CHAPTER 2

GENERAL MATERIALS AND METHODS

2.1 INTRODUCTION

Development of effective and economical technologies for mass rearing of prey and predators are key issues for successful biological control programs. Nutritional quality for prey and predatory arthropod rearing is a fundamental unit in biological control. Inadequate nutrition can cause comprehensive changes in the metabolism, behaviour and other characteristics of arthropods. Rearing entomophages is generally more complex than rearing phytophagous species, especially if the species is an obligate predator or parasite. In such cases prey must also be reared, which may in turn require the rearing of host plants for the prey. All these activities must be synchronised.

A number of different systems for rearing predatory coccinellids have been developed. The ladybird *Hippodamia sinuatua* Mulsant, was reared in plastic petri dishes and supplied either greenbugs or corn leaf aphids (Michels & Behle 1991). *Stethorus* spp. were reared on orange fruits in 15.46 x 28 hard ware-cloth trays, which held 138 orange fruit of 55 size. These trays were maintained in a linting box (Scriven & Fleschner 1960). Daniel (1976) reared *S. keralicus* in microcages made of cork and glass sheets (5 x 5 cm) into which a portion of a mite infested leaflet could be inserted. Adults and larvae of *S. pauperculus*, were reared in the laboratory in petri dishes (10 cm diameter) inverted one above the other and held in place by a paper band along the rim. Fresh leaves bearing heavy populations of the mite *Tetranychus cucurbitae* were provided daily in these petri dishes (Puttaswamy & ChannaBasavanna

1977). Richardson (1977) used two methods for culturing *S. nigripes* rearing. In the first method he maintained mite on orange fruit in a close container; in a second he brushed mites into 250 ml waxed paper cups in which predators were located. Methods used for mass rearing are not those commonly used for detailed biological studies. Richardson (1977) for example used 2 cm diameter Munger cells for individual stage rearing or biological studies.

2.2.Culture

2.2.1. Stethorus

The stock culture of the ladybird *Stethorus vagans* was started with adults caught from the field. Thirty potted (15 cm diameter pots) French bean plants infested heavily with *T. urticae* > 100 /leaf, were placed at 10 sites around the Centre for Horticulture and Plant Sciences, University of Western Sydney, Hawkesbury, Richmond (33^0 36'S, 150^0 44 E), New South Wales, Australia. Beetles collected weekly from these sites were examined under a binocular microscope to identify them to species. In general two species of *Stethorus* were caught from the field, namely *S. vagans* and *S. nigripes*. The number of *S. vagans* caught each week was consistently three times more than *S. nigripes*. After identification *S. nigripes* were released in the field, while *S. vagans* were retained and cultured on detached French bean leaves infested with *T. urticae*, that were placed over wet sponge in ventilated plastic boxes with (dimension 30 x 12 x 6 cm). The plastic boxes were covered with nylon cloth (mesh 70 μ) and their upper lids had a rectangle hole of dimensions 22 x 7 cm cut out (Fig 2.1). The culture was maintained as a stock colony in an illuminated incubator (Thermoline Industries, Thermoline Scientific Equipment Pty Ltd., 40 Blackstone Street, Wetherill Park, NSW 2164, Australia) with internal dimensions 153 x 60 x 61 cm at 25 ± 0.10°C with a photoperiod of L: D 16: 8 hour and relative humidity 44-66 % ± 10%.

Fig 2.1 Rearing of *S. vagans*.

2.2.2 Two spotted mites

T. urticae were used as prey throughout the three year study period and were obtained from a culture maintained in a heated glasshouse with temperature ranged 21-35°C and daily mean temperature 27 ± 5°C. The French bean *Vicia faba* (cv Redland Pioneer) host plants were grown in plastic trays 60 x 30 x 6 cm in a composted seed potting mix with a basal fertiliser added, and were infested with *T. urticae* one week after emergence. These plants were transferred to 15 cm pots with the same potting mixture and kept in the insectary to use either as food for the culture or for field collecting predators.

Infested potted bean plants were changed every second week in the field, while infested leaves were supplied every day to the *Stethorus* rearing culture.

Fig.2.2. Rearing of two-spotted mite cultures.

