	25°C	30°C	21.4°C (12.7-32.1°C)
Female	Pre-oviposition	12.5 ± 0.5	9.0 ± 0.5	5.4 ± 0.6	2.4 ± 0.2	1.1 ± 0.3	4.1 ± 0.2
	Oviposition	100.0± 7.5	60.9 ± 3.9	34.5 ± 3.8	28.2 ± 2.7	18.3 ± 2.5	25.1 ± 2.0
	Post-oviposition	13.1 ± 0.6	9.7 ± 0.4	8.2 ± 0.85	2.6 ± 0.2	1.3 ± 0.3	4.1 ± 0.2
Female longevity		125.7 ± 11	79.6 ± 5.7	48.1 ± 1.8	33.3 ± 3.7	20.7 ± 3.5	33.7 ± 3.1
Male longevity		125.6 ± 11	72.6 ± 6.7	39.4 ± 5.1	26.6 ± 2.3	16.3 ± 4.1	28.9 ± 1.8
Adult longevity		125.7?? ± 7.6	76.2 ± 4.4	42.5 ± 4.3	29.8 ± 2.1	17.9 ± 2.2	30.04 ± 1.6
Total life cycle		190.9 ± 10.0	111.0 ± 9.0	60.1 ± 6.0	41.9 ± 3.0	27.1 ± 2.0	45.4 ± 4.5
Mean generation time (egg-egg)		77.8 ± 1.7	43.8 ± 1.5	23.6 ±1.12	15.5 ± 0.8	10.3 ± 0.6	19.4 ± 0.5

4.4.2.Reproductive Biology

4.4.2.1 Sex ratio and mating behaviour

The number of larvae successfully reaching adulthood in all constant temperatures was 164; these comprised 82 males and 82 females, showing a sex ratio of 1:1. At fluctuating temperatures 32 beetles were raised of which 16 were males and 16 were females; again giving a 1:1 sex ratio (Table 4.3).

Mating was observed at all times of the day and throughout adult life. The mean lifetime mating frequency generally increased with increasing temperature (except for 30°C), ie. 5.2 ± 0.4, 9.7 ± 0.6, 11.1 ± 0.5, 14.6 ± 0.7, 10.3 ± 0.6 respectively at 12-30°C constant, and was 15.5 ± 0.7 at fluctuating temperatures (12.7-32.1). However the mean copulation period decreased with increasing temperature, ie. from 157.2 minutes at 12°C to 51.0 minutes at 30°C and 120.2 minutes at fluctuating temperatures (Table 4.3).

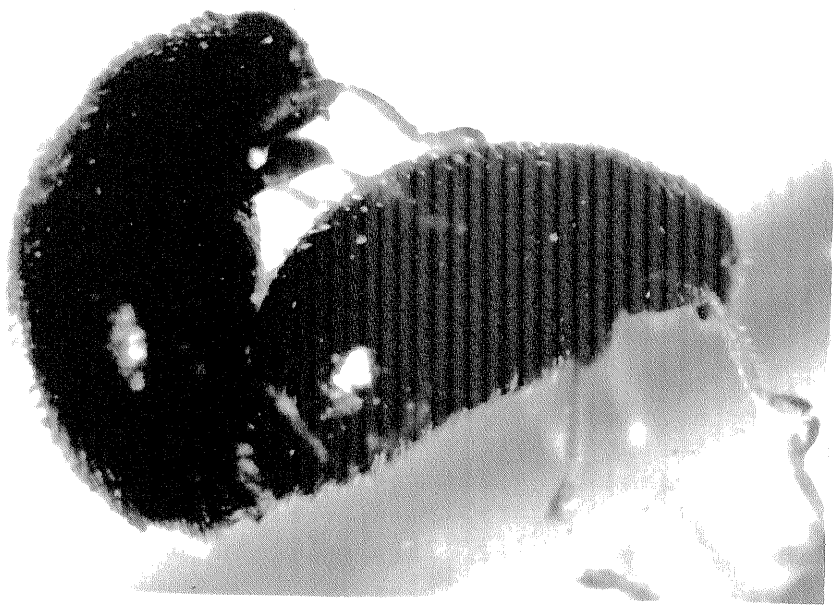

Fig 4.5 A mating pair of *S. vagans*.

