#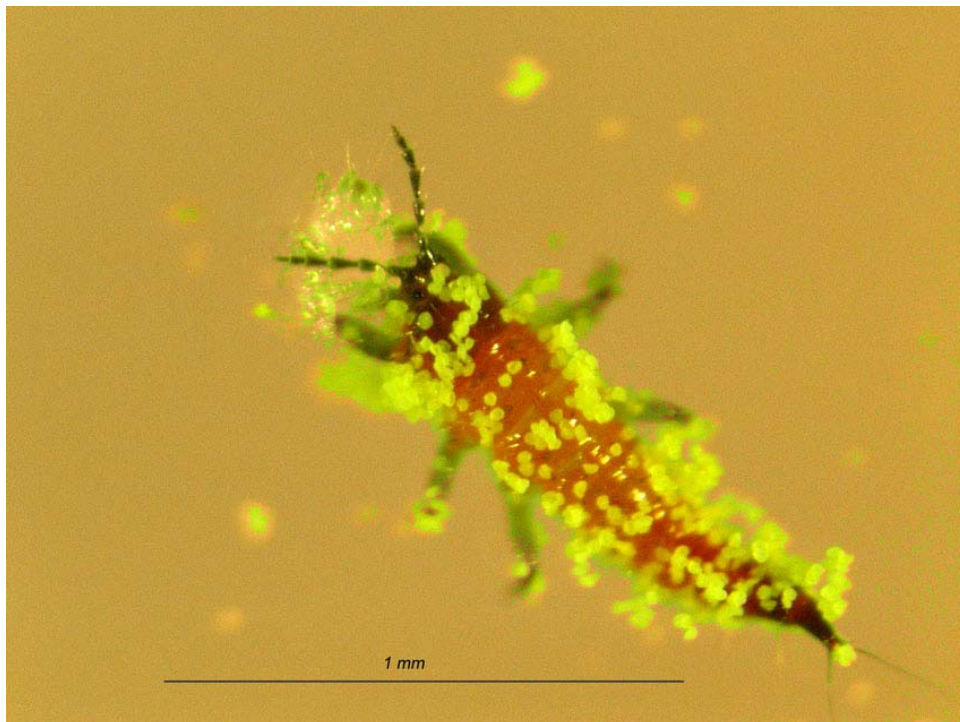 INVESTIGATION INTO ASPECTS OF THE BIOLOGY OF TUBULAR BLACK THRIPS, *Haplothrips victoriensis* BAGNALL (THYSANOPTERA: PHLAEOTHRIPIDAE), IN SOUTH AUSTRALIA

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The first instar larva of tubular black thrips with "pollen disguise" feeding on *Tyrophagus* mite.

The thesis submitted for the degree of Master of Science

Discipline of Ecology and Evolutionary Biology

THE UNIVERSITY OF ADELAIDE AUSTRALIA

SUB CRUCE LUMEN

April, 2008

DECLARATION

This thesis contains no material that has been accepted for the award of any other degree or diploma in any university. To the best of knowledge and belief, this thesis contains no material previously published or written by another person, except where due reference is made in the text of the thesis.

I consent to this thesis being made available for photocopying and loan when deposited in the University Library.

Signature: Date:

(changing this page by a yellow blank sheet here)

Dedicated in loving memories

to my mum & older brother

Pham Thi Tung   *(10/01/1945 - 08/10/1998)*

Le Cao Nguyen   *(22/06/1971 – 29/12/2005)*

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THESIS SUMMARY

Haplothrips victoriensis Bagnall (Thysanoptera: Phlaeothripidae) is an indigenous thrips of southern Australia. It is known as a predator of two-spotted mite. This project investigated various biological characteristics of *H. victoriensis* as a precursor to its potential use in biological control. In addition, *H. victoriensis* is very difficult to distinguish from other *Haplothrips* species in terms of morphology, especially as there are no keys to larvae. DNA barcoding and morphology of the larval stages are used to address these issues.

The thesis is divided into seven chapters comprising an introduction, four main research chapters, conclusion and reference chapters.

Chapter 2 covers the culturing techniques for *H. victoriensis.* Some new cases and rearing processes were designed to culture *H. victoriensis* and its prey. These were following series of rearing techniques investigated including rearing *H. victoriensis* for egg collection, *H. victoriensis* larvae and adults for biological and morphological observations, mass rearing of *H. victoriensis,* WFT and *Tyrophagus* mites, and rearing WFT for egg collection. Predatory *H. victoriensis* was reared in the laboratory with various diets of honey, pollen, *Tyrophagus* mites and western flower thrips (WFT).

Chapter 3 investigates the biological development of *H. victoriensis,* including egg, larva, prepupa, pupa and adult stages. *Haplothrips victoriensis* fed with 10% honey solution, mixed pollen and *Tyrophagus* eggs took about 16-22 days to develop from eggs to adults at 25 $^{\circ}$C. Developmental times of immature *H. victoriensis* varied when fed on different food sources. The longevity of *H. victoriensis* was significantly different with different diets.

Chapter 4 examines predatory and cannibalistic characteristics and consumption of *H. victoriensis* on various prey. *Haplothrips victoriensis* was found to feed on many kinds of common prey; Two spotted mite (TSM), *Tyrophagus* mite, WFT, spotted alfalfa aphid, greenhouse whitefly and diamond back moth but not on ash whitefly. Importantly, *H. victoriensis* was also found to be cannibalistic which might affect population fluctuations in the laboratory and in the field.

Chapter 5 examines the ecology and hosts of *H. victoriensis* in South Australia and taxonomy of *H. victoriensis* and some other *Haplothrips* species being similar to *H. victoriensis*. *Haplothrips victoriensis* was found to live on flowers from a range of different plant families. Morphological characteristics of the immature stages of *H. victoriensis* and a key developed to separate common *Haplothrips* in S.A. As well, COI was used to possibly identify *H. victoriensis* and its morphologically similar taxa and investigate whether *H. victoriensis* is a single species or perhaps contains cryptic species. None of the specimens of *H. victoriensis* included in the analyses showed any variation in COI indicating a single species for horticultural areas in S.A.

Chapter 6 provides an overall discussion of the main results and indicates areas of future research.

Table of contents

Contents	Page

CHAPTER 1

GENERAL INTRODUCTION

1.1. Thysanoptera – general review

1.2. Common pest thrips in SA

      1.2.1. Western flower thrips (WFT)

      1.2.2. Plague thrips

      1.2.3. Other pest thrips

1.3. Current control(s)

1.4. *Haplothrips* species in Australia

1.5. *Haplothrips victoriensis* Bagnall (Thysanoptera: Phlaeothripidae)

1.6. COI Barcoding

1.7. The aims of the study

CHAPTER 1

General Introduction

1.1. Thysanoptera – general review

Thrips were named *Physapus* when De Geer first described them in 1744. In 1758, these insects were called "Thrips" and Linnaeus included just four species belonging to one genus. The genus was raised to an order by Haliday, the English entomologist, in 1836 and the name "Thrips", used for both singular and plural forms, was applied to insects of this order (Lewis, 1997a). Thrips are very small insects, mostly a few millimeters long and of slender shape, which makes them difficult to recognise using the naked eye. Although they are minute insects, almost all thrips have two pairs of wings; some are however wingless. Each wing has two bands of cilia arranged symmetrically. Therefore their order was called Thysanoptera meaning "fringed winged" (Lewis, 1973).

300 μm

a   b
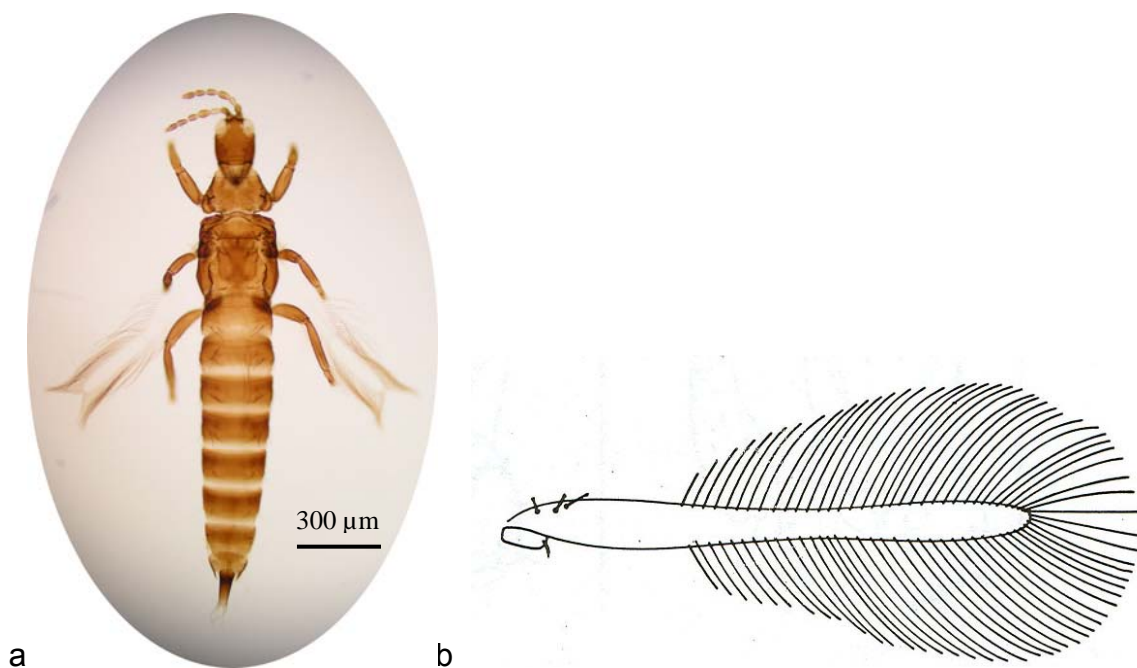
Figure 1.1. a: *Haplothrips victoriensis*; b: the fore wing of thrips (Pitkin, 1973).

There are approximately 5800 species described (Mound and Minaei, 2007) arranged into eight families belonging to two suborders Terebrantia and Tubulifera (Mound *et al.*, 1980). Thrips are distributed worldwide and pest thrips are very quickly able to extend their range to other places via wind, gardening tools, freight or agricultural machinery. As well, they are involuntarily scattered by human activities in company with plants or agricultural products. They are cosmopolitan and occur abundantly in tropical and temperate zones in a range of habitats including forests, grasslands, bushes, leaves and flowers (Lewis, 1973), litter (Mound, 1972), and galls (Mound, 1971).

Approximately half of all thrips species feed on fungi, with others feeding on leaves, branches, pollen or fruits of plants, and some are known as predators of mites and other thrips (Mound and Marullo, 1998). The latter includes *Haplothrips brevitubus* Karny (Kakimoto et al., 2006), *Haplothrips victoriensis* Bagnall (Bailey and Caon, 1986) and *Aeolothrips intermedious* Bagnall (Lewis, 1973).

Because of their feeding behaviour, About 50 hundred species of thrips are pests. Pest thrips have a detrimental effect on the agricultural economy both directly and indirectly. They cause serious damage to a diverse range of crops such as apple, capsicum, cucumber, eggplant, onion, orange, tobacco and wheat by their feeding action which reduces productivity and the quality of agricultural products. They also known as vectors of viruses such as the impatiens necrotic spot virus and the tomato spotted wilt virus, which attack crops (Mound and Kibby, 1998).

1.2. Common pest thrips in South Australia

Thrips was initially studied from the 1910s in Australia (Mound and Tree, 2007) and the Australian thrips fauna has been investigated extensively since the 1960s (Mound, 1996), with 445 species of Australia thrips identified and described (Mound,

1996). By 2003 more than 550 species were recorded on the website http://www.ento.csiro.au/thysanoptera/Ozthrips/Ozthrips.html (Mound, 2004a) and about 700 thrips species have now been described (Mound and Tree, 2007). The life-history and biology of the thrips fauna in South Australia has not been intensively investigated; as Mound (2004b) mentioned "the southern thrips fauna is both less species rich and less studied, although some species are shared with territories further south". Some of the research on thrips in South Australia includes *Karphothrips dugdalei* Mound & Walker and *Physemothrip*s recorded from Australia by Mound (2004b) but originally from New Zealand, and *Cartomothrips manukae* Stannard from New Zealand and Tasmania. Mound and Morris (2001) investigated the Phlaeothripine fauna on *Acacia aneura* in Australia and recorded nineteen genera and twelve species belonging to *Dunatothrips* and *Sartrithrips*. Of these, only one species, *Dunatothrips armatus,* was found in South Australia.

In South Australia, although there have not been any recently published studies on common pest thrips, both endemic and exotic or their biodiversity and distribution. Many thrips cause serious economic losses on flowers, fruits, ornamental plants and vegetable crops (especially cucurbits) by their feeding. Common pest species include western flower thrips (*Frankliniella occidentalis* Pergande), Plague thrips (*Thrips imaginis* Bagnall), tomato thrips (*Frankliniella schultzei* (Trybom)), onion thrips (*Thrips tabaci* Lindemann), Kelly's citrus thrips (*Pezothrips kellyanus* Bagnall) and gladiolus thrips (*Thrips simplex* (Morison)).

1.2.1. Western flower thrips (WFT)

Western flower thrips (WFT), *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae) is one of the most serious global thrips pests. It originates from western North America and was first recorded in 1895 (Driesche, 1998; Mound & Marullo,

1996). It occurrs in almost all regions of North America (Beshear, 1983) where it has continued to outbreak in glasshouse crops. It became a major worldwide crop pest in Asia, and Africa, New Zealand by 1992, and Australia by 1993 (Kirk and Terry, 2003). Kirk and Terry (2003) estimated that WFT outbreaks dispersed at the rate of 229 ± 20 km/year. In Australia, WFT was initially recorded in *Chrysanthemum* flowers in a glasshouse in Western Australia in April 1993 (Malipatil *et al.*, 1993). It spread to Queensland and New South Wales in the same year. It was later found in Tasmania and South Australia (1994) and Victoria (1996) (Kirk and Terry, 2003). According to CSIRO (2006), WFT is now present in almost all parts of Australia except the Northern Territory.

Like others thrips, WFT's shape is slender, long, hairy is about 1.6 mm in length. Adult males are usually smaller than females and have a light yellow body, whereas females appear in a range of colours from yellow, light yellow, yellow with brown splotches, to dark brown (Mound & Marullo, 1996; Parrella, 1996). Each female adult lays from 150-300 eggs but this varies depending on environmental conditions and nutrients. It oviposits its eggs into plant tissues of leaves, buds and petals. Larvae emerge after seven day and lack wings. Post emergence they spend two instars feeding on leaves and flowers, one to two days in the first instar and two to four days in the second. After this time the larvae stop feeding, change to prepupae and drop to the soil or hide in leaf litter to pupate. Winged adults emerge from one or two day later. Depending on temperature, WFT's life cycle lasts from one to two weeks (University of Massachusetts, 2003).

WFT is known as a serious worldwide greenhouse pest. It not only feeds on flowers and foliage of crops, leaves, buds, flowers and fruits but also transmits viruses to plants. WFT are known to damage many vegetable crops as well as a

range of fruit trees such as olive, apple and stone fruits including nectarine and peach. In Australia, they significantly reduce the productivity of strawberries (Steiner and Goodwin, 2005). WFT is known to transmit two kinds of Tospovirus, the impatiens necrotic spot virus (INSV) and the tomato spotted wilt virus (TSWV). Only the first instar larvae of WFT acquire Tospovirus from viruliferous plants and transmit to other plants when they become adults (Paliwal, 1976; Ullman, 1996; Mound, 2002). These viruses can infect over 600 species of crop plants, for example tomatoes, cucumbers or capsicums, as well as various ornamental and weed species. The symptoms of plants infected by these viruses can include brown, black, or white spots; necrosis on the leaf petiole; yellow mottling or variegation; death of young plants or death of terminal meristems of older plants; stunting; brown or black cankers on the stem; veinal necrosis; concentric ring spots; mosaics; and line or zonal patterns (Daughtrey *et al.*, 1995). The infected plants may also collapse completely (Sakimura, 1963; Ullman, 1996).

1.2.2. Plague thrips

Plague thrips (*Thrips imaginis* Bagnall) (Thysanoptera: Thripidae) was initially investigated at the Waite Institute in the early 1930s by Davidson and his team, and later by Andrewartha, who undertook Davidson's unfinished work to study the biology and ecology of the thrips. Eggs of *T. imaginis* are usually oviposited in tissues of flowers, and larvae and adults may inhabit flowers of various plants (Davidson and Andrewartha, 1948). Kirk (1984) showed that *T. imaginis* larvae have to move and live in specific flowers such as *Echium plantagineum*, (with many common names including salvation jane, Riverina bluebell, blue weed and purple bugloss), so that they can complete their development. When they have completed the larval stage, they frequently move to soil or litter to pupate (Davidson and Andrewartha, 1948).

Their life cycle lasts only 9 days at 25°C from eggs to adults (Steiner and Goodwin, 2005). There are two colour forms of *T. imaginis* depending on the season, dark colour in winter and pale yellow during spring (Kirk, 1985).

