CHAPTER 6

HOST LOCATION BY *S. VAGANS* AT DIFFERENT PREY DENSITIES

6.1 ABSTRACT

Host location by *S. vagans* was observed at four densities of its prey *T. urticae*, commencing at a nominal 10, 20, and 50 mites per plant in the field. Leaves from potted French bean plants located in the field and infested with the respective number of mites were examined daily for presence of *S. vagans*. The mean number of adult *S. vagans* recorded was 0.54, 2.5 and 5.18 at low, medium and high mite densities respectively, while none were found on mite free plants. Immature stages of *S. vagans* were only found on plants with high mite density. The mean number of all stages of *T. urticae* counted on leaves at the time of *S. vagans* collection was 4.27, 19.5 and 490.4 mites at low, medium and high densities respectively.

Both sexes of adult *S. vagans* were assessed in the laboratory in relation to their prey searching ability using a Y-tube olfactometer. Both satiated and starved adults were tested with and without an air current flowing from the prey chamber. No adults in any treatment were able to locate their prey in this device.

Host detection by *S. vagans* was also observed in a controlled temperature room at 25°C. For each observation, 4 adults (2 males and 2 females) were released in the middle of potted French bean plants, one infested with *T. urticae* and the other untreated as a control. All released adults, both satiated and starved, were able to located their prey. Adults located their prey at higher densities significantly faster than at medium or low densities. The mean time taken by satiated adults was longer than by starved adults, viz. 259.0 ± 5.3, 144.1 ± 2.5, 50.2

± 1.3 minutes and 199.5 ± 3.4, 93.3 ± 2.0 and 20.2 ± 1.1 minutes at low, medium and high prey densities respectively.

6.2 INTRODUCTION

How adult coccinellids find their prey was been the subject of some controversy. Most authors are not in agreement even on their method of prey detection. For example, Thompson (1951) reported that coccinellids detect their prey by sight, whereas Dixon (1959) using *Adalia bipunctata* concluded that both sight and scent are involved. Stubbs (1980) and Nakamuta (1984) supported this conclusion while working with *Coccinella septempunctata.* Sabelis & van de Baan (1983) reported that *Stethorus* larvae might use kairomones to detect phytoseiid hosts. Still other authors have confirmed the importance of physical searching in locating prey, including Colburn & Asquith (1970) who worked with adult *S. punctillum.* Hodek (1973) reported that adult coccinellids found their prey by actual contact, while Dixon (1959) and Kesten (1969) indicated that ladybirds walk upwards when searching for prey, because they are both positively phototaxic and negatively geotaxic. This chapter reports laboratory and field studies to investigate detection of their primary prey, *T. urticae* by adult *S. vagans*, because in the field the adult is the only stage capable of locating prey over any distance.

6.3 MATERAL AND METHODS

6.3.1 Host Finding

To determine whether *S. vagans* could find their prey, *T. urticae*, over a range of densities, investigations were conducted in the field, in the laboratory and in a constant temperature room.

6.3.1.1 Field experiment

Initially, French bean plants (cv. Redland Pioneer) were germinated in trays then transferred to 15 cm diameter plastic pots in potting mix after one week. The potted plants were infested with the required number of gravid adult female *T. urticae*, which had been cultured in a greenhouse at a mean temperature of 27 ± 2°C (Chapter 2). The mites were transferred and evenly distributed on each plant with a fine camel hair brush. Four treatments, three with mite densities: **low** (10 mites/plant), **moderate** (20 mites/plant), **high** (50 mites /plant) and a **control** (plants without mite) were set up at the same time with each density replicated on four potted bean plants. Thus, a total of 4 treatments x 4 replicates were exposed in the field at four different sites, a total of 64 plants. The sites were in four representative habitats: under pine (*Pinus radiata*) trees, in an ornamental area comprising shrubs and various flowers, in a peach (*Prunus persica*) orchard and in an orange (*Citrus sinensis*) orchard at the Centre of Horticulture and Plant Sciences, University of Western Sydney, Hawkesbury, Richmond (33^0 36'S, 150^0 44 E) in the central coast of NSW, Australia. The site for each density (treatment) was randomly changed weekly and the old plants were replaced with new ones. The distance between potted plants was 5 metres within the same density (replicates) and more than 150 metres between the different densities (treatments). These field-exposed plants were monitored daily and each plant was carefully inspected for at least five minutes. Leaves on