2.3.Identification of the Species

The identification of both predators and prey species were confirmed by the Entomology Section of the Biological and Chemical Research Institute Rydalmere; NSW Agriculture and later by the Australian National Insect Collection (ANIC), Canberra. ANIC also provided named specimens of *Stethorus* spp to assist in later identification.

2.4 Methodologies for Biological Studies

A range of experimental systems were evaluated prior to biological studies. These were initially selected on the basis of previously reported successful methodologies.

2.4.1 Munger cell

For life history, rate of development, fecundity, mortality and adult longevity modified Munger cells (2 cm diameter) were trialed. Munger (1942), Huffaker (1948) and Laing (1968) have described the construction of and use of Munger cells. There were 10 cells in each set, which were modified by drilling 10 holes in each cell (10 x 1 mm holes) in the upper lid to reduced relative humidity in the cell. A small moist cotton ball was placed in each cell to maintain leaf disc freshness. A pair of *S. vagans* was released in each cell to study their biology. This method proved unsuccessful, because the leaf discs commonly had free water on their surface probable from the humidity associated the moist cotton balls and the prey become wet and some time died. Both the prey and leaf surface were unacceptable for the *Stethorus*, resulted in high mortality.

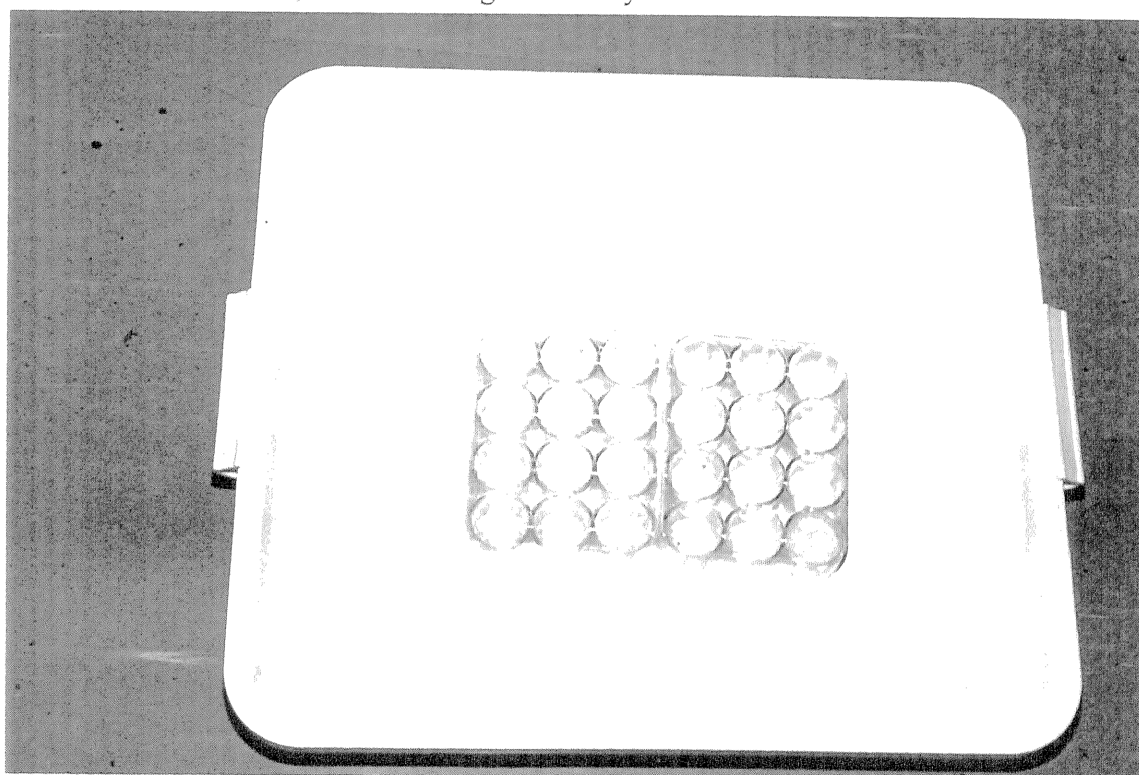

Fig.2.3. Munger cell trialed for *S. vagans* rearing.