Wilson et al. (1996) indicated that *T. imaginis* is also an opportunistic predator of two-spotted mites. However, its damage on apple products had a huge effect on the Australian agricultural economy in the 1900s and 1930s. *Thrips imaginis* is endemic in southern Australia and was found in apple blossom (Davidson and Andrewartha, 1948). They found the number of *T. imaginis* increases dramatically in spring, peaks in December and decreases in February each year. In their abundant period, they damage buds and flowers of apple crops leading to significant economic losses, particularly in the period 1900 to 1940 in Western Australia, South Australia, Victoria and New South Wales because there was a lack of information about *imaginis* populations and damage prevention methods. Until 1950, pesticides such as DDT and parathion were initially sprayed on apple crops (Andrewartha and Kilpatrick, 1951). Plague thrips also cause damage to the flowers of hydroponic strawberries in Australia generally and in the Adelaide Hills in South Australia, particularly in 1998 (Steiner and Goodwin, 2005).

1.2.3. Other pest thrips

As well as WFT and plague thrips, there are some other common pest thrips in South Australia including both native and exotic species.

Onion thrips, *Thrips tabaci* Lindemann (Thysanoptera: Thripidae), an exotic pest thrips, is found throughout Australia, It is a polyphagous insect and attacks many kinds of plants such as cotton, cucurbits and onions. It is also thought to be originated in western Asia (Mound, 1976). Onion thrips is also a vector of TSWV which causing quick damage on crops of tomato, tobacco, pepper and

chrysanthemum (Jenser et al., 2003). TSWV was stored in second instar larvae of onion thrips when they had fed on an infected plant, then adults emerging from the viruliferous larvae transmit the virus to other plants during their life (Jenser et al., 2003).

Kelly's citrus thrips (KCT), *Pezothrips kellyanus* Bagnall (Thysanoptera: Thripidae), was recorded in the 1990s in SA. It is known whether KCT is endemic or exotic to Australia (Mound and Jackman, 1998). It is a serious pest of citrus such as grapefruit, lemons, navel and Valencia (Baker et al., 2000; Vassiliou, 2007). Their eggs were oviposited in fruit and all parts of citrus flowers, especially in petals so they are abundant in flowering season in spring (Baker et al., 2000).

Thrips australis (Bagnall) (Thysanoptera: Thripidae), which is indigenous to Australia occurs in all territories of Australia and is also present in New Zealand, Africa, America, Asia and Europe. Its host-plants include eucalypts and other Myrtaceae and non-native Rosaceae (Kirk, 1987; Mound, 1968; Mound & Masumoto 2005).

Another species is *Thrips simplex* (Morison) (Thysanoptera: Thripidae), known to attack gladiolus cultivars. It lives on gladiolus flowers and species of *Kniphofia* and *Dianthus* (Nakahara, 1994; Mound & Masumoto, 2005). Gladiolus thrips originates from Africa and has been found in South Australia, Victoria, Western Australia and Queensland (Kirk, 1987; Mound & Masumoto, 2005).

1.3. Current control(s)

Pest thrips are currently controlled by either chemicals or using integrated pest management (IPM). Pesticides have been used to kill thrips since the middle 1940s, and comprise a wide range of inorganic and organic compounds such as α-cypermethrin, permethrin, bifenthrin, methomyl, and methiocarb. However the

pesticides needed to be applied in increasingly heavy doses due to the development of resistance in thrips. Advanced synthetic insecticides in the 1960s and 1970s and modern synthetic insecticides in the 1980s provided temporary control for some pest thrips (Lewis, 1997b). Currently, insecticides have been mainly used in lettuces to treat seeds before cultivation in nurseries so that chemicals, absorbed and existing in germs and seedlings, can temporarily prevent the attack of pest thrips.

Integrated pest management (IPM) is a strategy that maximizes natural enemies of pest populations and uses other options only if needed and with minimum effects on the surrounding environment when the pest population exceeds an economic injury level (Davis and McMurtry, 1979). IPM programs aim to involve maximum reliance on natural pest population controls with a combination of techniques that may contribute to suppression such as cultural methods, insect pathogens, resistant crop varieties, sterile insects, attractants, augmentation of parasites or predators, or chemical pesticides as needed (Cate and Hinkle, 1994). Natural enemies as biocontrol agents are often an important tool in any IPM program.

IPM has gained much more attention in terms of its environmental benefits and the development of sustainable agriculture. Many factors have been investigated as potential controls for pest thrips, such as host plant resistance, mechanical and cultural practices, postharvest and biological control. With biocontrol methods, biologists have explored the advantages of natural enemies (Hajek, 2004) such as insect and mite predators (*Orius tristicolor, O. laevigatus*, *Amblyseius cucumeris*, *A. limonicus* and *Haplothrips brevitubus*), parasitoids (*Ceranisus menes* and *C. americensis*) (Sabelis and Rijn, 1997), parasitic nematodes (Steinernematidae and Heterorhabditidae, *Thripinema nicklewoodi*) and fungal pathogens (*Beauverria bassiana* and *Verticillium lecanii*) (Loomans *et al.*, 1997) to manage and reduce pest

thrips to densities below economic thresholds. Kakimoto et al., 2006 investigated *H. brevitubus* and its potential to control *Pseudodendrothrips mori* (mulberry thrips) in Japan and found that it consumed up to 30 *P. mori* larvae in a day.

Biological control is an effective tool to reduce populations of pests naturally. This powerful control method involves the integration of agricultural technology and biological science to restore population balance in ecological systems (Bellows, 2001). Biological control has a potential role in terms of pest thrips management resulting in ecologically sustainable pest control. There are many natural enemies worldwide which can be used to contribute to the control of pest thrips. Many genera and species from a number of orders are listed as predators of adult thrips, including lacewings (Neuroptera: 16 species), flies (Diptera: 23 species), wasps (Hymenoptera: 16 species), beetles (Coleoptera: 27 species), mantids (Mantodea: one species), crickets (Orthoptera: 2 species), bugs (Heteroptera: 60 species), predatory thrips (Thysanoptera: 24 species) and mites (47 species) (Sabelis and Rijn, 1997). These authors also record 32 parasitic eulophid wasp species (Hymenoptera: Eulophidae) on thrips larvae, 23 species of egg parasitoids (Trichogrammatidae), ten of which were previously unidentified. Fungi also have promising potential to control thrips, and parasitic nematodes have also been recorded. Goodwin and Steiner (1996) carried out a survey with the purpose of recording native natural enemies of thrips in Australia. They surveyed 650 sites and recorded that 36 species of phytoseiid mites were predators of thrips, and that *Ceranisus menes* (Walker) (Hymenoptera: Eulophidae) was a common parasite of thrips in Australia. Although there is a large amount of information in regard to the natural enemies of thrips, this information is fragmented and comes largely from anecdotal records. To fully recognise the

potential of these natural enemies, a more thorough and complete study must be undertaken.

1.4. *Haplothrips* species in Australia

Thripidae and Phlaeothripidae contain about 93% of all species (Mound, 1976; Mound and Kibby, 1998). Phlaeothripidae, a single family of the suborder Tubulifera comprises 530 genera and 3500 species, and is the largest family of Thysanoptera (Mound and Morris, 2007), of which *Haplothrips* is the largest genus containing 250 species worldwide (Mound and Minaei, 2007). They are mainly found on the flowers of Compositae and grasses (Mound and Kibby, 1998), and on dead leaves and branches (Pitkin, 1973). Pitkin (1973) included 11 Australian *Haplothrips* species. However, the number of *Haplothrips* taxa in Australia has recently been updated to 24 species by Mound and Minaei (2007) (see Table 5.1). The key for Australian Haplothripini of Kelly and Mayne (1934) was updated by Pitkin (1973). This key has been used to identify Haplothripini species, even though a few species included by Pitkin were derived from central and southern America (Mound, 1996). Mound and Kibby (1998) published a second revised edition "Thysanoptera: an identification guide" which provides a key common thrips genera, and the new version key to Australian Haplothripini has recently been published by Mound & Minaei (2007).

1.5. *Haplothrips victoriensis* Bagnall (Thysanoptera: Phlaeothripidae)

Haplothrips victoriensis was first described in 1918 by Bagnall and belongs to the Phlaeothripidae family (Bagnall, 1918). It is endemic to Australia and is commonly collected on flowers of lucerne, grape vines and roses, and orchards in South Australia. Immature morphologies were described initially by Steele (1935) but developmental times were not investigated. Andrewartha (1936) measured the

developmental rate of *H. victoriensis* for a range of temperatures. However developmental time of each stage was unknown. Bailey & Caon (1986) noted that a mean development time from egg to first instar was 5 days and from the first instar to adult was 9.9 days at 25°C. Otherwise, aspects of the life cycle of *H. victoriensis* remain unclear and other information about their biology and ecology needs to be considered.

Haplothrips victoriensis lives in flowers and feeds on pollen and nectar. It has been reared in the laboratory with snapdragon stamens (Andrewartha, 1936), and with pollen and honey (Bailey and Caon, 1986). Bailey and Caon also found that *H. victoriensis* was an omnivorous thrips predator on eggs of two-spotted mites (*Tetranychus urticae*). As well, *H. victoriensis* has been found as a predator of melon thrips, *Thrips palmi* Karny on eggplant in Northern Territory (Young and Zhang, 2001) but according to Mould and Minaei (2007), the predatory thrips used by Young and Zhang (2001) is *H. haideeae* sp. nov. instead of *H. victoriensis* and *H. victoriensis* has never been found in Darwin. There were also five records from a survey of Goodwin and Steiner (1996), and they doubted that *H. victoriensis* might be predatory on western flower thrips, a serious worldwide pest in the field and in greenhouses (Yudin *et al.*, 1986). However, *H. victoriensis* was truly a predator of TSM (Bailey and Caon, 1986). *Haplothrips victoriensis* may therefore have some potential for biological control as a predator of two-spotted mite and various pest thrips species. This gives rise to the question "What is the potential of *H. victoriensis* as a predator on two-spotted mite, western flower thrips, plague thrips, onion thrips and similar crop pests?".

Haplothrips victoriensis is morphologically and taxonomically difficult to compare with its taxa in the same genus, and if it is to be used in biological control, it needs to

be easily identified. Although tubular black thrips was recognised in 1918 and included in the key to Tubulifera by Kelly and Mayne (1934), according to Pitkin (1973), the Tubulifera key of Kelly and Mayne contained many errors. Pitkin's key (1973) was more substantial, and he included four *Haplothrips* species (*H. victoriensis, H. froggatti, H. anceps* and *H. angustus*), all of which were difficult to distinguish from each other. This was the situation when the current work commenced. However, Mound and Minaei (2007) have since described additional *Haplothrips* species in Australia, bringing the number to 24 species. There is still no key to larvae of *Haplothrips* species. Therefore, the taxonomy of the larvae of *Haplothrips* requires further elucidation in order to easily identify these thrips in the field. Key questions for this are suggested: "Are the *Haplothrips* found in South Australia definitely *H. victoriensis,* or is it a similar species, or perhaps part of a species complex?" and "Can this group of species be separated by DNA barcoding?"

1.6. COI Barcoding

DNA barcoding is a technique used to identify and compare closely related species. It is based on a short genetic sequence of mitochondrial DNA (mtDNA) which exists in almost all eukaryote cells (Harrison, 1989; Lunt *et al.*, 1996; Wikipedia-contributors, 2006). Cytochrome oxidase I (COI), a gene of mtDNA, contains hereditary information involving evolutionary features of animals (Lunt et al., 1996). Taxonomists currently exploit COI as a powerful taxonomical key to study the evolution of insects. In order to determine the COI sequence, the DNA is extracted, and the COI gene is amplified, and then sequenced (Penton and Crease, 2003). The extraction process uses Proteinase K, which allows for the DNA to be extracted without destroying the exoskeleton of the specimen so it can also be used to make slides to observe the morphology (Morris and Mound, 2004). The COI gene is

decoded and amplified by Polymerase Chain Reaction (PCR) using specific primers. The PCR products will be run on an agarose gel to isolate all the COI sequences. The COI sequences are purified by centrifugation and sequencing reactions take place in a themocycler. The DNA sequence database of experimental species are then edited and aligned and a phylogenetic tree is constructed for examine relationships within species and a group.

1.7. The aims of the study

IPM is an ecologically based management tool that relies on natural mortality factors and utilizes alternative control options which are compatible with these factors (Flint and Van Den Bosch, 1981). An understanding of the taxonomy, biology and ecology of *H. victoriensis* is a requirement for its successful inclusion in an IPM program for both WFT and two-spotted mite, and possibly other insect/mite pests. The project will involve a combination of field and laboratory-based work to investigate, *inter alia*, the following questions: i) are the thrips found in SA *H. victoriensis* or is it a similar species or perhaps part of a species complex?; ii) what is the life-cycle of the thrips in SA? iii) what is the predatory behavior of the thrips in SA? This project will have a systematics/taxonomy component (molecular taxonomy), as well as biological, ecological and behavioural components. As there are implications for IPM of western flower thrips, *Thrips imaginis,* as well as two-spotted mite, the eventual outcome of this work, although beyond the scope of this project, would be to construct a model IPM strategy based on tubular black thrips.

Thus, the aims and objectives of this project can be summarised as follows:

- To investigate whether *H. victoriensis* can be reared in the laboratory.
- To investigate aspects of the biology (life cycle, feeding, longevity, etc) of *H. victoriensis* (field and laboratory).

- To investigate predatory characteristics of *H. victoriensis.*

- To determine the current distribution, as well as host plants of *H. victoriensis* in SA.

- To investigate the larval taxonomy of *Haplothrips* in SA, and their identification at larval and adult stages by DNA barcoding.

CHAPTER 2

CULTURING TECHNIQUES FOR

H. victoriensis AND ITS PREY

2.1. Introduction

2.2. Materials and Methods

    2.2.1. Rearing prey

        2.2.1.1. Rearing *Tyrophagus* mites

        2.2.1.2. Rearing western flower thrips (WFT)

    2.2.2. Culturing *Haplothrips victoriensis*

        2.2.2.1. *Haplothrips victoriensis* culture – for egg collection

        2.2.2.2. *Haplothrips victoriensis* culture – rearing larvae for observations

        2.2.2.3. *Haplothrips victoriensi*s culture – rearing adults for observations

        2.2.2.4. *Haplothrips victoriensis* population culture

2.3. Results and Discussion

CHAPTER 2

Culturing Techniques for *H. victoriensis* and its prey

2.1. Introduction

To date, there are no published techniques on the mass rearing of predatory tubular black thrips *(Haplothrips victoriensis),* although other thrips species have been successfully reared, for example, by Milne and Walter (1997), Steiner and Goodwin (1998), Murai and Loomans (2001), and Murai (2001). Two previous studies showed that *H. victoriensis* would feed on *Antirrhinum* (snapdragon) stamens (Andrewartha, 1936), and two-spotted mite (TSM) eggs (Bailey & Caon, 1986). Andrewartha (1936) investigated the developmental rate of *H. victoriensis* on snapdragon at different temperatures and Bailey and Caon (1986) investigated predatory characteristics of the thrips on TSM. The present research attempts to create a basic culturing technique for *H. victoriensis* in order to study various aspects of its biology and behaviour. Also, the results obtained in this study will be benefit the future development of mass rearing techniques for *H. victoriensis,* which shows some potential for biological control (see Chapter 4).

There are two main questions arising in this study of *H. victoriensis*. Firstly, which host can be used and secondly what type of rearing cages might be used to culture the thrips. In performing culturing trials, *H. victoriensis* was found to feed on many different kinds of prey (see Chapter 4). However, at the time of this rearing study, it was known that *H. victoriensis* is a predator on western flower thrips (WFT), *Tyrophagus* mite (from trials of this project) and TSM (Bailey and Caon, 1986). TSM was not used for the culturing technique in this project because it is a serious pest which is very difficult to control on glasshouse plants. Escaping

TSM may have damaged other crops and experiments in the Waite insectary where the trials were conducted. WFT is more common and easier to collect than *Thrips palmi* in the field. Therefore, *Tyrophagus* mites and WFT were used as the main prey source for culturing *H. victoriensis* in this study. Many preliminary trials were performed in the laboratory to explore suitable cage designs, and these are outlined below.

2.2. Materials and Methods

2.2.1. Rearing prey

WFT and *Tyrophagus* mites were reared in the laboratory for use as prey for *H. victoriensis*. A culturing method for each prey is described in the following sections (2.2.1.1 and 2.2.1.2).

2.2.1.1. Rearing *Tyrophagus* mites

In the field, it was observed that *Tyrophagus* mites were often found together with *H. victoriensis* when collected on rose flowers. In the laboratory it was often observed that the mite was being preyed upon by *H. victoriensis*, indicating that *Tyrophagus* mites would be a potential prey item for rearing *H. victoriensis*. *Tyrophagus* mite was also observed to develop well on mixed pollen (this is made up of many different varieties of pollen and can be purchased from health food shops) which was used to culture *H. victoriensis* in a thrips rearing cage. Therefore mixed pollen was used to mass-rear *Tyrophagus* mite in this technique.