which adult *S. vagans* were observed were detached, then sealed separately in bottles and brought back to the laboratory. Each leaf was examined under a binocular microscope (at 20 X magnification) (Carl Zeiss, Germany) and all stages of *S. vagans*, *S. nigripes* and *T. urticae* were counted. All *S. vagans* were subsequently transferred to the laboratory culture. As the mite density was reduced as result of this leaf detachment, the plants were replaced weekly with new plants infested with the appropriate number of adult mites. The meteorological data during the collection period was obtained from a weather station located less than 1km from the experimental site on the campus of University of Western Sydney, Hawkesbury, Richmond. These investigations were conducted from March 1997 to March 1998.

6.3.1.2 Y-tube olfactometer

A Y-tube olfactometer was used to test the ability of adult *S. vagans* to locate the prey in the laboratory. The technique was similar to that used to assess olfactory responses of other predacious species to tetranychid mites (Dicke & Groeneveld 1986; Dong & Chant 1986). The apparatus consisted of a Y-tube with arms 30 cm long and square in section (30 mm x 30 mm). Each arm was lined with absorbent paper to provide a non-slippery surface for the beetles. The absorbent paper was replaced after each trial. Each arm terminated in a 10 x 11 x 5.5 cm chamber with an open lid at the top.

Adult *S. vagans* of both sexes were randomly selected from the founder colony, maintained at 25 ± 2°C in the laboratory. Some of the adults were starved for 24 hours, while others (satiated) were used in the Y-tube olfactometer immediately after selection. There were six treatments each replicated three times. A replicate was comprised of 10 adults (5 males and 5 females). The beetles were tested with and without airflow in the Y-tube. The air current was blown into each of the target chamber by a double outlet aquarium pump, exiting via the

release chamber. The temperature and relative humidity during the investigations was 21.5-27°C and 45-70% respectively, while light intensity during the investigation was supplied by two 40 Watt fluorescent tubes.

The treatments were:

1. Bean leaves with all stages of *T. urticae* vs bean leaves without *T. urticae*
2. Bean leaves with all stages of *T. urticae* vs damaged (by hand) bean leaves without *T. urticae*
3. Bean leaves with all stages of *T. urticae* vs empty cage
4. Bean leaves without *T. urticae* vs damaged bean leaves without *T. urticae*
5. Bean leaves without *T. urticae* vs empty cage
6. Damaged bean leaves without *T. urticae* vs empty cage

On each occasion 10 adult *S. vagans* (5 males and 5 females) were released in one chamber. The beetles were observed at hourly intervals for 8 hours then left over night (16 hours) and re-examined the next morning to determined their ability to locate their host.

6.3.1.3 Controlled temperature room

The host finding behaviour of adult *S. vagans* was also studied in a constant temperature room (Defensor® Axir Ltd. WMH Walter Meier Holding Co. Switzerland) with dimensions 2 x 3 x 2.4 m. The walls and roof of the control room were white and the room was equipped with a refrigerator unit, a humidifier (Atomizer 505 S) and two white fluorescent tubes at a height of 1.2 m from the floor. The experimental conditions for these investigations were 25 ± 2°C with RH 50 ± 10% and 80 W /m^2 light intensity without any air movement.

French bean plants (cv. Redland Pioneer) were grown in trays and later transferred to pots as described in Section in 6.3.2. Adult female *T. urticae* were released uniformly on these plants at rates of 20, 50, and >100 mites /plant, one hour before the investigation commenced. Each experiment was conducted by simultaneously exposing two plants (treatments) at a distance of 3 m from each other in the room from the following treatment combination, providing a choice for the released *Stethorus* adults:

1. Bean plant without mites vs Bean plant with damaged (damaged by hand) leaves
2. Bean plant without mites vs Bean plant with 20 mites /plant.
3. Bean plant without mites vs Bean plant with 50 mites /plant.
4. Bean plant without mites vs Bean plant with >100 mites /plant.