Table 4.3 Sex ratio, mating frequency and duration of copulation of adult *S. vagans* at constant and fluctuating temperatures.

		Constant Temperature					Fluctuating Temperature
		12°C	15°C	20°C	25°C	30°C	12.7-32.1°C
Sex Ratio	Male	14.0	17.0	22.0	16.0	13.0	16.0
	Female	14.0	18.0	23.0	15.0	12.0	16.0
	Male: Female	1: 1	1:1.06	1:1.05	1:0.94	1:0.92	1: 1
Mating	Frequency	5.2	9.7	11.1	14.7	10.3	15.1
	Mean Duration of copulation (min)	157.2	136.5	120.5	105.4	51.0	120.2

4.4.2.2 Reproductive period

There were significant differences in the length of pre-oviposition, oviposition and post-oviposition periods between the different temperatures (Table 4.2). The mean duration of these periods decreased as temperature increased.

The mean pre-oviposition period was 12.5 ± 0.5 days at 12°C and 1.1 ± 0.1 days at 30°C. The mean oviposition period ranged from 18.3 ± 2.5 days at 30°C to 100.2 ± 7.5 days at 12°C. The longest oviposition period maintained by an individual female was 175 days at 12°C and the shortest recorded was 5 days at 30°C. Oviposition ceased 1.3 ± 0.4 days before death at 30°C and 13.1 ± 0.6 days at 12°C. There were no significant differences between female and male longevity. Mean adult longevity was 125.7 ± 11.0 days at 12°C and 27.1 ± 2.0 days at 30°C (Table 4.2). The longest adult longevity recorded for an individual *S. vagans* was 197 days at 12°C.

4.4.2.3 Fecundity and egg hatchability

More eggs were produced at 20 and 25°C than at the other three constant temperatures (Table 4.4). The highest mean total eggs laid per female throughout adulthood was 189.7 ± 20.6 at 25°C and the lowest were 96.6± 9.0 at 12°C and 96.3 at 30°C. The highest mean daily fecundity per female was 6.6 ± 0.5 eggs per day at 25°C, while on several occasions individual females laid more than 10 eggs per day at this temperature. The lowest number of eggs oviposited was 0.95 egg/female/day at 12°C. The oldest reproductive age was 175 days at 12°C. The greatest number of eggs laid by an individual at 25°C was 327 (33 days) and the lowest was 52 eggs (10 days). The highest percentage of egg hatch was 84.1 ± 1.7% at 25°C and the lowest 46.6 ± 2.3% at 12°C (Table 4.4).

Table 4.4 Reproduction of *S. vagans* reared on *T. urticae* at different constant temperatures

Temperature	Number of females (n)	Duration of preoviposition (days) Mean ± SE	Duration of oviposition (days) Mean ± SE	Total eggs laid Mean ± SE	Eggs /day Mean ± SE	Total number eggs hatched Mean ± SE	Percent egg hatchability Mean ± SE
12°C	14	12.5 ± 0.5	100.2 ± 7.5	96.6 ± 9.0	1.0 ± 0.3	57.3 ± 6.0	58.7 ± 2.3
15°C	17	9.0 ± 0.4	60.9 ± 3.9	127.0 ± 10.5	2.1 ± 0.2	86.5 ± 7.0	68.9 ± 2.0
20C	22	5.4 ± 0.2	34.5 ± 3.4	148.3 ± 16.0	4.3 ± 0.2	114.2 ± 12.9	75.8 ± 1.5
25°C	15	2.4 ± 0.1	28.2 ± 2.7	189.7 ± 20.6	6.6 ± 0.3	161.9 ± 18.5	84.1 ± 1.7
30°C	12	1.1 ± 0.1	18.4 ± 2.5	96.3 ± 14.0	5.5 ± 0.4	72.7 ± 10.8	75.1 ± 2.1

4.4.2.4 Effect of male: female ratio on oviposition and egg hatchability

The mean oviposition rate of females allowed to mate continuously with only one male was significantly higher ($p \leq 0.0001$) than for unmated females, those mated only once and those confined continuously with two males (Table 4.5). There was no significant difference in the fecundity of females mated only once or those confined with two males permanently, although both were significantly higher than in unmated females. All females were able to lay eggs throughout their life span, except for unmated females, which laid very few eggs only in the first week after emergence.