Tyrophagus mites were collected from rose flowers on the Waite Campus and cultured in Petri dish cages (Fig. 2.1a) at 25 °C. The simple Petri dish cage consists of two different sized Petri dishes glued together so that the larger Petri

dish (d= 82 mm, h= 17 mm) contains a smaller one (d= 45 mm, h= 10 mm). Mixed pollen and *Tyrophagus* mites were placed on a triangle of filter paper in the small Petri dish. Tap water was poured into the space between the two Petri dishes so that the water around the small Petri disk acted as a barrier, preventing escape of the mites (Fig. 2.1a). A Petri lid with a hole (d = 15 mm) covered by fine curtain mesh fabric, was placed on the top of the Petri dish cage. *Tyrophagu*s mites developed quickly for 3-5 days on the mixed pollen diet (Fig. 2.1 b) and could be collected for the rearing of *H. victoriensis* after one week.
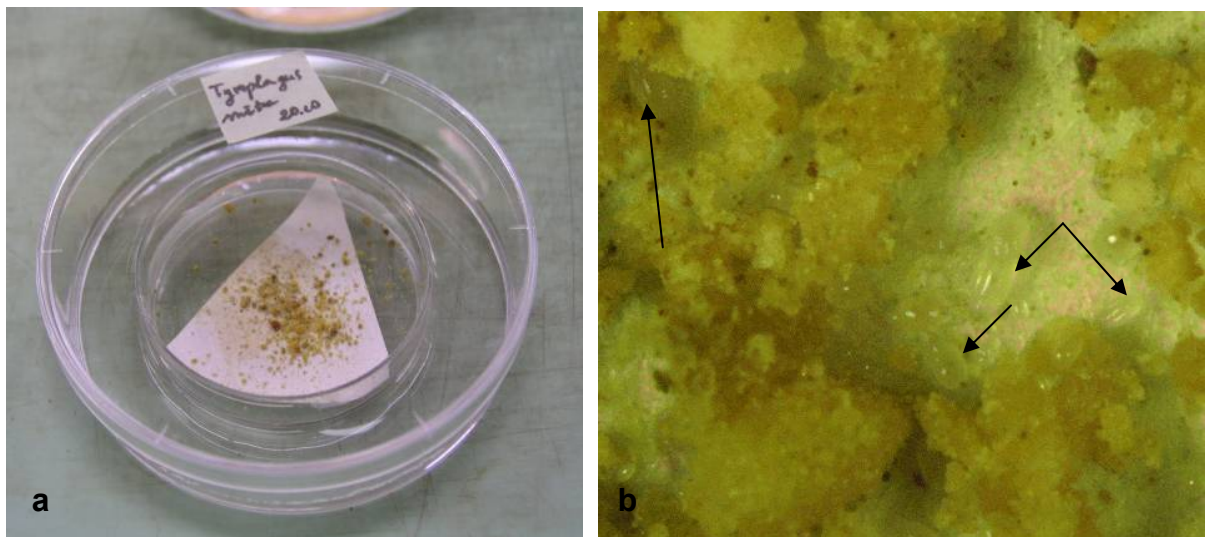
Figure 2.1. a. Petri dish rearing cages. b. *Tyrophagus* mites on mixed pollen.

2.2.1.2. Rearing western flower thrips (WFT)

WFT is a global pest on glasshouse plants and other ornamental plants, and laboratory cultures of WFT have been studied in many laboratories worldwide. WFT can be reared on a variety of media including green bean pods, immature capsicum, cucumber fruit (Steiner and Goodwin, 1998), leaf powder or broad bean seeds (Murai and Loomans, 2001), and cucumber leaves (Hulshof *et al.*, 2003). All of these methods used pollen and honey as supplements to feed WFT.

Biological aspects of Haplothrips victoriensis in SA

As a part of these project requirements, WFT was reared in laboratory and used as prey for *H. victoriensis*. WFT rearing is divided into two parts: mass rearing and collection of WFT eggs. Each of these methods was adapted from existing techniques.

(1) Mass rearing of *WFT*

The rearing process essentially followed the rearing technique for WFT which was developed by South Australia Research & Development Institute (SARDI). Some steps were adapted to simplify the method and to make it more suitable for these trials. Décor@ 8.5L plastic containers were used as mass rearing cages. Two square holes (2.5 cm x 10 cm) covered with fine mesh (ϕ =100-135 µm) were made on opposite sides of the cage. Several folded pieces of VIVA paper towel on which several parallel cuts were made, were placed in the cage so that adult, larval and especially pupal thrips, could hide within the folds. The paper covered about half of the bottom of the cage. Wire mesh bent into a U shape was placed over the folded paper towel. The wire mesh was used as a flexible base for containing upright cucumber fruits and provided gaps between the folded paper towels where thrips could hide. If cucumbers are placed on the paper towel, moisture at the contact point between the cucumbers and the paper towel causes mould. Two or four Lebanese cucumbers were dipped in tap water for 30 minutes, to reduce possible pesticide residue and fungi, then rinsed twice and dried before being placed upright on the wire mesh in the cage. A piece of filter paper containing mixed pollen was placed on the remaining half of the cage bottom. A plastic pill cup or small Petri lid containing moist cotton rolls was placed in the corner of the cage to keep humidity high within the cage. One or two pill cups containing 10% honey solution with diluted 0.1% sorbic acid (sorbic acid was used

to prevent development of mould) were covered with transparent wrapping film. A small piece of paper towel was folded and cut to form a long wick. One end of the wick was placed into the plastic pill cup containing the honey solution, through the transparent wrapping film. The other end was divided into two strands which were directly placed onto the cucumbers. The wick was moistened with honey solution and acts to irrigate the cucumbers with honey solution (Fig. 2.2a). About 50-100 WFT adults collected from a lucerne crop were placed in the cage. The cage was covered by two layers of transparent wrapping film and secured with a rubber band. All manipulations were conducted in a constant temperature room at 25 ± 2°C and experiments were conducted in an environmental incubator at 25°C with 16h light and 8h dark. Humidity in the cages was maintained at about 65 %RH by spraying additional purified water every two days. Female WFT oviposit their eggs into the cucumber fruits and larvae emerge after 3-4 days. Cucumbers were replaced weekly. Mixed pollen, honey and moistened cotton rolls were regularly checked for mould and replaced if necessary. About 300 to 500 larvae emerged per cage per week. The larvae were separated from adults by gently lifting and lightly blowing on the cucumber, so the adults dropped off the cucumber. Half of the larvae were used to feed predatory thrips, one quarter of the larvae were returned to the mass rearing cage and the remaining larvae with cucumbers were moved to an incubation cage (Fig. 2.2b). Further WFT larvae emerged on surface of the cucumbers for another week and these were allowed to become adults in the incubation cage. These adults provide stock for mass rearing or for egg collection (described below). A single rearing cage can be operated for 1-2 months depending on contamination by mould and the fitness of the adult WFT population.

Figure 2.2. (a) Mass rearing cage and (b) incubation cage for culturing WFT.

(2) Egg collection of WFT

Thrips belonging to the suborder Terebrantia generally lay their eggs into leaves, branches or fruits by inserting their ovipositors into the plant and depositing an egg (Moritz, 1997). The egg is completely deposited into the plant such that the head end of the egg is about level with the internal plant surface. When emerging, larvae move to the surface of the plant. To collect WFT eggs, a technique was designed based on, and adapted from, a procedure for mass-rearing of thrips (Loomans and Murai, 1997; Murai and Loomans, 2001) (Fig. 2.3).
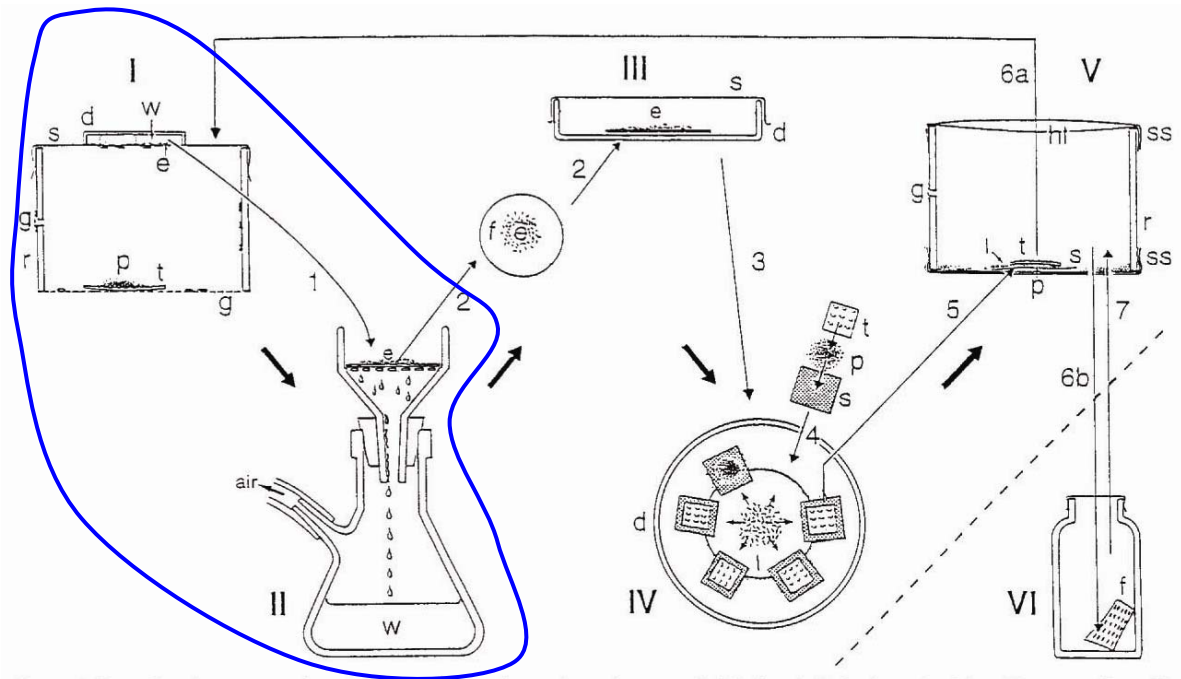
Figure 1. Procedure for mass-rearing of thrips and natural enemies using an artificial film. I. Units for oviposition, II. egg-washing, III. incubation, IV. trapping, V. rearing and VI. storage. Eggs are laid in water through stretched film (1), and are washed in a waterfilter on filterpaper (2) and subsequently incubated at high humidity conditions. Hatched larvae are trapped (3, 4) with pollen and reared (5) on pollen + 10% honey solution until maturity and then are transferred to unit I (6) for culture maintenance or stored as pupa (7). d = petri dish, e = eggs, f = filter paper, g = gauze, h = honey solution, l = larvae, p = pollen, r = ring cage, s = sealon film, t = tissue paper, w = water (modified from Loomans and Murai (1997).

Figure 2.3. Steps I & II (circled) of the mass-rearing procedure of Murai & Loomans (2001) were used and adapted for WFT egg collection.

A round plastic container (d1= 80 mm, d2= 87 mm, h= 62 mm) was used as a laying cage. A hole (d= 12 mm) was made on the side of the cage and covered by 100-135 µm cotton mesh fabric to reduce humidity levels to below 65%RD. Mixed pollen was placed on a piece of filter paper in the cage and 10% honey solution was provided to the thrips in a small Petri dish covered with transparent wrapping film. Paper towel wicks were stuck on the wall of the cage instead of on the surface of cucumber as previously described. Many small folds of paper towel were provided for the thrips to hide in. Seventy to hundred WFT adults of good fitness (characterized by visually being of brighter colour, larger and active) were placed each the cage. A Petri dish lid (d= 90 mm) filled to the brim with purified water to avoid air bubbles inside was covered by stretched Parafilm and this was

used as an oviposition site for WFT. The Petri dish lid with purified water covered by stretched Parafilm was upturned and covered on the cage (Fig. 2.4).
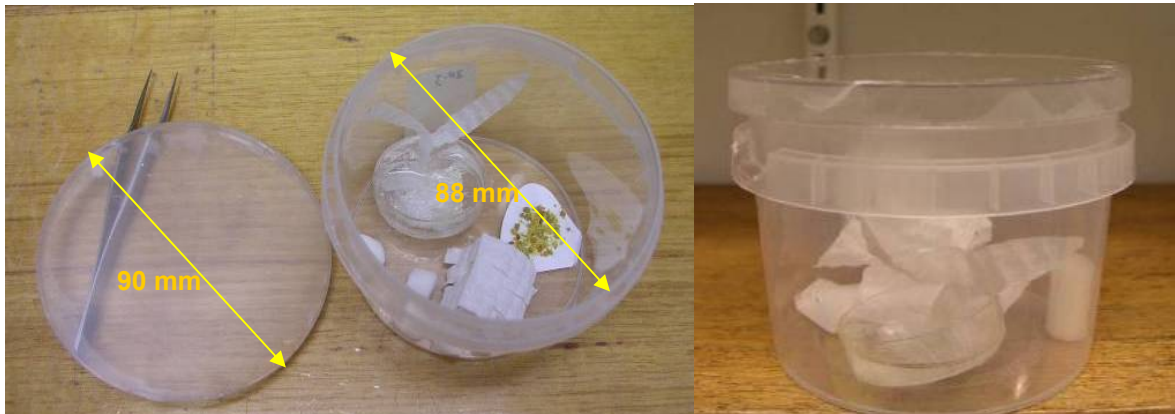
Figure 2.4. Materials being prepared for WFT oviposition (left). The egg collecting cage (right).

Figure 2.5. a & b: A female WFT is inserting her ovipositor into water through stretched Parafilm. c & d: an egg is oviposited into water.

Female WFT insert their ovipositor into the water through the stretched Parafilm and lay one egg into the water at a time (Fig. 2.5). About 50-150 eggs were laid in the water over 2-3 days (Fig. 2.6a) before the Petri lid was changed.

The eggs in the water were collected using a plastic pumping bottle to wash the eggs through black filter paper in a Buchner filter system (Fig. 2.6b). The white colour of the WFT eggs clearly contrasted with the back filter paper (Fig. 2.6c). The Petri lids with eggs were collected 4-5 times for each cage. Viable eggs laid in water (Fig. 2.6a) could be stored in a refrigerator at 4-5 $^{\circ}$C for more than one month.

Figure 2.6. (a) WFT eggs were laid in water. (b) the Buchner filter system. (c) WFT eggs on black filter paper (each egg is about 210 µm in length).

2.2.2. Culturing *Haplothrips victoriensis*

The culturing of *H. victoriensis* is divided into two main parts, firstly the rearing of *H. victoriensis* to provide eggs, larvae and adults to make observations and conduct experiments on life history, feeding behavior, ovipositing behavior, prey consumption and longevity, and secondly as a preliminary step to mass rearing.

2.2.2.1. *Haplothrips victoriensis* culture – for egg collection

The idea for collecting *H. victoriensis* eggs in the laboratory arose when several eggs of *H. victoriensis* were found on the base of a rose flower. The eggs were not oviposited into plant material but were scattered on soft hairs or anthers of stamens of the flower (Fig. 2.7). The question arose as to whether an artificial material somewhat like the base of the flowers could act as an oviposition site for

H. victoriensis. I conducted many trials in the laboratory before a basic technique was established.
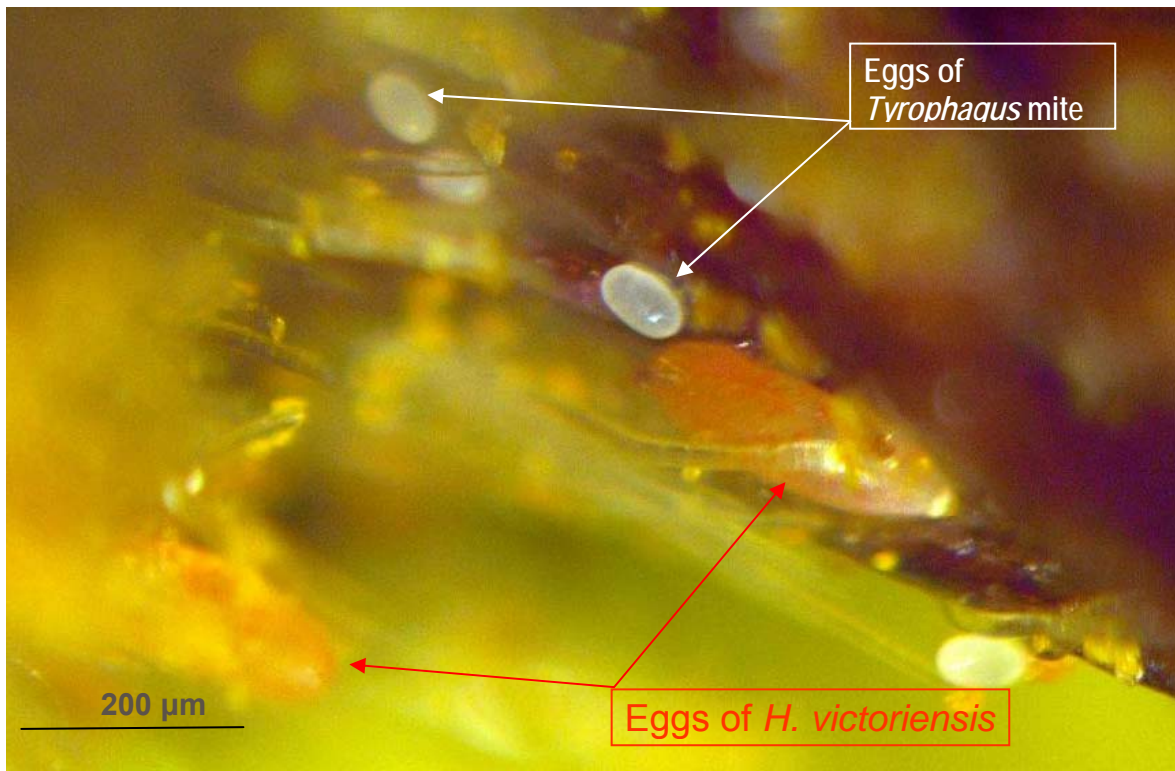
Figure 2.7. *Haplothrips victoriensis* eggs oviposited on the base of a rose flower.