For each trial 4 *S. vagans* adults (2 males and 2 females) were randomly selected from the mass culture and simultaneously released mid way between the two plants (i.e. 1.5 m from each plant). This was repeated 6 times for both satiated and 24 hour-starved beetles for each treatment. The time between each treatment was 16 hours, while the position of the plants was changed after each test (replicate). Beetles were marked with different florescent powder (Radiant® Colour Division, Imperial Colour and Chemical Department, Hercules Inc., 2800 Radiant Ave., CA. 94804) before their release to assist in their recognition when recording their movements. They were observed continuously until they located their host (in the case of highest densities), or for a maximum five hours. The beetles were used only once to prevent previous experience influencing results.

6.3.4 Data Analysis:

The hypothesis was that *S. vagans* is able to locate populations of two-spotted mite, *T. urticae* at very low densities.

Costat Statistical Package (CoHort Software, Minneapolis, MN 55419, USA) was used to analyse the data. Treatment effects were compared by using Analysis of Variances (ANOVA). Least significant differences and standard errors were calculated by Duncan's multiple range test. Graphical representations were made using Origin 4.1 (Software for Technical Graphics and Data Analysis for Windows).

6.4 RESULTS

6.4.1 Host Finding:

6.4.1.1 Field Experiment:

Adult *S. vagans* were first observed on the field exposed plants on the 6th, 4th, and 2nd day after exposure at light, medium, and heavy mite treatments respectively. No predators were found on any mite-free (control) plant throughout the investigations. Only those leaves on which adult *S. vagans* were observed were collected from the infested plants. After 8 weeks of collection, the mean number of *Stethorus* recorded on high mite density plants was significantly greater than on medium and low density plants. The mean number of *Stethorus* was twice as high as that which occurred on plants with medium mite density and almost three times more than on plants with low mite density (Table 6.1). The mean number of all mite stages recorded from these treatment plants was 490.4 ±20.5, 19.5 ± 2.0, and 4.27 ± 0.5 respectively. No immature stages of *S. vagans* were found on plants with light or medium mite infestation. However in the high mite infestation treatment both eggs and larvae of *S. vagans* were found. Analysis of the data confirmed that mite density on plants significantly influenced the number of *S. vagans* found on them.

The number of all stages of *T. urticae* was also counted on each leaf on which adult *S. vagans* were observed. The mean number of eggs, nymphs, and adults were 49.5 ± 8.0, 18 ± 3.5 and 14.3 ± 2.1 respectively in the light mite density, 188 ± 13.2, 90.25 ± 7.4 and 94.5 ± 2.5 for medium and 757.25 ± 32.1, 673.25 ± 26.4 and 699.5 ± 18.3 for high density treatment.

Table 6.1 Mean number of leaves containing mites and *S. vagans* on plants exposed in the field.

Treatments (Nominal mite numbers)	n	Number of leaves with *S. vagans*	Number of TSM / leaf	Number of *S. vagans*
0 mite/plant (control)	16	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}
10 mites/plant	16	0.4 ± 0.1^{a}	4.3 ± 2.0^{b}	0.5 ± 0.5^{b}
20 mites/plant	16	1.7 ± 0.4^{b}	19.5 ± 5.0^{b}	2.5 ± 1.0^{c}
50 mites/plant	16	3.7 ± 0.6^{c}	490.4 ± 21.5^{b}	5.2 ± 3.0^{d}

* n = Number of plants in each treatment

* Numbers followed by different letters are significantly different ($p \leq 0.05$) using Duncan's multiple range test