Viability was significantly higher ($p \leq 0.0005$) in eggs laid by females confined with one male than for females that were unmated, those mated only once and those that were confined with two males. Egg hatchability from females mated once and females confined with two males did not differ significantly from each other, but both were significantly greater than for unmated females (Table 4.5). The egg viability in females that had mated at least once was >76% compared with 0% in unmated females.

Table 4.5 Mean fecundity and egg hatchability of unmated females, females mated once, and females confined with one or two males.

Treatments	n	Eggs laid/• /day	Eggs hatched	
		Mean ± SE	Mean ± SE	Percent hatch
1 • (Unmated)	20	1.9 ±0.3[a]	0.0 ± 0.0[a]	0.0[a]
1• (Mated once)	20	5.3 ± 0.5[b]	4.1 ± 0.5[b]	77.4[b]
1• +1 •	20	6.3 ± 0.5[c]	5.1 ± 0.6[c]	81.0[c]
1• + 2 •	20	5.6 ± 0.5[b]	4.3 ± 0.4[b]	76.8[b]

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

4.4 6 Diapause

No diapause was observed in any stage of *S. vagans* at any of the treatments assessed, as determined by developmental times and reproductive rates at different constant temperatures and photoperiods (Table 4.7). All females oviposited when exposed to either short (6L: 18D) or long (18L: 8D) days with low (11 ± 0.5°C) or high (32 ± 0.5°C) temperatures. There was no significant difference in oviposition rates in females reared at the same temperature but different photoperiods.

The mean total number of eggs laid per female under the treatments ranged from 37.9 ± 1.2 to 39.2 ± 1.3 and egg viability ranged from 60.1 to 62.9%. Analysis of the data showed that neither temperature nor daylength had a significant influence ($p \geq 0.87$) on the mean fecundity and egg hatchability. A minimum of 65% *S. vagans* preimaginal stages completed their development at all treatments tested.

No diapause in any stage of *S. vagans* was observed in the field, as indicated by the continuous collection of all stages of *S. vagans* over a two-year period (Table 4.8). Populations were highest in autumn, spring and summer and lowest in winter. However throughout the year, the relative proportion of different life stages remained fairly constant, which further indicated that diapause in the field was unlikely.

Table 4.6 Effect of temperature and day length on oviposition and egg hatchability of *S. vagans*.

Treatment	Males and Females	Oviposition		Egg hatching	
	n	Total eggs	Mean ± SE	Total eggs	Percent hatchability
Short day and Low temperature	20	758	37.9 ± 1.2 [a]*	484	62.9 ± 3.4 [a]*
Short day and High temperature	20	783	39.2 ± 1.3 [a]	471	60.8 ± 4.0 [a]
Long day and Low temperature	20	761	38.1 ± 1.3 [a]	441	60.1 ± 4.2 [a]
Long day and High temperature	20	777	38.9 ± 1.1[a]	467	61.0 ± 3.3 [a]

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

Table 4.7 *Stethorus* species collected from the field during a two year period (March 1996-March 1998)

Season	*Stethorus vagans*					*Stethorus nigripes*				
	Adult	Egg	Larva	Pupa	Total	Adult	Egg	Larva	Pupa	Total
Autumn 96	301	70	27	14	412	98	38	14	9	161
Winter 96	101	13	8	9	131	38	12	4	3	57
Spring 96	272	76	32	19	399	96	40	20	14	170
Summer 96-97	137	35	20	12	204	37	15	10	7	69
Autumn 97	304	82	34	14	461	105	50	17	10	182
Winter 97	83	15	4	8	110	27	9	4	2	42
Spring 97	247	108	49	18	422	73	32	13	7	125
Summer 97-98	187	29	8	6	230	58	21	7	4	90