A round plastic container (d1= 80 mm, d2= 87 mm, h= 62 mm) with mixed pollen and honey was set up in the same manner as for the egg collection of WFT. A piece of sterilised cotton wool or some pieces of cotton roll soaked in 10% honey solution and left to dry, were lain on a piece of Velcro (w= 20 mm, l= 30 mm). The Velcro with the cotton wool or cotton rolls was placed in the cage (Fig. 2.8 b&c). About 100 WFT larvae were transferred using a hand aspirator and dropped in the cage to feed *H. victoriensis* every 2-3 days. The cage was completely sealed by its lid. *Haplothrips victoriensis* adults were often found hiding in the Velcro (Fig. 2.8a). Females oviposited their eggs into the cotton wool or cotton rolls which were collected every 2-3 days and placed in the Petri dish cage for incubation and hatching.

a   b   c
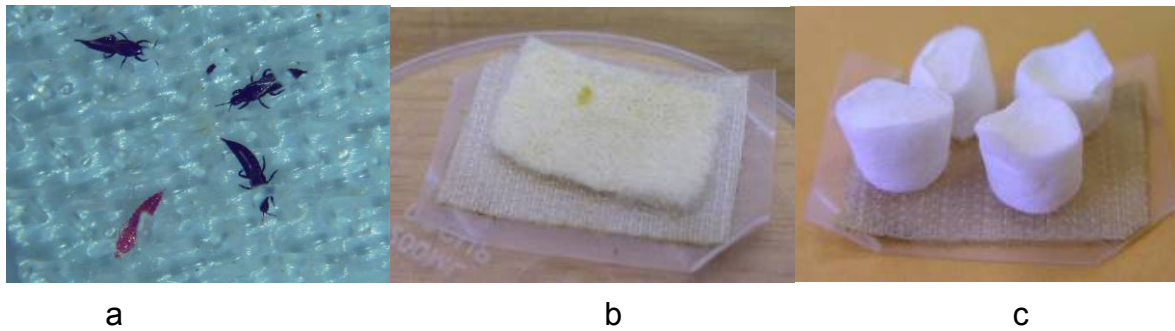
Figure 2.8. (a) *Haplothrips victoriensis* in Velcro; (b & c) cotton wool and cotton rolls are placed on a piece of Velcro.
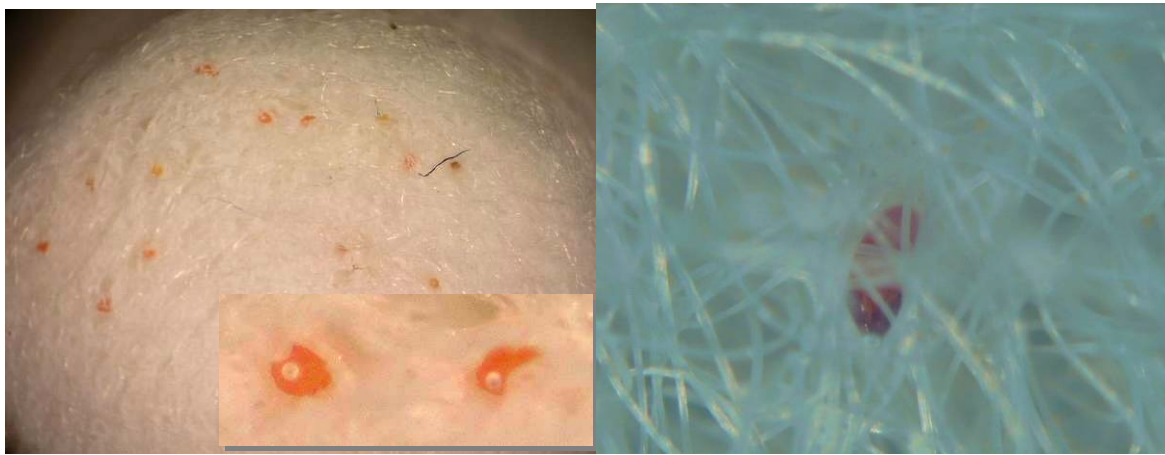
Figure 2.9. *Haplothrips victoriensis* eggs in cotton roll (left) and cotton wool (right).

2.2.2.2. *Haplothrips victoriensis* culture – rearing larvae for observations

Thrips are tiny insects (1-2 mm) and they usually live in flowers or hide in litter (Lewis, 1973). They can escape easily from cages if the cages are not tightly closed, or they can become stuck in the gaps between a cage and its lid becoming easily injured or killed when the lid is opened for feeding or observation.

This rearing technique was developed to conduct various experiments such as life history, development, prey consumption, and behavior of larvae (see Chapters 3 and 4). Rearing cages were as used previously described (Fig. 2.1a & 2.10a). Honey solution (10% honey with 0.1% sorbic acid) absorbed into one to two pieces of cotton roll and mixed pollen were placed in the Petri dish cage to provide food for newly emerged larvae. The cotton wool or rolls with *H. victoriensis* eggs

collected from the egg collection technique were placed in the Petri dish cage. A Petri dish lid with a ventilated hole (d= 15 mm) covered by fine mesh was placed on the top of the cage (Fig. 2.10b) and it was essential that it covered the base of the Petri dish cage without any gap. This tight seal can prevent any thrips adults from entering which may have escaped from other cultures. The eggs were incubated at 25 °C and 16L:8D in an environmental incubator. After the larvae of *H. victoriensis* emerged, *Tyrophagus* mite eggs or WFT eggs were added as prey items. A piece of paper towel (about 12 mm x 12 mm) was also added for larvae to hide in. Larvae were moved to other cages using a very fine camel hair brush. Additional honey solution was dropped onto the piece of cotton roll every two days. Larvae could be separated into small groups or individuals depending on the experimental requirement.
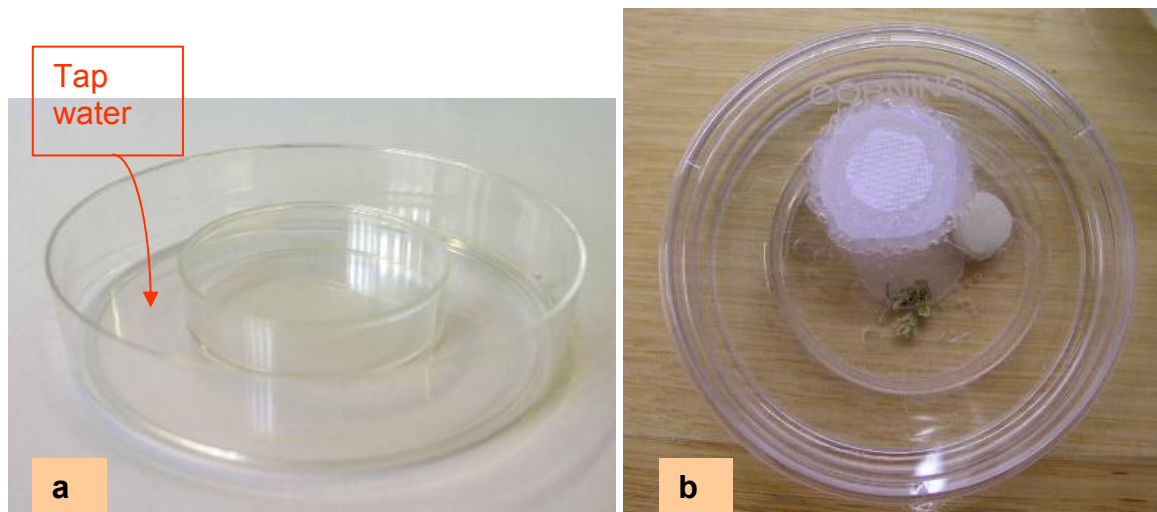
Figure 2.10.   (a) Preparing the rearing cage, (b) The Petri cage to rear *H. victoriensis* larvae.

2.2.2.3. *Haplothrips victoriensi*s culture – rearing adults for observations

Mixed pollen on a piece of filter paper and honey solution absorbed in one or two cotton rolls were provided to *H. victoriensis* adults in the same way as for rearing larvae. However, in this case, a rearing cage was a round plastic jar (h= 55

mm, d= 45 mm) covered by stretched Parafilm (Fig. 2.11). Twelve small holes (d= 200-250 µm) needed for ventilation were made in the stretched Parafilm using an entomological pin. A piece of paper towel (about 12 mm x 12 mm) was also placed in the cage for thrips to hide in. *Tyrophagus* mite or WFT larvae or eggs were used as prey and added to the thrips cage. Every three days, honey solution was added to the cage and the cage was checked for mould. This technique is used to observe the feeding of thrips, longevity experiments, consumption prey experiments, and other observations.

Figure 2.11. The jar cage for observations on *H. victoriensis* adults.

2.2.2.4. *Haplothrips victoriensis* population culture

In addition to the rearing techniques developed, I also intended to mass rear *H. victoriensis*. This is important for several reasons. Firstly, in the field in South Australia, *H. victoriensis* populations only "explode" in summer, so mass rearing *H. victoriensis* in the laboratory is essential for the demands of other studies. Secondly, *H. victoriensis* is a predator of TSM (Bailey and Caon, 1986) and many other pest species (see Chapter 5), so mass-rearing of these thrips as a potential

tool for biological control is important. Although *H. victoriensis* mass-rearing was unsuccessful in producing thousands of offspring, a small scale population culture for *H. victoriensis* can be used to maintain an experimental population in the laboratory.

For the laboratory culture in this instance, the rearing cage was a plastic container (l =18 cm, h =18 cm, w =14 cm) with two ventilation holes covered with two layers of cotton net. Folded paper towel was placed on half the cage bottom to allow thrips to hide in and under the folds, especially in the pupal stage. As above (Section 2.2.1.2) wire mesh was placed over the folded towel paper. Two cucumbers with about 400-600 WFT larvae collected from the mass-rearing WFT (Section 2.2.1.2) were placed on the mesh. Mixed pollen, 10% honey solution and cotton rolls moistened with purified water in a pill cup were also added to the cage. Four pieces of Velcro (w= 20 mm, l= 30-40 mm) were glued around the walls of the cage and four pieces of cotton wool were stuck on the four Velcro pieces. This was to create "homes" and ovipositing sites for *H. victoriensis*. *Haplothrips victoriensis* eggs could be also collected from the cotton wool. About 200 *H. victoriensis* adults collected from lucerne were placed in the cage and the cage was sealed with a lid. Besides the moist cotton rolls contributed to humidity in the cage, purified water was also sprayed every day to maintain the relative humidity at around 65 %RH. Fungal contamination of mixed pollen, honey and cotton rolls was checked every three days and these were replaced if necessary. The cucumbers were replaced every week by two other cucumbers with WFT larvae on their surface. The culturing trial was conducted in an environmental incubator at 25 $^{\circ}$C and 16L:8D.

2.3. Results and Discussion

Although the main purpose was to culture *H. victoriensis*, I was also able to rear prey including *Tyrophagus* mite and WFT in the laboratory. Five different types of rearing cages were designed and adapted from previous studies to successfully rear *Tyrophagus* mite and different stages of *H. victoriensis* and WFT.

Tyrophagus mite was unintentionally collected on rose flowers and reared with *H. victoriensis* in some pilot trials. I found that *H. victoriensis* fed on the mite, so the mite is a natural prey of thrips. As the mite lives and oviposits on rose flowers (Fig. 2.7), it was thought that the mite might be feeding on pollen and/or nectar.

Tyrophagus mites were successfully reared using mixed pollen as an artificial diet (Section 2.2.1.1). *Tyrophagus* mite could also be mass-reared on sugar powder (Keller, 2007 pers. comm.). Populations of *Tyrophagus* mite developed quickly from about 20 mites to several thousand mites per cage. As the mite is very tiny (about 100–800 μm long) and its population explodes in a short time, the rearing of the mites should be attended to frequently. The Petri dish cage satisfied the requirement of confining the population of mites. The water barrier should be checked every two days and kept full. If it is not controlled, the number of mites increase, flood into the water, and will escape from the cage. To protect other lab cultures from mite infestation if the population of mites become too large, the mite population can be killed by applying 70% alcohol.

Tyrophagus mite and its eggs are prey of *H. victoriensis*. However, *H. victoriensis* also become prey of the mite when *H. victoriensis* has just emerged as new adult from a pupa (Fig. 2.12). Therefore *Tyrophagus* mites were not used as prey when mass-rearing *H. victoriensis*. Only eggs and inactive *Tyrophagus* mites were provided to feed *H. victoriensis*.

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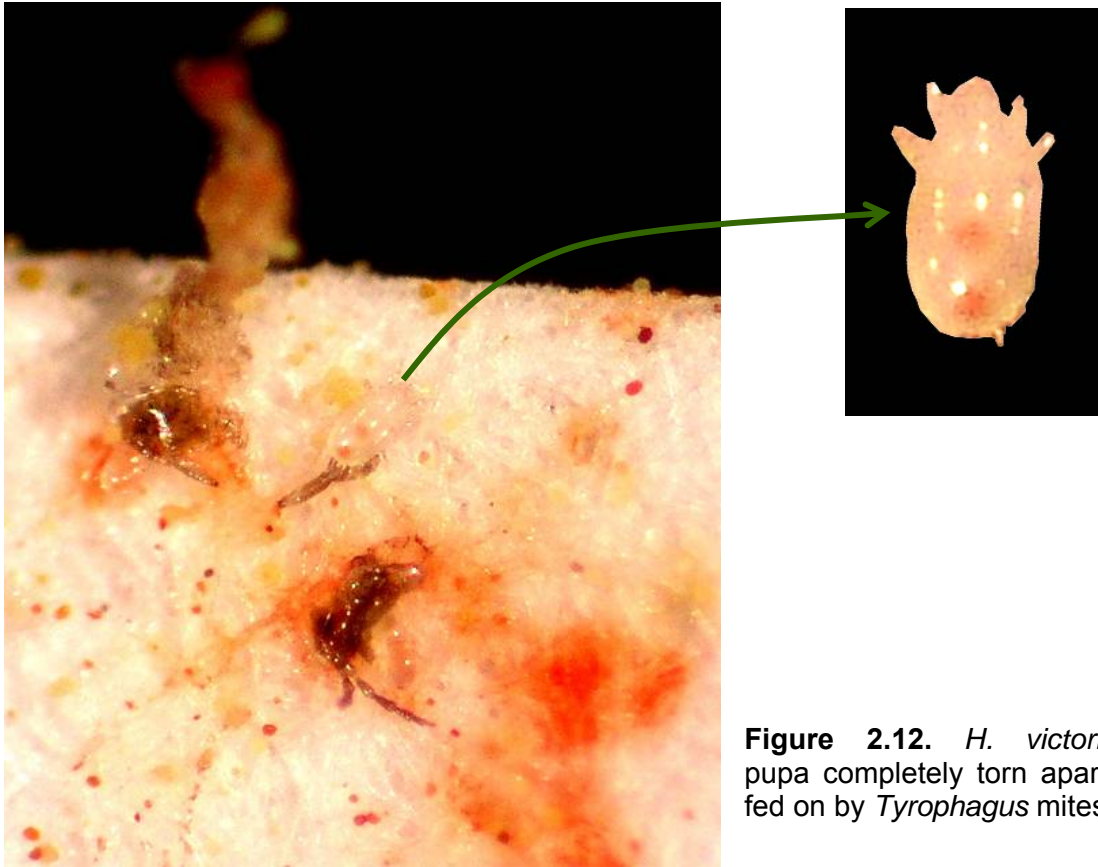
Figure 2.12. *H. victoriensis* pupa completely torn apart and fed on by *Tyrophagus* mites.