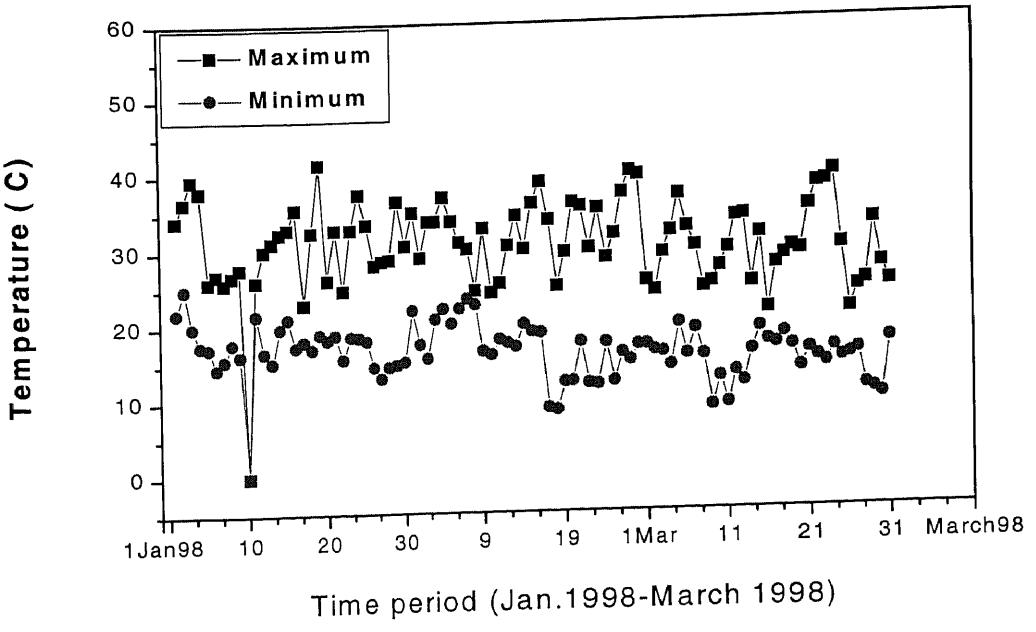

Fig. 6.1 Maximum and minimum temperatures recorded in the field during *S. vagans* host detection studies.

6.4.1.2 Y-Tube Olfactometer:

No *S. vagans*, whether starved or satiated were able to locate their host in the Y-tube olfactometer within an initial period of 8 hours, nor when left overnight (an additional 16 hours). The adults were observed walking from the release chamber up in the tube and trying to fly in the chamber, but were still not successful in locating their prey in the opposite chamber over the 24 hour investigation period.

6.4.1.3 Controlled temperature room:

Both starved and satiated adult *S. vagans* were able to locate their prey, *T. urticae*, at all three prey densities in the control temperature room. No beetles moved onto any mite-free bean plant (whether or not they had damaged leaves). They generally flew directly upwards and sat on the roof of the room immediately after their release in the middle of the two plants (ie. infested or damaged and untreated control). However after several minutes, they commenced searching by walking in the vicinity. This walking movement continued for approximately three to four minutes, after which the adult made a short flight and landed in an area approximate 5-10cm from the area already searched, and recommenced searching. With each flight and search they moved closer to the infested plant until the final short flight when they alighted on the plant. None moved in the direction of the identical mite-free plant. The mean searching time to locate their host plants decreased significantly ($p \leq 0.05$) as mite populations increased, for both satiated and starved beetles (Table 6.2). However beetles starved for 24 hours located their prey significantly faster ($p \leq 0.003$) than did satiated beetles. There was no significant difference between male and female searching times, whether satiated or starved, in any treatment.

Table 6.2 Mean searching time for satiated and starved adult *S. vagans* at different mite densities.

Treatment	n	Mean time to locate host (min.) *S. vagans* adults (Satiated)	Mean time to locate host (min.) *S. vagans* adults (Starved)
Control undamaged plants (No mites /plant)	12	> 300[a]	>300[a]
Plants with damaged leaves (No mites /plant)	12	> 300[a]	>300[a]
Low (20 mites /plant)	12	259.0 ± 5.3[b]	199.55 ± 3.4[b]
Medium (50 mites /plant)	12	144.08 ± 2.5[c]	93.24 ± 2.0[c]
High (100 mites /plant)	12	50.16 ± 1.3[d]	20.22 ± 1.1[d]

* n = number of plants used for each treatment.