2.4.2 *Small cages*

A second method evaluated was a cage with a frame made of 5-6 cm diameter aluminium rods and covered with nylon mesh (70μ), except for the front sleeve (12 cm long) which was tied by a rubber band. The volume of each cage was 30 x 30 x 15 cm and a total of 10 cages were used. The base of each cage had a shallow metal tray 25 x 12 x 4 cm, which was regularly filled with water to maintain nutrient levels in potted plants. In each cage a potted strawberry (*Fragaria ananassa*) plant heavily infested with *T. urticae* was placed. A pair of *S. vagans* was released on each plant to study their biology. This method was also not successful because observation of the immature stages under a stereo microscope was very difficult and disturbed them.

2.4.3 *Small petri dishes*

Sealable petri dishes (5.0 x 0.9 cm) Falcon® (Becton and Dickinson Labware, Becton Dickinon & Co. Franklin Lake, New Jersey, USA) were also evaluated. Leaves were detached from mite infested French bean plants (cv Redland Pioneer) and punched into 2.5cm diameter discs with a metal cork-borer. These discs were placed upside down on 4cm diameter wet sponge on the base of each petri dish. A pair of *S. vagans* was placed on each leaf disc to assess this method for reproduction. However vapour condensation on the upper lid of the dishes resulted in high mortality of adult *S. vagans* due to drowning. In an alternative method infested leaf discs were placed on dry sponge, but these discs dried rapidly, again resulting in high mortality of *S. vagans*.

2.4.4 *Modified petri dishes*

A number of modifications were made to the sealable petri dishes (Section 2.4.3) to reduce problems with condensation. Initially 30 x 1 mm diameter holes were drilled in the upper lid,

but this did not sufficiently reduce relative humidity. A second modification involved drilling a 3 cm diameter hole in the upper lid and covering it with fine nylon mesh (70μ). This method was successful in reducing the humidity, but the problem was now to maintain sufficient humidity to prevent desiccation of prey and predators in the forced air temperature chambers. The dishes were placed in 35 x 30 x 6 cm plastic containers with the upper lid drilled with 60 x 1 mm diameter holes. This method resulted in low mortality and satisfactory completion of life cycles. The dishes also allowed for adequate observation of *S. vagans* with minimal disruption. This methodology was therefore used to study rates of development, mating behaviour, fecundity, longevity, diapause, time partitioning, and rate of prey consumption.

Leaf discs (2.5 cm diameter) infested with *T. urticae* were placed on wet sponge (4 cm diameter) in these modified petri dishes for experiments with adult *S. vagans*, while dry filter papers (4.7 cm diameter) in the same dishes were used for immature stages. Immature stages were reared on dry filter paper for two reasons: first, dry filter paper provided a large surface area with similar characteristics to leaves; second the last instar larvae become impaled on trichomes on the surfaces of French bean leaves. All stages of *T. urticae* were supplied daily to *S. vagans* larvae by brushing infested French bean leaves with a mite brushing machine (Moreton 1969; Helle & Sabelis 1985b) (Fig 2.5).