The mass-rearing of WFT provided sufficient numbers of prey to satisfy the predation requirements of *H. victoriensis*. The rearing technique for WFT egg collection contributed significantly to success of the project and will benefit other studies in the future. Although the WFT egg collection technique was adapted from the technique of Murai and Loomans (2001), it was refined for egg collection using a Petri dish lid filled with purified water coverd by stretched Parafilm as an oviposition site. WFT eggs are easily and simply collected by removing the Petri lid from the top of the rearing cage whereas according to Murai & Loomans (2001), WFT eggs collected from a few mL of water dropped onto the stretched film, which seems more difficult and complicated. Also, in my opinion, some WFT eggs may be lost in the process. WFT eggs in the Petri dish lid were able to be stored under refrigeration for over a month to supply further experiments. Oviposition behaviour was clearly observed through the transparent, continuous and homogeneous

environment of thin glass and water of the Petri dish lid. It was very useful to observe egg laying behaviour of WFT. This method may also lead to opportunities for future studies on Terebrantia thrips, as almost all of these species are only known to insert their eggs into plant material when ovipositing. Future studies in areas such as oviposition behaviour, fecundity and egg collection for other predators and parasitoids of thrips, might be enhanced using this technique.

The various trials showed that *H. victoriensis* can be reared using several techniques for different life-stages.

The oviposition technique using Velcro, cotton rolls and cotton wool as an oviposition site could also be applied to rear other Tubulifera species which live on flowers. Similarly the Petri dish cage used to rear larvae could be applied to other thrips larvae. It provided ideal conditions to rear and control larvae concisely and strictly. It also created a stable environment with about 65 RH%, ideal for the thrips. This also appears to have reduced contamination by mould.

The adult rearing cage was successfully used to rear adults for consumption and longevity experiments (see Chapters 3 and 4). These cages created a suitable environment for *H. victoriensis* adults in terms of diet, prey, accommodation, relative humidity (about 65%) and temperature. Although the effect of different humidities in rearing cage has not been examined in detail, the design seems to provide appropriate conditions to restrict mould which usually appears from mixed pollen when it gets wet. The adult rearing cage technique may be also used for studies on predatory thrips and others thrips in future.

The preliminary mass-rearing of *H. victoriensis* was trialled in a laboratory situation. Although *H. victoriensis* was known as a cannibal (adults and larvae feed on eggs, adults and second instars feed on first instars, and a bigger larva feeds

on a smaller larva if the same instar – see Chapter 4), supplying enough prey reduced the rate of cannibalism of *H. victoriensis*. In the laboratory a population of about 200 adults remained relatively stable for up to four months, although after that the population decreased in terms of population fitness. A population of this size was sufficient to provide *H. victoriensis* samples for other small-scale studies in the laboratory such as those on morphology and feeding behaviour. However, this technique did not prove to be successful for the type of mass rearing that would be necessary for further biological control studies on *H. victoriensis*. If warranted, mass-rearing *H. victoriensis* would require further study.

CHAPTER 3

BIOLOGICAL DEVELOPMENT OF

H. victoriensis

3.1. Development and life history of *H. victoriensis*

    3.1.1. Materials and Methods

    3.1.2. Results

    3.1.3 Discussion of development and life history

3.2. Effect of different food sources on development and longevity of *H. victoriensis*

    3.2.1. Effect of different food sources on *H. victoriensis* development

        3.2.1.1. Materials and Methods

        3.2.1.2. Results

    3.2.2. Effect of the addition of WFT to a basic diet on longevity of *H. victoriensis*

        3.2.2.1. Materials and Methods

        3.2.2.2. Results

3.3. Discussion of the effect of different food sources on *H. victoriensis* development and longevity

CHAPTER 3

Biological Development of Tubular Black Thrips

Tubular black thrips *(Haplothrips victoriensis)* has previously been cultured in the laboratory to measure developmental rate for a range of temperatures (Andrewartha, 1936), for identification and biological information (Steele, 1935), and for predatory studies on two spotted mite (Bailey and Caon, 1986). However, developmental times and most of the biological characteristics are largely unknown. The object of this part of the project was to obtain detailed knowledge of the biological development of *H. victoriensis*, particularly its life history, to examine relationships between thrips development on various food sources, and thrips longevity. Results of these observations will directly support research that follows in this project and the information will contribute to knowledge of thrips biology.

3.1. Development and life history of *H. victoriensis*

Thrips like other homometabolous insects have four main life-history stages; egg, larva, pupa, and adult. They have just two larval instars, as well as prepupa, and pupa in the developmental stage (Moritz, 1997). The development of immature thrips can be divided into two groups, each of which corresponds to the suborders of Thysanoptera: Terebrantia and Tubulifera. Terebrantia have four developmental stages consisting of first instar, second instar, prepupa, and pupa whereas Tubulifera have five stages including first instar, second instar, prepupa, first pupa, and second pupa (Palmer *et al.*, 1989). Six developmental stages of *H. victoriensis* from egg to second pupa were described by Steele (1935) and developmental times of larval and pupal periods were investigated by Andrewartha

(1936) at a range of temperatures (12, 16.5, 18.7, 20.8, 22.5 and 26°C). However, the developmental time for each stage has not yet been investigated. In this research section, I attempt to observe the developmental times of the various life history stages with the aim of building a life history of *H. victoriensis.*

3.1.1. Materials and Methods

To observe the egg stage, about 50 *H. victoriensis* adults were collected from the field and reared in an egg collecting cage using the technique for egg collection outlined in Section 2.2.2.1. These were checked daily, and eggs oviposited in cotton rolls were moved to a Petri dish cage. When the eggs hatched, hatching time and new larvae were recorded. Newly emerged larvae (n=9) were moved to individual Petri dish cages for the second part of the observations. As well as a 10% honey solution and mixed pollen as a food supplement, each larva was provided with *Tyrophagus* mite eggs and inactive *Tyrophagus* mites as a food source. Larval development was observed and recorded daily. When each larva developed to a prepupa or pupa, individuals were moved to a new Petri dish cage using a very fine camel hair brush. When each individual became an adult, it was moved to and reared in a jar cage (see Section 2.2.2.3) for further observation. The development of each adult was observed and recorded until it became black (from preliminary rearing studies I found that a newly emerged adult is red and it changes to brown, dark brown and black over time, but black is its consistent colour). The life history of *H. victoriensis* as observed in this study and each thrips developmental stage was recorded carefully.

3.1.2. Results

Seven developmental stages were identified in *H. victoriensis*; egg, instar I, instar II, instar III (prepupa), instar IV (first pupa), instar V (second pupa), and adult (Fig. 3.2). The first instar emerges from the egg 3-4 days after being laid, lives for 3-5 days, and then moults to the second instar larva. The second instar develops and then moults to a prepupa after 5-7 days. The developmental time of the prepupa is from 12 hours to a maximum of one day. There are two pupal stages in the pupal development. The developmental times are 1-1.5 days for the first pupal stage and 3-4.5 days for the second pupal stage. The new adult rests for 2-3 days before it starts moving and feeding. The life history of *H. victoriensis* is between 16-22 days at 25°C with a diet of honey, pollen and *Tyrophagus* mites.

The morphological development of *H. victoriensis* including changes in shape, size and body colour pattern distribution is shown in Fig 3.3.

The egg is about 380 µm long and red or pink, and is laid on the base of flowers (Fig. 2.7), in stamens (Fig. 3.1c) and, in the laboratory, in cotton rolls or cotton wool (Fig. 2.9). Sometimes *H. victoriensis* eggs are scattered on Velcro (Fig. 3.1d), on paper towel (Fig. 3.1a), or in the gap between the lid and base of the rearing cage (Fig. 3.1e). The eggs may be oviposited as a group or singly (Fig. 3.1).

The size of *H. victoriensis* larvae is variable. The first instar is about 500-600 µm long with a slender body, is bright red (Fig. 3.2), and is active and searching for food immediately after emerging. Antennae and legs are colourless to transparent grey and the joints of the femorae and tibiae on all legs are grey. The pronotum is reddish brown, broad, and appears harder than the mesonotum, metanotum and abdomen. The posterior half of abdominal segment 9 and

abdominal segment 10 are dark brown. A long pair of terminal setae are on the apex of the posterior tube of the abdomen. They are about one third length of the body and look like two tail setae. The body of larval instar I quickly elongate and reaches about 1000 µm before moulting to the second instar.

The new second instar begins with a bright red body. The head, antennae and legs are transparent and then change to opaque and transparent grey within a few hours. The legs are also grey, similar to the first instar. The pronotum is dark brown, cuticular and divides medially into two halves appearing as two scales. These appear strong and hard. The mesonotum and metanotum are large and increase in size after feeding, but the abdomen still looks slender. Abdominal section 9 and 10 are dark brown and there are two short dark brown stripes at both sides of the posterior abdominal segment 8. The two terminal setae are shorter than those of the first instar. They are from one fifth to one fourth of its body length. The second instar larva is very active, ferocious and voracious. It feeds on many prey items and develops quickly. Its body enlarges from about 1200 µm to 1500 µm and becomes red as it develops. The opaque colour of the legs, head, antennae and the ninth and tenth abdominal segment also become darker. At the end of the second instar, the larva stops feeding, the red exoskeleton is broken, and it moults to the prepupal stage.

500 µm
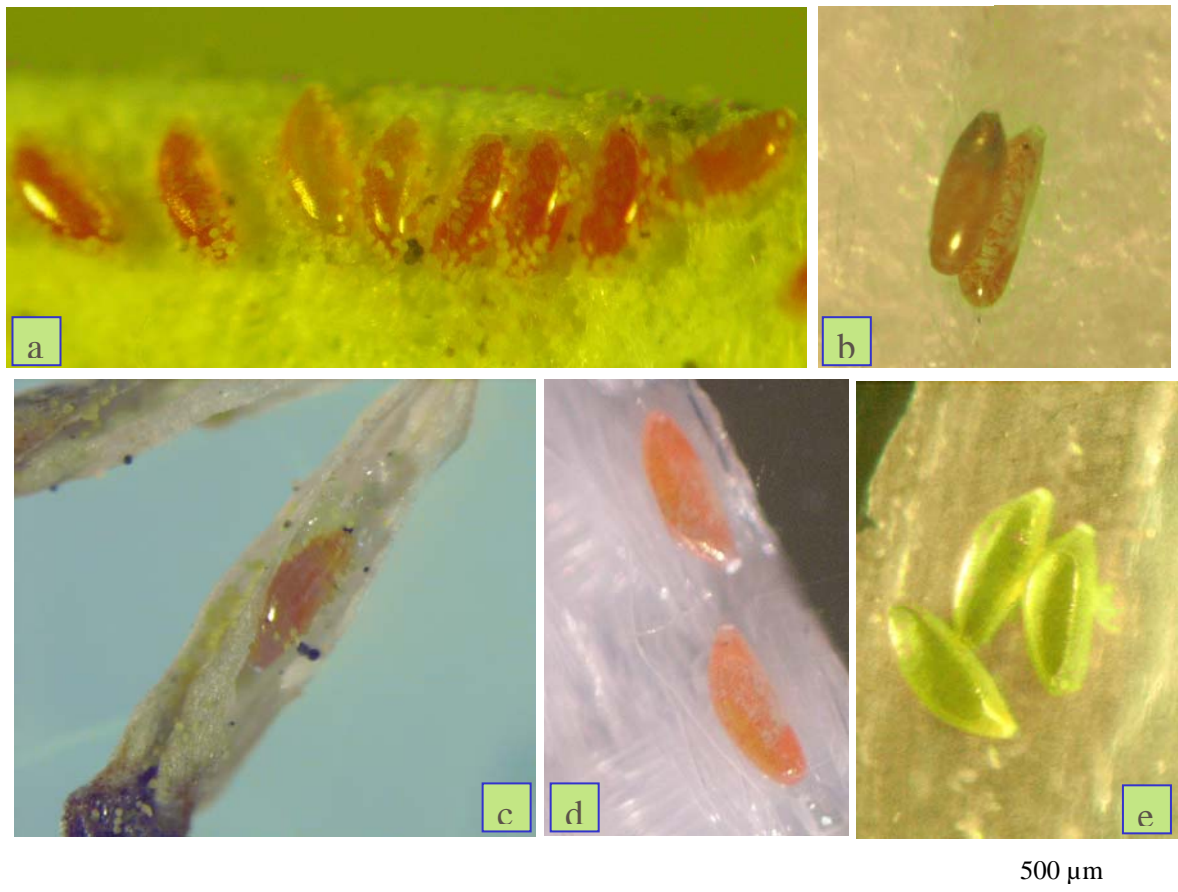
Figure 3.1. The eggs of *H. victoriensis* were laid (a) on paper towel, (b) on cotton wool, (c) in stamen, (d) on Velcro, and (e) between two plastic walls of a rearing cage.
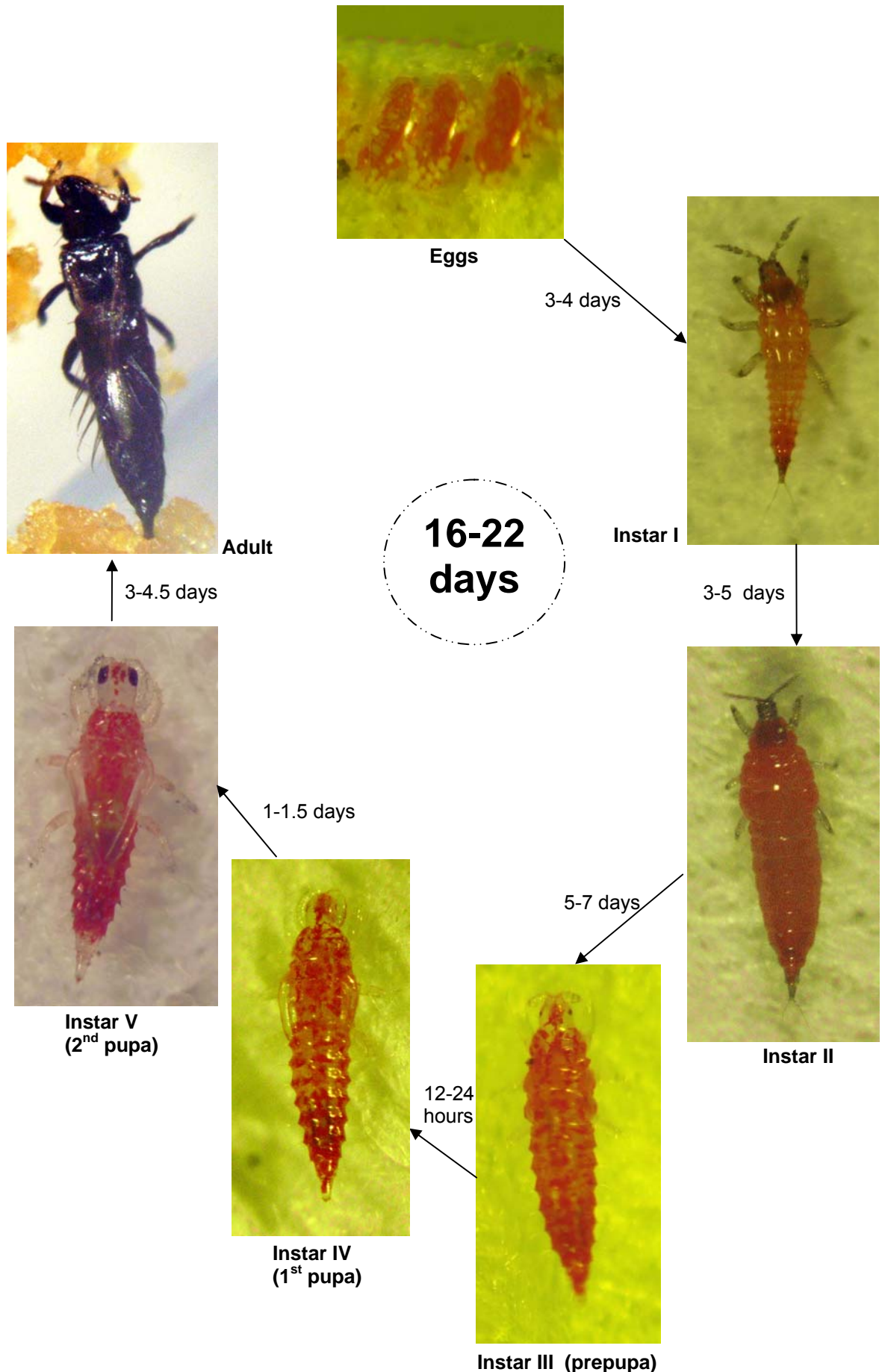
Figure 3.2. Life history of *H. victoriensis* fed with mixed pollen, 10% honey and *Tyrophagus* mites at 25°C.
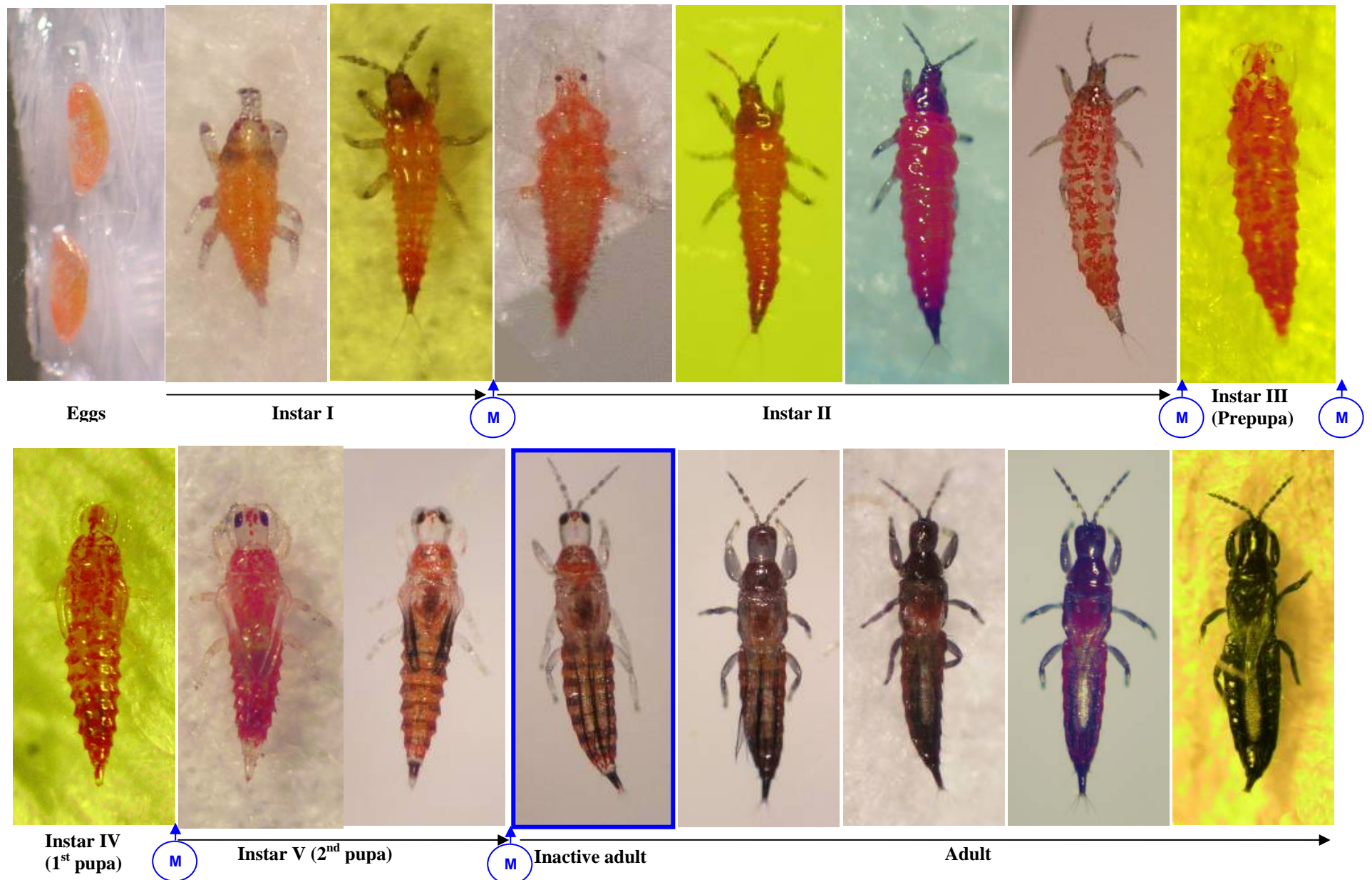
Figure 3.3. The developmental patterns of *Haplothrips victoriensis* (M = moult).