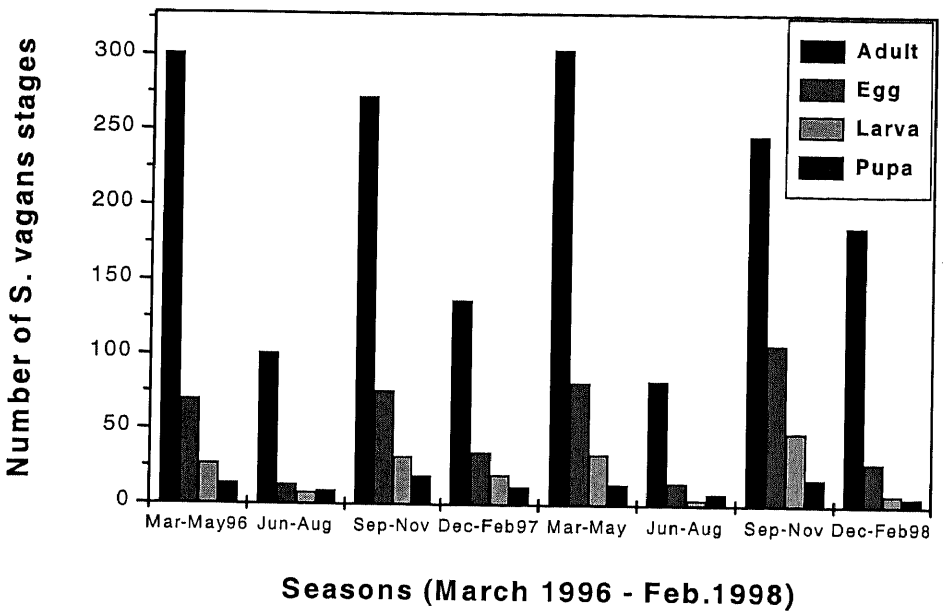

Fig. 4.7 Field collected stages of *S. vagans* over two years (March 1996-March 1998).