*Numbers followed by different letters are significantly different ($p \leq 0.05$) based on Duncan's multiple range test

6.5 DISCUSSION

6.5.1 Host finding

6.5.1.1 Field experiment

The results clearly demonstrate that the adult *S. vagans* are capable of detecting and locating their prey from short and medium distances (Section 6.4.1.3) at densities at least as low as 10 mites per potted French bean plant. The number of *S. vagans* found on infested plants increased as mite densities increased. It therefore seems reasonable to assume that *S. vagans* are able to regularly establish on field grown plants infested with low populations (minimum 10) adult *T. urticae* per plant. Our results are strongly supported by Raworth (1990) and Zadeh *et al.* (1995), who reported that *S. punctum* and *S. gilvifrons* were active at a very low prey densities (1 mite /leaf) in the field. Congdon *et al.* (1993), Helle & Sabelis (1985b), Hull *et al.* (1977b), Richardson (1977) and Readshaw (1973) all reported that *Stethorus* spp. were able to find *T. urticae* in small isolated patches with one mite or less /leaf. Our investigations did not assess mite densities at this level. The view that *Stethorus* spp. are "high prey density dependent predators" has relied mostly on the study of random leaf samples (Congdon *et al.* 1993), and is not supported by our results, if this view is based at least in part, on their ability to locate prey populations population at low density.

Female *S. vagans* only oviposited in high-density mite populations, and no eggs were found at medium or low mite densities. However the leaves containing adult *S. vagans* were detached from plants at one-day intervals. This may not have been allowed females sufficient time to adjust to the new plant conditions and respond via ovipostion. Hull *et al.* (1977) reported that *S. punctum* laid its eggs on mite infested leaves which had 0.3-1.9 mites /leaf,

while a similar investigation by Congdon *et al.* (1993) reported that *S. punctum picipes* deposited a few eggs at very low prey density (1 mite /leaf).

6.5.1.2 Y-tube olfactometer

The investigations in the laboratory using the Y-tube olfactometer were not successful. None of the predators whether satiated or starved were able to find their host through the tube. However they were observed walking from the release chamber up in the tube, but invariably returned to same chamber. Most attempted to fly in the release chamber as well as in the tube, but there was insufficient room for successful flight. We also conducted some trials using fans to blow air down the tubes from the prey, but this was also not successful. Even when the olfactometer was placed perpendicular (90^0) to the bench top, none of the beetles were able to locate their prey. This was initially surprising, given their reported negative geotaxic behaviour (Dixon 1959; Kesten 1969). Based on the information discussed in the Section 6.5.1.3, it is hypothesised that for successful host location adult *S vagans* must make short flights, then search by walking. Such behaviour was not possible in the closed Y-tube olfactometer.

6.5.1.3 Controlled temperature room

In the enclosed room, all satiated and starved male and female *S. vagans* were able to find their prey at all mite densities, while none were attracted to mite-free plants even when they had been mechanically damaged. Starved beetles took significant less time to locate their prey at all mite densities than did satiated beetles. The time taken to locate their prey increased with reduced mite density for both satiated and starved predators. When the beetles were first released, they flew and sat on the roof of the room for few minutes and then commenced searching. Each beetle (whether satiated or starved) made a low short flight and

sat to search their vicinity by walking. This process continued until they located their host, with each flight bringing them closer. A possible reason is that *T. urticae* emit some chemical e.g kairomone, which attracts predators, and this attraction is not merely associated with plant feeding damage. Sabelis & van de Baan (1983) reported that larval stages of *Stethorus* were attracted to *T. urticae* by kairomones, as has also been reported for predatory mites (Dong & Chant 1986). It is likely that adult *Stethorus* are also attracted by kairomones. This may explain reports that adult *Stethorus* can fly actively to locate even isolated mite colonies (Helle & Sabelis 1985b).

Based on the results presented above, it is likely that adult *Stethorus* have two modes of searching to locate their prey, ie. from a distance they locate by smell, and initially fly in the direction of their prey. To actively locate prey, they search an area by walking which may rely on sight, direct encounters and /or perhaps smell. After a period of unsuccessful searching, they fly again in the direction of their prey, landing and searching in a new area.