Three moulting times from prepupal stage to adult stage were not observed because they took place in a very short time and no exoskeletal skin was found in this period. The immature thrips of this period might only partly moult at the head, antennae, wings and posterior abdomen. Basically, during the experimental observation, I found that there are three main pupal shapes depending on the major changes of head, antennal buds and wing buds in this period. They were divided into prepupal, first pupal and second pupal stages.

The prepupa (instar III) is about the same size as the second instar (1500-1600 µm long) and has a red body. The head is triangular in shape and the compound eyes are not well developed, being present as two dark spots. The two antennal buds are small, unsegmented, and transparent. The legs and most of the head are transparent (Fig. 3.2). Only the posterior half of abdominal segment 10 is transparent at the prepupal stage, with the remainder of the abdomen red.

In the first and second pupal stages, the colour and the length of the body are similar to the prepupa and the head changes to a cylindrical shape. The compound eyes are still black spots in the first pupal stage but they become larger and develop fully in the second pupal stage (Fig. 3.3). The transparent antennal buds also become longer, curve down and lie along the cheeks. Several very fine hairs appear on the antennae which disappear when the thrips complete the pupal stage. Antennal segments in the second pupal stage become more defined than in the first pupal stage. The two pairs of wing buds initially appear and are transparent in the first pupal stage. The wing buds are about one third the length of its abdomen in the first pupal stage and about two

third that length in the second pupal stage. Legs, antennal buds, wing buds and abdomen segments 9 and 10 are still transparent in the second pupal stages, but wing buds and abdominal segments 9 and 10 continuously change in the same stage (Fig. 3.3). Abdominal segment 9 changes to dark red (the same as the body colour) and abdomen segment 10 changes to dark brown by the end of the second pupal stage. A short light black band appears on the anterior edges of each wing bud and it becomes longer and darker on all the wing edges (Fig. 3.3). The prepupa and pupa tend to stand and rest for most of the time while important metabolic and internal changes occur inside the body. However, they can move and hide when they recognise a danger, such as other predators, and they respond to the touch of a very fine brush.

From second pupa to adult, the body shape of thrips changes considerably. In adults, the body becomes dark red and a little bigger and longer than the pupa. It is approximately 1700-1850 µm long. Horizontal light black stripes appear on the abdomen which defines the abdominal dorsal segments clearly. The body colour of adults changes from red, to dark red, then to brown, dark brown, and then black (Fig. 3.3). However, the colour change of *H. victoriensis* in its development in the adult stage may be influenced by food source (see Section 3.2). The colour of head, legs and antennae change from transparent to opaque, light black, and then black except fore tarsi, apices of fore tibiae, and the base of antennal segment 3 which change to yellow. The antennae elongate and extend straight; the antennal segments become more defined. The wings develop in full with long feathery hairs initially appearing on both sides of the elongated wings where they grow from the edges of the wings. On each side of the body, the wings lie together in parallel with the other two opposite side

wings. After 2-3 days, the wings overlap along the middle of the dorsal abdomen. The inactive period occurs when *H. victoriensis* has just become an adult and lasts about 2-3 hours.

Colour changes, and the time these take, may also be affected by temperature. This was observed during the course of the study but was not investigated experimentally.

3.1.3. Discussion of development and life history

The egg of *H. victoriensis* is fairly large (about 380 µm) compared with WFT eggs (about 210 µm). *Haplothrips victoriensis* eggs were laid on many different surfaces which were convex and concave or were a mass of soft hairs or fibres. However, from my observations, *H. victoriensis* usually preferred to lay their eggs into soft and fibrous substances such as cotton rolls, cotton wool, or the base of flowers, or certain cracks. For example, the eggs were often laid in the crack between two plastic walls of the rearing cage where other predators can not reach these eggs to feed. This characteristic apparently helps the eggs to avoid predation. This is similar to the observations of Andrewartha (1936) when he reared *H. victoriensis* in a vial and noted that: "The adults nearly always lay their eggs on the surface of the cotton-wool plugs".

The developmental time from first instar larvae to adults reared on the diet of honey, mixed pollen and *Tyrophagus* mite eggs was approximately 13-18 days at 25°C and 16L:8D. This is similar to the developmental time of 14.53 days when larvae were reared on *Antirrhinum* (snapdragon) stamens at 26°C (Andrewartha, 1936). The larval developmental time seen in this study (average of 10 days) is similar to the previously observed average of 9.9 days of larval

development when feeding on lucerne flowers observed by Bailey and Caon (1986) at 25°C.

The inactive period is very important in the final stage of development of *H. victoriensis* in spite of the short time of about 2-3 hours. It is likely that there are still significant changes happening inside the body of the inactive adult. This inactive period could also be considered a "weak point" in the life history of *H. victoriensis* because inactive adults could be attacked easily by their enemies. However, from my observations in the laboratory *H. victoriensis* pupae usually hide at the bottom of the rearing cages, and so avoid being exposed as young, inactive adults.

Depending on the rearing technique (Chapter 2), these studies provide a general synopsis of the development of *H. victoriensis* (Fig. 3.3). The images recorded in this study (Fig. 3.3) may also be used by investigators to quickly classify the various developmental stages of *H. victoriensis* in the field before identifying them with more precision in the laboratory using keys.

These experiments were conducted at only one constant temperature of 25°C in laboratory, so they have not given any initial evaluation about the survival of *H. victoriensis* in the field where the temperature is highly variable. Although the survival of *H. victoriensis* observed in laboratory 77.1-91.9% was not affected at the temperatures of 12°C, 16.5^{o}C, 18.7^{o}C, 20.8^{o}C, 22.5^{o}C, 26.0^{o}C (Andrewartha, 1936), a wider understanding of the viability of *H. victoriensis* in the laboratory and field over a wider range of temperatures is required.

3.2. Effect of different food sources on development and longevity of *H. victoriensis*

Development of insects, including thrips, is affected by many environmental factors especially temperature and food. For instance, the developmental rates of *Thrips hawaiiensis* linearly decreased in correlation with a decrease in rearing temperature (Murai, 2001). The developmental time of predatory *Frankliniella schultzei* was significantly different on a diet of cotton leaf tissue compared with a diet of mite eggs and cotton leaf tissue (Milne and Walter, 1997). The F3 and F4 generations of predatory *Aleurodothrips fasciapennis* were still maintained when they fed on the eggs of *Corcyra cephalonica* but larval mortality increased, whereas the F3 and F4 population of this predatory thrips reduced and stopped when feeding on eggs and crawlers of *Chrysomphalus aonidum* (Watson et al., 2000). The rearing trials in this study also showed that types of diets affected its development. In this section, the objective was to investigate aspects of different food sources on the development and longevity of *H. victoriensis* in the laboratory.

3.2.1. Effect of different food sources on *H. victoriensis* development

To answer the question, "how do the thrips develop with different food sources?", two parameters were investigated: developmental rate, which is developmental capacity of thrips per day and developmental time, which is the number of days thrips develop in a considered period. The experiments also evaluated the quality of different diets on the development of *H. victoriensis*.

It is previously known that *H. victoriensis* could feed on *Antirrhinum* (snapdragon) stamens (Andrewartha, 1936), lucerne pollen and two-spotted mites (Bailey and Caon, 1986), mixed pollen, honey, *Tyrophagus* mites and

western flower thrips (WFT) (see Chapter 2), and in these experiments the latter diets was used.

3.2.1.1. Materials and Methods

The experiment was established with three food sources as treatments:

A. mixed pollen and 10% honey solution

B. mixed pollen, 10% honey solution and *Tyrophagus* mite

C. mixed pollen, 10% honey solution and WFT eggs

Developmental time of immature *H. victoriensis* in each treatment was observed for first instar, second instar, prepupa and pupa. The two pupal stages were considered together. When the thrips became adults, they were observed continuously in the period from newly emerged, inactive adults to black, active adults. There were two experimental parameters: developmental rate (per day) and developmental time (days) of life history stages from first instar to adult. The number of replications of each stage is given in table 3.1. The experiment was conducted at 25°C and at about 65% RH in an environmental incubator with the light controlled 16L:8D. Results of the experiment were statistically analysed and evaluated by using theory SPSS version 15.0 (Pallant, 2007). Levene's test was used to check equality of variances of the three treatments. If the results of Levene's test do not violate the assumption of homogeneity of variance, using one-way ANOVA test of parametric methods checks a significant difference between treatments. Significant differences between each pair of treatments were investigated using homogeneous subsets tests. Kruskal-Wallis and Mann-Whitney nonparametric tests were used when the assumption of homogeneity of variances was violated. Kruskal-Wallis indicates

a significant difference between three or more treatments and Mann-Whitney indicates a significant difference between two treatments.

Table 3.1. Numbers of replications used in the experiment on the effect of food sources on the development of *H. victoriensis*.

Observation parameters	Number of replications		
	Treatment A	Treatment B	Treatment C
First instar larvae	10	12	11
Second instar larvae	9	9	11
Prepupae	9	11	11
Pupae	9	11	11
From first instar to adult	9	9	11

3.2.1.2. Results

Developmental rates (per day) of *H. victoriensis* were compared for each developmental stage (Fig. 3.4). The first instar larvae feeding on honey and pollen (treatment A) had a significantly lower developmental rate compared with the first instar larvae feeding on honey and pollen plus either *Tyrophagus* mites or WFT eggs (Appendix 1, Tables C & D). The developmental rates of the second instar stage were significantly different (95% confidence interval) for the three treatments (Appendix 2, Table C). These treatments are significantly different in each pair and developmental rate of second instar larvae is fastest for treatment C and lowest for treatment A (Appendix 2, Table D). Developmental rate of second instar larvae in group A is significantly lower than that of groups B and C with 99% statistical confidence (Appendix 2, Table E). The thrips in treatment B and C had the same developmental rate in the pupal stage but significantly higher than pupae in treatment A (Appendix 3, Tables C

& D). Thrips spent about 0.5-1 day in the prepupal period in all three treatments (Fig. 3.4).

Aside from the statistical analysises, it was worth noting that *H. victoriensis* larvae feeding on only honey and pollen were weaker and smaller and apparently had lower fitness than those in the remaining treatments. About 25% of these larvae did not develop to the adult stage and a possible reason for this could be a nutrient deficiency which prevents moulting of larvae. There was an isolated case, where a larva feeding on honey and pollen developed slowly and spent a long time in the first instar stage, and then progressed to second instar. It developed slowly and died in the second instar stage apparently because it could not moult. Three other cases recorded where the larvae reared with honey and pollen took longer than usual but they moulted immediately after being fed a WFT larva, a *Tyrophagus* mite, or some WFT eggs.

In general, larvae fed on honey and pollen developed significantly slower in all stages except the prepupal stage compared with larvae fed on the diet which included prey.

Table 3.2. Effect of different food sources on the developmental time of *H. victoriensis*.

Stages	Mean developmental time (days) ± SD		
	Treatment A	Treatment B	Treatment C
First instar	10.3 ± 2.7	4.0 ± 0.7	3.7 ± 0.8
Second instar	18.2 ± 4.5	6.6 ± 0.7	5.8 ± 0.6
Prepupa	0.8 ± 0.2	0.8 ± 0.2	0.8 ± 0.2
Pupa	5.7 ± 0.9	4.4 ± 0.5	4.5 ± 0.5

    A. Mixed pollen and 10% honey solution

    B. Mixed pollen, 10% honey solution and *Tyrophagus* mite

    C. Mixed pollen, 10% honey solution and WFT eggs
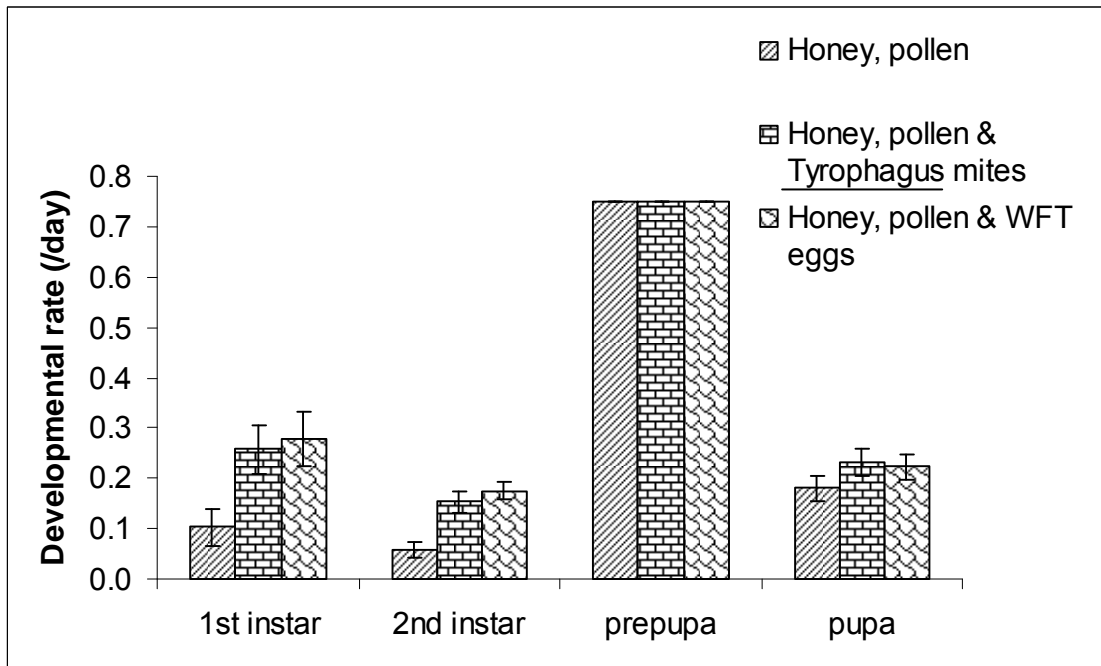
Figure 3.4. Effect of different food sources on the developmental rate of *H. victoriensis.*
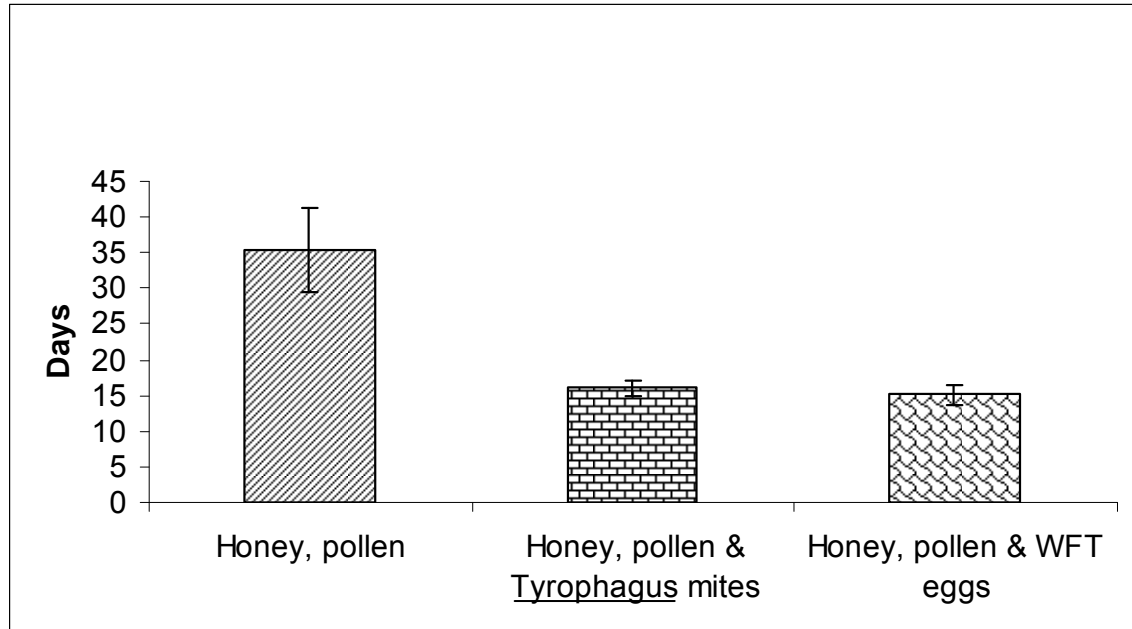
Figure 3.5. Effect of food sources on overall developmental time of *H. victoriensis* from first instar to adult (mean ± SD).