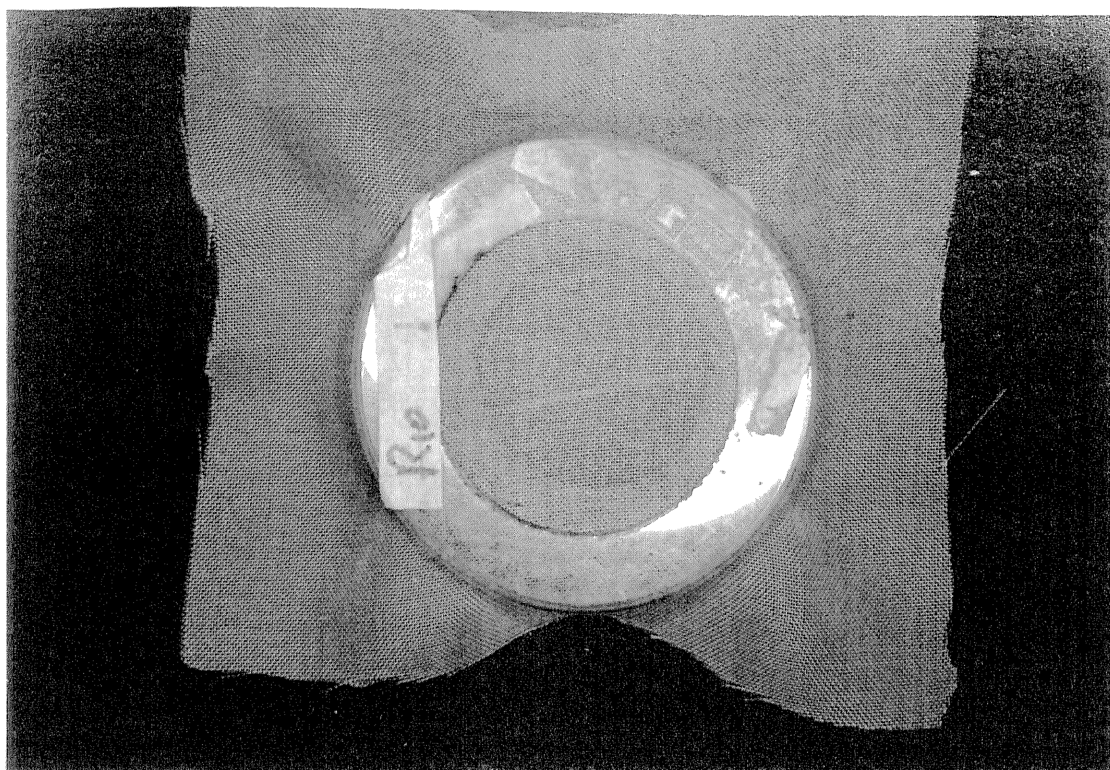

Fig 2.4 Modified sealable petri dishes used for biological studies of *S. vagans*.

Fig 2.5 Mite brushing machine.

Assessment of alternative hosts sources was undertaken in the same petri dishes, but with several differences. Wet cotton balls (concentration of distilled water and honey of 50: 50 v/v) were placed on the mesh screen; while fresh pollen from pecan (*Carya illinoensis*) were supplied in a small plastic lid (3 mm diameter) placed on the filter paper in the base of petri dish. All other potential prey were either provided on 2.5 cm leaf discs of their respective host plants or removed from the leaf with a fine camel hair brush and placed directly on the filter paper. The moist cotton balls were replaced daily, while pollen was replaced every second day to prevent fungal contamination. For living hosts, their number was counted at the time of introduction and removal, and they were changed daily.

2.5 Constant Temperature Incubator

Thermoline® incubators (internal dimension 122 x 52.5 x 43 cm) (Catalogue number RI 250, Thermoline Scientific Equipment Pty Ltd., 40 Blackstone Street, Wetherill Park, NSW 2164, Australia) were used to investigate effects of temperature, relative humidity and photoperiod on the development of *S. vagans*. These were operated at constant temperatures of 10, 15, 20, 25, 30, and 35°C ± 0.1°C with 16 hour photoperiod.

The temperatures in the incubators were automatically controlled. Engineering parameters were configured to provide stable temperature control with minimum overshoot and quick recovery times. However, temperature and RH were regularly confirmed with a digital thermometer and RH probes placing inside in the plastic container beside the petri dishes.

Light intensity was provided by 3 x 30 watt white fluorescent tubes in the door of incubators, which were controlled by a 24 hour time clock. There were air vents located in the top and bottom of the door enclosure to minimise the effect of heat generated by the fluorescent tubes, while most internal surfaces were white to maximise lighting.

2.6. Climate Controlled Growing Room

Host detection behaviour was investigated using infested French beans plants in a temperature control room 2 x 3 x 2.4 m (Bondor® James Hardie Building Systems, Sydney, Australia). The room contained low intensity of light 3 x 30 fluorescent tubes, a refrigerator unit and a humidifying unit (Defensor 505, Axair AG 88108 Plaffiker, Switzerland). The temperature and relative humidity were controlled automatically. The temperature was set at 25 ± 5°C with relative humidity 44-66 % ± 10% and confirmed with a digital thermometer and RH probes.