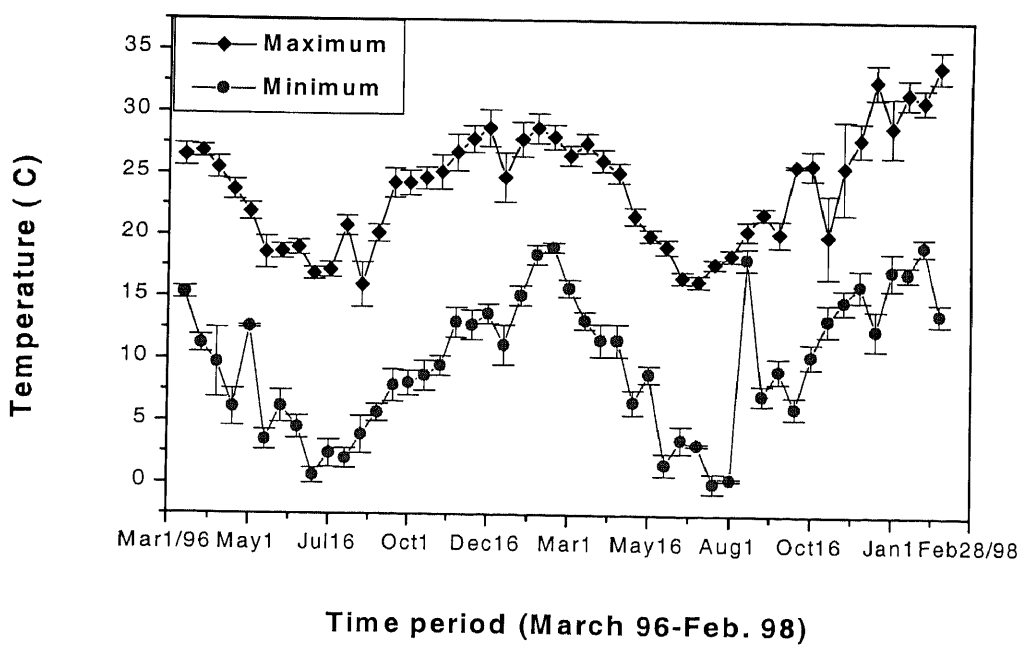

Fig. 4.8 Maximum and minimum temperatures during field collection of *S. vagans* (March 1996 - Feb. 1998).

4.5 DISCUSSION

4.5.1 Life Cycle

4.5.1.1 Egg

S. vagans appears to have a similar egg morphology and development as that reported for a number of other *Stethorus* species, such as *S. punctillum* (Putman 1955b, *S. gilvifrons* (Mathur 1969; Ahmed & Ahmed 1989) *S. keralicus* (Daniel 1976) *S. pauperculus* (Puttaswamy & ChannaBasavanna 1977) and *S. nigripes* (Richardson 1977). However the mean egg size of *S. vagans* (0.36 x 0.19 mm) is relatively small compared with *S. punctum* (0.43 x 0.33 mm) (Colburn & Asquith 1971), *S. keralicus* (0.37 x 0.32 mm) (Daniel 1976) but similar to *S. pauperculus* (0.38 x 0.20 mm) (Puttaswamy & ChannaBasavanna 1977). No such measurements have been reported for *S. nigripes*.

More than 60% of eggs hatched within the normal time period at ≥ 15°C, after a long exposure (61days) to 10°C (Chapter 3). This confirms that *S. vagans* eggs are tolerant of low temperature (10°C) and can survive during winter without loss of viability. The data obtained from field collected eggs over a two year period also confirmed this. At higher temperatures (30 and 35°C) and lower relative humidity, eggs of *S. vagans* lost viability; however with higher humidity (70-85%) many remained viable at 30°C. At the higher relative humidity at 35°C, however, eggs appeared to develop completely but the larvae were unable to emerge. Similar results were reported for *S. nigripes*, which retained egg viability up to 35°C (Richardson 1977), but which decreased greatly above that temperature. Based on the data obtained for both fluctuating (12.7-32.1°C) and at high constant temperatures (30 and 35°C), its appears that normal field development of *S. vagans* is likely to occur under the climatic conditions experienced in the Hawkesbury district (see Fig 4.7), especially given that in crop

plants, microclimates are commonly created where temperatures are lower and relative humidity is higher than ambient conditions.

4.5.1.2 Larva

We recorded four larval instars for *S. vagans*, the same as that recorded for most other coccinellids including *Stethorus* spp., although five instars have been recorded for the *Chilocorus bipustulatus* (Yinon 1969). The larvae were similar to that described by Britton & Lee (1972) and Houston (1980). The larval instars were differentiated by body length and width as well as head capsule width (Table 4.1). *S. vagans* (i.e 1^{st}, 2^{nd}, 3^{rd} and 4^{th} instar larvae had dimensions of 0.64 x 0.12, 0.91 x 0.16, 1.14 x 0.22 and 1.84 x 0.41 mm respectively) which is smaller than *S. punctum*, {ie. 1^{st} larval instar 1.03 x 1.57 mm and 4^{th} larval instar 2.5 x 2.2 mm) (Colburn & Asquith 1971), but similar to *S. pauperculus* (0.61 x 0.20, 1.0 x 0.32, 1.2 x 0.46 and 1.8 x 0.68 mm, for 1^{st}, 2^{nd}, 3^{rd}, and 4^{th} larval instars, respectively) (Puttaswamy & ChannaBasavanna 1977).

We differentiated larval instars from one another by the presence of shed exoskeletons as well as by head capsule size. Head capsule measurements are the most reliable method of differentiating between larval instars (Dyar 1890). However, its appears that apart from Daniel (1976) who reported head capsule measurements for 1^{st} instar *S. keralcus*, no such measurements have been taken for other *Stethorus*.