The effect of food sources on overall developmental time of *H. victoriensis* from first instar larvae to adults is illustrated in Fig. 3.5. A Kruskal-Wallis Test

revealed a statistically significant difference in developmental time across three different food sources (Treatment A, n=9: honey and pollen diet; Treatment B, n=9: honey, pollen and *Tyrophagus* mites; Treatment C, n=11: honey, pollen and WFT eggs, χ^2 (2, 29) =19.76, P =0.000) (Appendix 4, Tables C & D). The thrips reared on honey and pollen plus *Tyrophagus* mites or WFT eggs took approximately 15-16 days to complete development from first larvae to adult. A Mann-Whitney *U* test revealed no significant difference in these two treatments *U* =28, z =-1.696, P =0.09 >0.05 (Appendix 4, Tables G & H). Thrips feeding on honey and pollen had the longest developmental time (average 35 $\pm$ 5.94 days) and this treatment was significantly slower than the other two treatments B and C (Appendix 4, Tables E, F, G & H).

3.2.2. Effect of the addition of WFT to a basic diet on longevity of *H. victoriensis*

The longevity of adult *H. victoriensis* is unknown and this experiment set out to try to determine its longevity. As it is expected that the inclusion of prey in the diet will influence longevity, the effect of providing WFT as prey to a basic diet of honey and pollen was examined.

3.2.2.1. Materials and Methods

Haplothrips victoriensis larvae collected from mass-rearing cages were kept in separate Petri dish cages and fed with mixed pollen, 10% honey solution and WFT eggs. The larvae were reared until adults in the Petri dish cages similar to the rearing technique for larvae (see Section 2.2.2.2), Adults were separated and reared individually in a jar cage using the adult rearing technique (see Section 2.2.2.3). These adults were divided into two groups, with each group

fed either 10% honey and mixed pollen (treatment A) with 10 replications or 10% honey, mixed pollen and WFT larvae (treatment B) with 6 replications.

The experiment was conducted in an environmental incubator at 25°C, and about 65%RH and 16L:8D. The computer program SPSS version 15 was used to statistically analyse the experimental data. Levene's test checks equality of variances of the two groups of the experiment. If an analysed result of Levene's test does not violate the assumption of homogeneity of variance, using independent-samples T-test checks for a significant difference between the groups. Nonparametric Mann-Whitney tests were used to indicate a significant difference between the two groups when assumption of homogeneity of variances was violated (Pallant, 2007).

3.2.2.2. Results

The effect of having WFT in the diet of *H. victoriensis* on its longevity is presented in Fig. 3.6. Thrips fed on a diet of 10% honey and mixed pollen plus WFT larvae live on average 109.7 $\pm$ 11.27 days whereas thrips fed on mixed pollen and 10% honey solution alone only averaged 59.7 $\pm$ 13.94 days. This is significantly different at 99% confidence levels (Appendix 5).
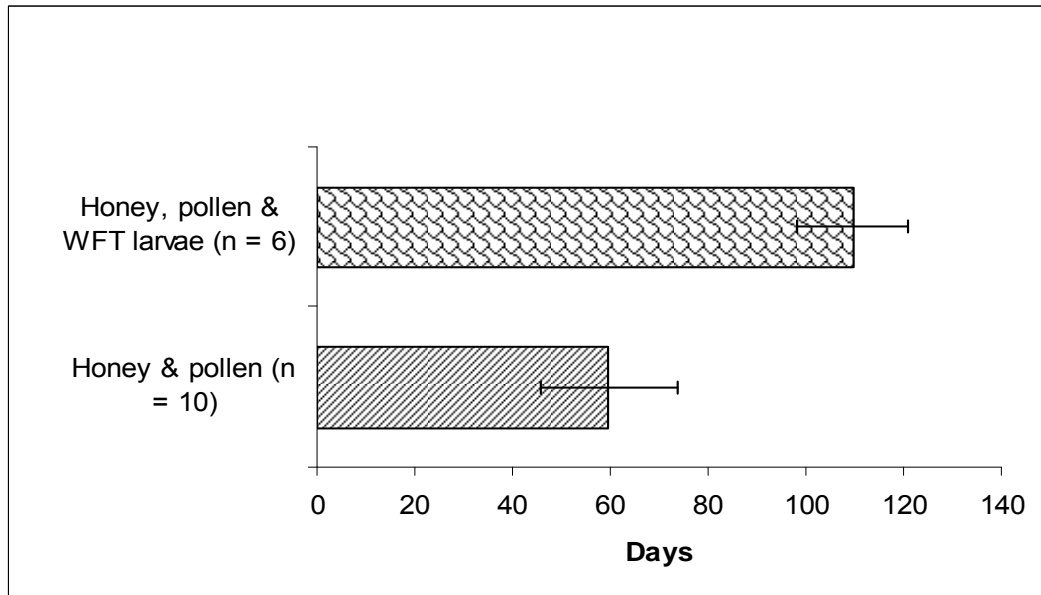
Figure 3.6. Effect of the addition of WFT prey to the basic diet on longevity of *H. victoriensis* (mean ± SD).

3.3. Discussion of the effect of different food sources on *H. victoriensis* development and longevity

Honey and pollen are only basic survival food for the thrips. So, it might be reasonably concluded that *H. victoriensis* is an obligate predator for its developmental stage and prey are an essential food source for *H. victoriensis* longevity. The experiments did not focus on analysing the nutritional status of the food sources, but rather was a simple investigation into the effect of diet on the development and longevity of *H. victoriensis* so that results could provide useful information for later mass-rearing experiments on *H. victoriensis*.

The developmental time of 10.6 ± 0.7 days of the larvae fed on honey, pollen and *Tyrophagus* mite eggs (Table 3.2) is similar to the results of Andrewartha (1936) and Bailey and Caon (1986). However, these show that larval *H. victoriensis* are not obligate predators.

Larvae develop much slower with 10% honey and mixed pollen than with *Tyrophagus* mites or WFT eggs in my experiments, whereas according to

Bailey & Caon (1986), second instar larvae on lucerne flowers developed slower than when second instar larvae were provided with two spotted mite eggs. This might be due to *Tyrophagus* mites, WFT eggs, lucerne flowers and snapdragon stamen containing similar essential nutrients for the development of *H. victoriensis* larvae, which are possibly lacking in two spotted mite eggs.

In the study by Bailey and Caon (1986) it is unclear if the *H. victoriensis* larvae were reared in groups or singly. *Haplothrips victoriensis* is a cannibal (see Chapter 4) which may have influenced their results. They hunt prey for their development and feed together if they are reared together in a cage so some data may be missed or lost. Also, it is unclear whether the lucerne flowers used in that study were young or old or whether they were free of eggs/larvae of other thrips or mites which might have provided a food source.

Laboratory observations also recorded that the time of changing colour of the emerged adults from red to dark red, brown, dark brown, and then black (shown in Fig. 3.3) is impacted by food sources. These changes take 5-10 days for *H. victoriensis* adults feeding on the diet with *Tyrophagus* mites or WFT which is significantly faster than the 10-15 days for thrips feeding only on honey and mixed pollen.

CHAPTER 4

PREDATION AND FEEDING OF

H. victoriensis

4.1. Introduction

4.2. Materials and Methods

    4.2.1. Observations of predatory characteristics of *H. victoriensis*

    4.2.2. Observation of cannibalism in *H. victoriensis*

    4.2.3. Consumption of prey by *H. victoriensis*

4.3. Results

    4.3.1. Predatory characteristics of *H. victoriensis*

    4.3.2. Cannibalism

    4.3.3. Consumption of *H. victoriensis* on *Tyrophagus* mites and WFT eggs

4.4. Discussion

CHAPTER 4

Predation and Feeding of Tubular Black Thrips

4.1. Introduction

Approximately half of all thrips species feed on fungi, with others feeding on leaves, branches, pollen or fruits of plants, and some are also known as predators of mites and other thrips (Mound and Marullo, 1996). Thrips use a single mandible which extends from a mouth cone on the underside of the head to pierce a hole into their food. They then feed by inserting a pair of maxillary stylets into the formed hole and the two maxillary stylets form a tube to suck up the liquid part of the food (Kirk, 1997). According to Kirk (1997), the mouth cones of both predatory and phytophagous thrips have a similar feeding mechanism.

Haplothrips species are usually found on flowers, feeding on pollen and nectar. Some, such as *Haplothrips victoriensis* (Bailey and Caon, 1986) and *H. brevitubus* (Kakimoto et al., 2006), have been identified as predators, the later as a predator of mulberry thrips (*Pseudodendrothrips mori* (Niwa)). *Haplothrips victoriensis*, a species endemic to southern Australia, lives mainly in grass and grass flowers (Bagnall, 1918; Pitkin, 1973; Mound and Minaei, 2007). It is a predator of two-spotted mite (TSM) on lucerne in South Australia (Bailey and Caon, 1986) and western flower thrips (WFT) in a survey of Goodwin and Steiner (1996). Two other important feeding characteristics of *H. victoriensis* noted by Steele (1935) were that "when in small glass vials, the second stage nymphs will feed on each other, and will also feed on unhatched eggs, even though plant food is present" and "in summer, this thrips occasionally damages peaches, feeding on the ripe fruit and causing white patches on the skin".

The next section of this research attempted to clarify the feeding characteristics of *H. victoriensis* by considering predation and cannibalism, and includes a discussion of the pest status of *H. victoriensis*. The questions for this research section are:

- What is the predatory capacity of *H. victoriensis* on other pests?

- What are the cannibalistic characteristics of both larval and adult *H. victoriensis* and does this change when all stages are present together?

- How much prey does *H. victoriensis* consume per day?

4.2. Materials and Methods

4.2.1. Observations of predatory characteristics of *H. victoriensis*

To investigate predatory capacities, *H. victoriensis* larvae or adults were trialled first on prey which has a close taxonomic relationship to the predator such as WFT and plague thrips, then on *Tyrophagus* spp, two-spotted mites, and then finally on other common crop pests (whitefly, aphid, diamondback moth) to explore their potential value for biological control. Predatory *H. victoriensis* were either fed with water only and starved for one day or fed with 10% honey solution for 2-4 days prior to the trial starting. Because *H. victoriensis* is an obligatory predator (see Chapter 3), both treatments still required them to seek prey. One to three *H. victoriensis* larvae/adults starved of prey were placed in a Petri dish cage with 5-15 target prey. Episodes of predation were then observed and recorded.

4.2.2. Observations of cannibalism in *H. victoriensis*

Cannibalism was often observed when rearing laboratory cultures of *H. victoriensis.* However, to understand the cannibalistic interactions of *H. victoriensis* between developmental stages or within the same stage, several different trials were conducted (Table 4.1). Experimental *H. victoriensis* were fed with 10% honey solution and pollen only for 1-2 days prior to the trials starting.

Table 4.1. Trials of *H. victoriensis* cannibalism.

No.	Predatory interactions	The number of experimental thrips				Rearing cage *
		Egg	1st instar	2nd instar	Adult	
01	First instar larvae versus eggs	5	3			Petri dish cage
02	Second instar larvae versus eggs	5		3		Petri dish cage
03	Adults versus eggs	5			3	Jar cage
04	First instar larvae		6			Petri dish cage
05	First instar versus second instar larvae		3	3		Petri dish cage
06	First instar larvae versus adults		3		3	Jar cage
07	Second instar larvae			6		Petri dish cage
08	Second instar larvae versus adults			3	3	Jar cage
09	Adults				6	Jar cage

(*) Petri dish cage and jar cage as described in Chapter 2.

Each predatory interaction trial was replicated one – three times. The second or third replication was not carried out if cannibalism had occurred in a previous test. Each rearing cage was observed for cannibalism events 4 times per day over a

total of 3 days. The tests were conducted in a constant temperature room at 25 $\pm$ 2°C and 16L:8D.

4.2.3. Consumption of prey by *H. victoriensis*

Here I tested the feeding consumption of *H. victoriensis* at various developmental stages (first instar, second instar, and adult) using inactive *Tyrophagus* mites and western flower thrips (WFT) eggs as prey. The experimental *H. victoriensis* were fed with water only for 24 hours prior to the start of experiments. The mites or the eggs were provided to the predators on a piece of black filter paper which provide a contrast and made observations easier. The experiments were conducted in an environmental incubator at 25 $\pm$ 0.5^{o}C and 16L:8D

Each experiment comprised three treatments based on three different feeding stages of *H. victoriensis*: first instar, second instar and adult. A single *H. victoriensis* individual was placed in each rearing cage so the number of experimental thrips is equal to the number of replications of each treatment and also to the number of rearing cages (Table 4.2).

Table 4.2. Feeding consumption test replications of three life stages of *H. victoriensis* feeding on inactive *Tyrophagus* mites and WFT eggs.

Haplothrips victoriensis	Experiment on *Tyrophagus* mite (no. of *H. victoriensis*)	Experiment on WFT egg (no. of *H. victoriensis*)	Rearing cage *
First instar	11	10	Petri dish cage
Second instar	10	11	Petri dish cage
Adult	9	11	Jar cage

(*) Petri dish cage and jar cage were described in Chapter 2.

4.3. Results

4.3.1. Predatory characteristics of *H. victoriensis*

Feeding observations showed that *H. victoriensis* was a predator on *Tyrophagus* mite (Fig. 4.1), WFT (Fig. 4.3), spotted alfalfa aphid (Fig. 4.4), greenhouse whitefly (Fig. 4.5), diamondback moth (Fig. 4.6) and plague thrips (Fig. 4.7). The predatory capacity of *H. victoriensis* on two spotted mite eggs (Fig. 4.2) was also confirmed the result of Bailey & Caon (1986). *Haplothrips victoriensis* did not consume any ash whitefly when they were used as prey in the trials.

Figure 4.1. *Haplothrips victoriensis* feeding on an inactive *Tyrophagus* mite.

Figure 4.2. Two spotted mite eggs (left); *Haplothrips victoriensis* feeding on a TSM egg (right).

Figure 4.3. *Haplothrips victoriensis* in different stages feeding on different stages of WFT. a: Second instar larva feeding on WFT pupa; b: First instar larva feeding on egg and larva of WFT c: Adult feeding on WFT egg; d: Second instar larva feeding on WFT eggs; e: Second instar larva feeding on WFT adult; f: Adult feeding on WFT larva.

Figure 4.4. *Haplothrips victoriensis* feeding on nymph of spotted alfalfa aphid, *Therioaphis trifolii* (Hemiptera: Aphididae) collected on lucerne.