4.5.1.3 Pre-pupa

The duration of the pre-pupal stage for *S. vagans* we recorded at 25°C varied from 8 to 13 hours, which is similar to that reported for most other species of *Stethorus*. For example Daniel (1976) recorded 10-15 hours for *S. keralicus* at 26-34°C, while Puttaswamy &

ChannaBassavanna (1977) reported 8 hours for *S. pauperculus* at 24-26°C. However it is much shorter than that reported for *S. nigripes* (24 hours) at 25-35°C (Richardson 1977).

4.5.1.4 Pupa

The pupal appearance was similar to that reported by Britton & Lee (1972) and Houston (1980) for the species. Its length and width was 1.06 x 0.74 mm, which is larger than *S. keralicus* (1.03 x 0.7 mm) (Daniel 1977), but smaller than *S. punctum* (1.36 x 0.97 mm) (Colburn & Asquith 1971) and *S. pauperculus* (1.8 x 1.1 mm) (Puttaswamy & ChannaBasavanna 1977).

The duration of the pupal stage was shorter than for *S. keralicus* (3.5-4.0 days at 26-30°C) (Daniel 1976) and *S. pauperculus* (3.8-4.0 days at 25.4-26°C) (Puttaswamy & ChannaBasavanna 1977), but longer than *S. gilvifron* (2.5 days at 35°C) (Ahmed & Ahmed 1989), *S. loi* (3.3 days at 23.8°C) (Shih *et al.* 1991) and *S. nigripes* (3.16 days at 25°C) (Richardson 1977). It possible that for the latter species, Richardson (1977) recorded an extended pre-pupal stage and a shorter pupal stage (see 4.5.1.3).

4.5.1.5 Adult

The colour of newly emerged adult *S. vagans* was light yellow, which turned to a uniform black-brown. Similar results have been described for *S. pauperculus* (Puttaswamy & ChannaBasavanna 1977) and for *S. nigripes* (Richardson 1977) as well as other ladybird genera (Majerus 1994). The males and females were easily distinguished on the basis of characteristics described by Britton & Lee (1972) and Houston (1980). Britton & Lee (1972)

also reported a mean length only for "adult" *S. vagans*, of 1.12mm, while our dimensions were 1.04 and 0.75mm for males and 1.15 and 0.79 mm for females, respectively. As with the immature stages, the adults are smaller than most other *Stethorus* spp., such as *S. pauperculus* (1.47mm length and 1.0 mm width) (Puttaswamy & ChannaBasavanna 1977) and *S. punctillum* (1.5 and 1.01 mm, respectively) (Gordon & Chapin 1983).

Adult longevity of *S. vagans* was less than that recorded for most other species of *Stethorus*, including *S. pauperculus* (30-61 days) at 24-26°C (Puttaswamy & ChannaBassvanna 1977), *S. punctillum* (32-53 days) at 24-28°C (Jiang *et. al.* 1982) and *S. loi* (48-57 days) at 24°C (Shih *et al.* 1991). This may be because *S. vagans* has a smaller body size than other *Stethorus* spp. and has a larger surface area to volume ratio, therefore requiring more energy to maintain normal physiological and behavioural functions such as location of prey.

4.5.2 Reproductive Biology

4.5.2.1 Sex ratio and mating behaviour

The sex ratio of *S. vagans* was 1:1, which is similar to observations made for most ladybirds and for many other insect species. For example Richardson (1977) reported a similar sex ratio in *S. nigripes*, as did Shih *et al.* (1991) and Kumar & Chakraborty (1997) for *Scymuns nubilus* (Coleoptera: Coccinellidae).

Frequent mating was observed in *S. vagans* throughout their life. This is similar to that recorded for other *Stethorus* spp. such as *S. gilvifrons* (Mathur 1969), *S. pauperculus* (Puttaswamy & ChannaBasavanna 1977) and *S. nigripes* (Richardson 1977). The duration of copulation in *S. vagans* was similar to that recorded for *S. pauperculus* (30 minutes) (Puttaswamy & ChannaBasavanna 1977) and *S. gilvifrons* (50 minutes to 6 hours) (Mathur

1969). However it was much shorter than that noted for *S. nigripes* (2-5 minutes) (Richardson 1977). It appears that frequent mating is not necessary for *S. vagans* to maintain fecundity up to 18 days. However based on the data presented in this thesis, some females needed to mate again to maintain high fecundity after this period. This phenomenon occurs in *S. nigripes*, where fecundity was reduced after 20 days and regained when males were placed with females (Richardson 1977). The duration of copulation appears to have no effect on oviposition and fertility in *Stethorus* species. For example the duration of copulation in *S. vagans* is substantially longer than for *S. nigripes*, but in both species, fecundity declined approximately 20 days after the initial mating.

4.5.2.2 Reproductive period

The pre-oviposition period of *S. vagans* was almost double that recorded by Richardson (1977) for *S. nigripes* for all temperatures tested (15, 20, 25 and 30°C), while the mean oviposition period was only half that recorded for *S. nigripes* at 25°C. The post-oviposition period has not been recorded for any other species of *Stethorus* other than *S. vagans*. As the mean daily oviposition rate for *S. vagans* and *S. nigripes* are similar, it appears that the critical factor influencing fecundity is adult longevity.

The mean adult female longevity of *S. vagans* was 33.3 ± 3.7 days at 25°C, much shorter than that recorded for *S. nigripes* (50.5 ± 5.3 days). One possible reason for the differences may be a result of the different methodologies used in recording their life cycle studies. We used (5 cm diameter) petri dishes, while Richardson (1977) used 2 cm diameter munger cells. As explained in section 4.5.1.5, *S. vagans* is smaller than *S. nigripes*, but the searching area in the comparative investigations for *S. vagans* was much greater than that for *S. nigripes*. Therefore more energy may have been expended searching by *S. vagans*, contributing to .

reduced longevity. Our methodology is likely to be more closely correlated with field conditions and may more accurately represent likely field activity. In addition, the same methodologies were used to rear immature stages of the respective species, and we recorded substantially lower mortality rates (Chapter 2). It therefore appears likely that our experimental conditions were better suited to maximising longevity of *S. vagans*.

4.5 2.3 Fecundity and egg hatchability

The mean total fecundity of *S. vagans* (189.7 ± 20.6 eggs) at 25°C was much less than that recorded by Putman (1955a) for *S. punctillum* (1290 eggs) and *S. pauperculus* (339 eggs) (Puttaswamy & ChannaBasavanna 1977) and slightly less than *S. nigripes* (281 eggs) (Richardson 1977). The most likely reasons are that the oviposition periods and adult longevity of the above species are almost double that recorded for *S. vagans*. A second reason for the lower fecundity may be the smaller relative size of *S. vagans* females 1.15 x 0.78 mm compared with *S. pauperculus* (1.47 x 1.0 mm) and *S. punctillum* (1.46 x 1.01 mm) and therefore inherently lower oviposition capacity. The mean daily fecundity of *S. vagans*, however was similar to that published for *S. punctillum* (Robinson 1953), *S. bifidus* (Collyer 1964b), *S. gilvifrons*, (Kaylani 1967), *S. picipes* (Sandness & McMurtry 1970) and *S. nigripes* (Richardson 1977).

The number of eggs produced by isolated female *S. vagans*, those mated only once and those paired continuously are similar to results obtained by Richardson (1977) for *S. nigripes*. However he did not assess fecundity of females maintained with multiple males. The egg rate decline in females confined with two males may be due to disturbance during copulation, because during mating the second male either attempted to ride on the first male or followed

the united pair. However, if mating interference occurred this was not reflected by reduced egg viability over the oviposition of life of females.

The egg viability in *S. vagans* at 25°C was slighter higher (84.1%) than that recorded for other ladybird species. For example Richardson (1977) recorded 80% in *S. nigripes* at 25°C, while Elhag & Zaitoon (1996) recorded egg hatchability of 81.8, 74.5, 72.8, and 68.5% for *Adonia variegate*, *Coccinella undecimpunctata*, *C. novemnotata*, and *C. septempuctata* at 25°C respectively. On the other hand, the females mated only once and confined singly or confined with two males had significantly reduced fertility. These results are supported by Richardson (1977) who observed isolated and paired *S. nigripes* at 25°C and Majerus (1994) who studied a number of other ladybird species.

4.5.2.4 Effect of male: female ratio on the oviposition and egg hatchability

We observed multiple mating in *S. vagans*. This is similar to that reported for *S. punctillum* (Putman 1955a), *S. gilvifrons* (Mathur 1969), *S. pauperculus* (Puttaswamy & ChannaBasavanna 1977) and *S. nigripes* (Richardson 1977). However a single mating was sufficient to produce fertile eggs for 18 days, presumably because viable spermatozoa could be maintained in the spermatheca for that period. *S. nigripes* can produced fertile eggs for 20 days (Richardson 1977) and *Adalia bipunctata* for three months after a single mating, while *Chilocorus renipustulatus* store sperm in their spermatheca throughout winter (Majerus 1994). There was a slightly reduction in the fertility of *S. vagans* as determined by egg viability after 18 days. Richardson (1977) obtained similar results for *S. nigripes* in which fertility was maintained by the introduction of males 20 days after the initial mating. All eggs produced by unmated females were not viable and failed to hatch, as was also reported by

Richardson (1977) for *S. nigripes*. Clearly, mating is essential both for egg production and egg development in these species.

4.5.4 Diapause

Diapause may occur in any insect life stage as a result of one or more factor(s) such as day-length, temperature and food availability (Waage *et al.* 1985). However in most insects it is induced by a specific day: night ratio with low or high temperatures (Daly *et al.* 1998). We conducted our investigations at selected photoperiods and temperatures. These combinations of parameters chosen were not exhaustive; however were considered to be practical and consistent with those reported by other authors. For example Swift (1987) studied diapause in the predatory mite *Amblyseius fallacis*, Canard (1990) in the lacewing *Nineta pallida* and Okuda & Hodek (1994) in *Coccinella septempunctata* all at 16L: 8D and 12L: 12D at 20 , 25 and 30 C.

No diapause was recorded in any stage in any temperature /day-length treatment, either in the laboratory or in the field. Rate of development was related to temperature alone. The laboratory data are supported by the results of the *Stethorus* collections from the field over a two-year period (Table 4.7 and 4.8). These results are also supported by Richardson (1972, 1977), who reported no diapause in the Australian species *S. nigripes* in Adelaide (South Australia) and California, and Readshaw (1975) who observed *Stethorus* spp. (including *S. vagans*) in Canberra, Australia, which experiences cooler winters than those recorded at either Richmond or Adelaide. While it is unclear whether *S. vagans* or other Australian *Stethorus* spp. diapause in the coolest regions in Australia (eg. Tasmania) in the field it appears unlikely, based on our results.

While the field observations confirmed the laboratory results that *S. vagans* do not diapause in winter (Table 4.7), their major prey *T. urticae* spider mites do undergo diapause (Helle & Sabelis 1985a). Although some *T. urticae* activity may continue in protected areas (eg. near walls, rocks or under trees and bushes) or in subtropical regions, further south or at elevation in central NSW or in unprotected areas, spider mite activity ceases and they diapause. Readshaw (1975) found adults and larvae of *Stethorus* spp (including *S. vagans)* in tree bands throughout the winter feeding on diapausing *T. urticae*. Active mite populations in protected areas and diapausing mites in crevices or under tree bark or in leaf litter, as well as availability of alternative hosts (Chapter 5) (Helle & Sabelis 1985a) may be sufficient to support populations of *S. vagans* and other *Stethorus* spp. to continue limited activity during winter.