Figure 4.5. *Haplothrips victoriensis* feeding on a larva of greenhouse whitefly, *Trialeurodes vaporariorum* (Hemiptera: Aleyrodidae).

Figure 4.6. A second instar larva of *H. victoriensis* feeding on eggs of diamondback moth, *Plutella xylostella* (Lepidoptera: Plutellidae) on canola leaf.

Figure 4.7. *Haplothrips victoriensis* larva feeding on a larva of plague thrips, *Thrips imaginis* (Thysanoptera: Thripidae).

4.3.2. Cannibalism

Cannibalism was commonly observed in *H. victoriensis* populations in the laboratory. *Haplothrips victoriensis* is an obligate predator (see Chapter 3), and therefore predation needs to occur for successful maturation and reproduction. From my observations, hungry *H. victoriensis* seldom miss any chance of predation when facing prey even if they encounter a conspecific. Cannibalism within a *H. victoriensis* population usually complies with the rule: "a stronger and hungrier *victoriensis* is the winner in an encounter". Laboratory observations showed that adults and larvae fed on eggs (Fig. 4.8 & 4.9); adults and second instar larvae fed on first instar larvae (Fig. 4.10); and in the same larval stage, a smaller larva was prey of a larger larva (Fig. 4.11). In comparison, second instar larvae appeared more voracious and violent hunters. It was also observed that a smaller second instar larva could chase off a bigger second instar larva that was satiated. However cannibalism did not result in this case because the bigger one is usually stronger and therefore was able to escape. There was no case recorded where an adult fed on a second instar larva. In prepupal and pupal stages, *H. victoriensis* move slowly and when under predation pressure tended to hide (for example, under paper towel) presumably to avoid their predators. They appeared inactive prepupae or pupae. However, if other insects approached, they waved the posterior end of their abdomen like a tail as a defense mechanism to chase off the insects or they quickly moved to a safe hiding place. There was not any instance observed where *H. victoriensis* prepupa or pupa was preyed on by larval or adult stages. Cannibalism usually happened during the vulnerable period when *H. victoriensis* has just emerged to become a new adult (see Chapter 3). Newly

emerged adults that are inactive were usually eaten by both larval stages (Fig. 4.12). No instances of cannibalism by adults on other adults were observed.

Figure 4.8. *Haplothrips victoriensis* cannibalism: adult feeding on eggs.
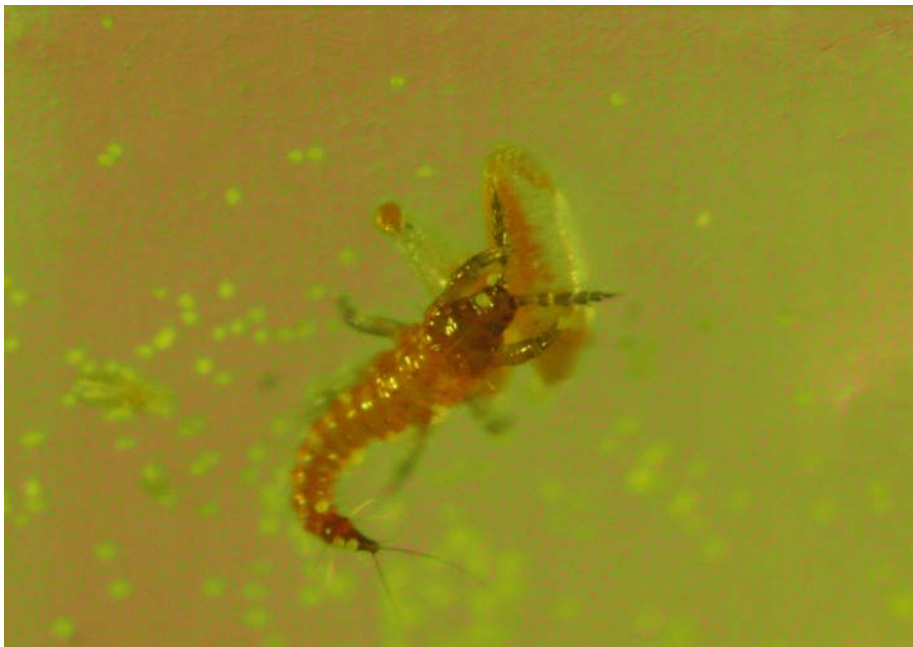
Figure 4.9. *Haplothrips victoriensis* cannibalism: first instar larva feeding on eggs.

Figure 4.10. *Haplothrips victoriensis* cannibalism: second instar larva feeding on first instar larva.
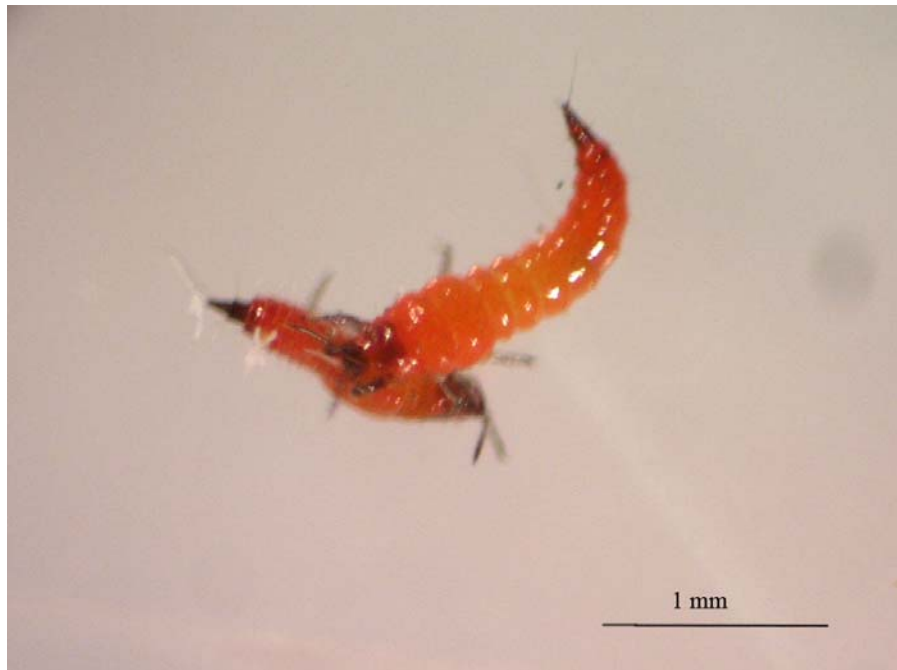
Figure 4.11. *Haplothrips victoriensis* cannibalism: second instar larvae feeding each other.

Figure 4.12. *Haplothrips victoriensis* cannibalism: second instar larva feeding on a newly emerged adult.

Figure 4.13. *Haplothrips anceps* cannibalism: second instar larva feeding on first instar larva.

Figure 4.14. Western flower thrips cannibalism: first instar larva feeding on egg.

Laboratory observations of cannibalism were also recorded for *Haplothrips anceps* [Phlaeothripidae] (Fig. 4.13) and *Frankliniella occidentalis* (WFT) [Thripidae] (Fig. 4.14). However cannibalism in WFT was not commonly observed.

4.3.3. Consumption of *H. victoriensis* on *Tyrophagus* mites and WFT eggs

Tyrophagus mites were consumed by first and second instar larvae and adults of *H. victoriensis* (Figure 4.15). A Kruskal-Wallis Test revealed a statistically significant difference between the three treatments (χ^2(2, 30) = 17.78, P = 0.000) (Appendix 6, Tables C & D). Two Mann-Whitney U tests (Appendix 6, Tables F & H) revealed that the consumption of *Tyrophagus* mite by the second instar larvae and adults was significantly higher than first instar larval consumption at the 99% confidence level.
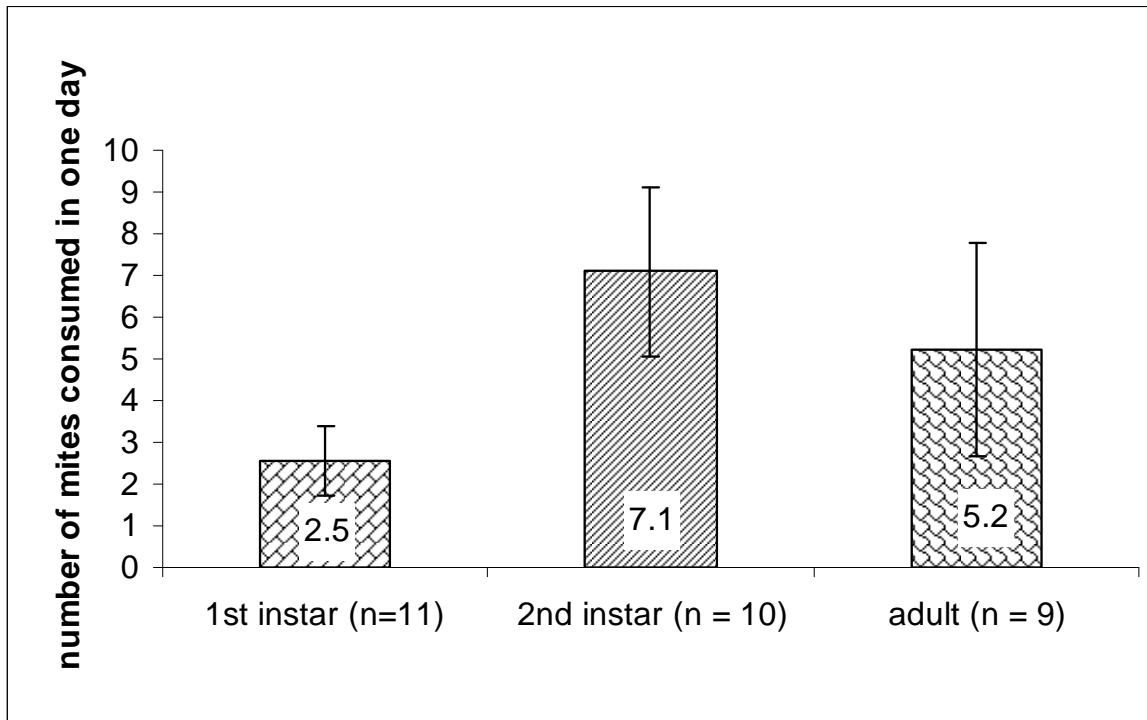
Figure 4.15. Mean ± SD consumption of inactive *Tyrophagus* mites by larvae and adults of *H. victoriensis*.
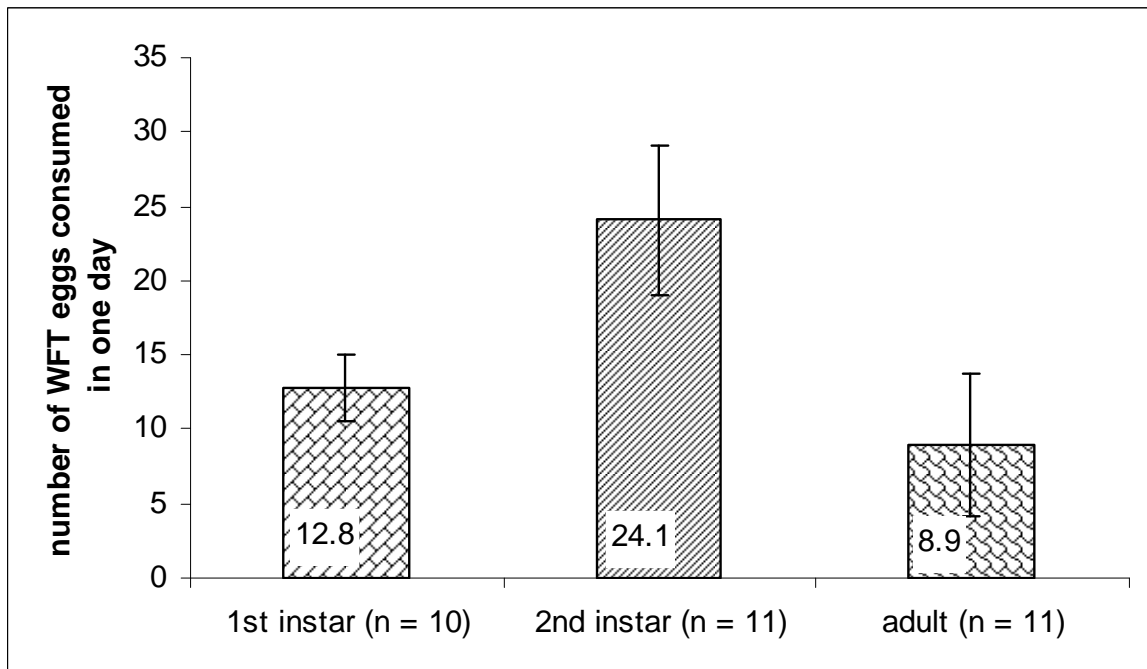
Figure 4.16. Average ± SD consumption of WFT eggs by larvae and adults of *H. victoriensis*.

WFT eggs were consumed by first and second instar larvae and adult *H. victoriensis* (Fig. 4.16). A one-way between-groups analysis of variance was

conducted to explore difference between the three levels of consumption of first instar, second instar and adults on WFT eggs (Treatment W1, n = 10: consumption of first instar larvae on WFT eggs; Treatment W2 n = 11: consumption of second instar larvae on WFT eggs; Treatment W3, n = 11: consumption of adults on WFT eggs) (Appendix 7, Table A). There was a statistically significant difference at $p < 0.01$ between the three treatments: F (2, 29) = 37.02, P = 0.000) (Appendix 7, Table C). Subset comparisons using Tukey's HSD test indicated the significant differences of each pair of treatments at p = 0.01 (Appendix 7, Table D). The number of WFT eggs consumed by the second instar larvae (Treatment W2: M = 24.1 $\pm$ 5.1 SD) was highest and double the number of WFT eggs consumed by first instar larvae (Treatment W1: M = 12.8 $\pm$ 2.3 SD) and adults (Treatment W3: M = 8.9 $\pm$ 4.8 SD). Consumption by first instar larvae and adults on WFT eggs were not significantly different.

Second instar larvae consumed the most prey in both consumption experiments. It is likely that larvae usually consume more prey than adults because larvae have a high nutrient demand for their development. In the consumption experiment by *H. victoriensis* on WFT egg, one instance was recorded where an adult did not feed on any WFT eggs indicating that an adult can survive without prey for a minimum of 2 days.

4.4. Discussion

Haplothrips victoriensis has potential as a biological control agent because it can feed on a wide range of prey including known serious pests of horticulture such as WFT (a vector of tomato spotted wilt virus (TSWV)), diamondback moth (a brassica pest), two-spotted mite (a greenhouse pest) and greenhouse whitefly. It

may feed on a wider range of prey than the trials of my observations, so this would need further testing. Given the results of its predatory capability, it is also possible that *H. victoriensis* could be a predator of many other pest thrips. Furthermore, *H. victoriensis* is a polyphagous species. Its consumption of prey was relatively high with a range of 2.5-7.1 *Tyrophagus* mites or between 8.9-24.1 WFT eggs per day respectively, although there could be a difference in the relative amounts of prey taken depending on whether the prey is inactive (eggs) or mobile (thrips larvae or adults). The second instar larva could consume up to 30 WFT eggs per day. By comparison, research by Young & Zhang (2001) showed that, a predatory *Haplothrips species* of their experiments consumed a maximum of only two *Thrips palmi* larvae per day. Milne & Walter (1997) looked at consumption of mites by *Frankliniella shultzei* (Thysanoptera: Thripidae) where they found that a second instar larva could feed on up to 20 TSM eggs per day. When we consider that these eggs are about 1/5 the size of WFT eggs, then we see that *H. victoriensis* can consume not only more prey but greater food volume than *F. shultzei.* Given its relatively high food intake shown in the laboratory experiments, *H. victoriensis* may help contribute to the pest suppression with its predation and consumption capacities in the field.

Another aspect which needs to be considered is the pest status of *H. victoriensis*. Steele (1935) noted that peaches were damaged occasionally by *H. victoriensis* feeding in summer which caused white patches on the skin of the ripe fruit. In contrast, from my observations this was not the case when *H. victoriensis* was cultured with WFT as prey on cucumber fruits (see Section 2.2.4). *H. victoriensis* adults and larvae usually moved and used their antennae gropingly touching on the surface of cucumber fruits step by step searching for WFT eggs

which were oviposited under the cucumber surface. When they were feeding on the WFT eggs it appeared as though *H. victoriensis* were feeding on the cucumber fruits but they were actually feeding on the WFT eggs. Oviposition of Terebrantia thrips can cause damage on apples, avocados, citrus, bananas, cherries, grapes, legumes, tomato and peas (Childers, 1997). Therefore the appearance of white patches on peaches noted by Steele (1935) might be caused by oviposition of other Terebrantia species rather than the feeding on thrips eggs by *H. victoriensis*. In this situation, *H. victoriensis* is still a predator but it is unclear whether its predation contributed to the damage on peaches. An alternative hypothesis could be that *H. victoriensis* feed on the fruit if there are no suitable prey. Consequently, it is suggested that before considering *H. victoriensis* as a pest, the mechanisms causing such damage need to be investigated more closely.

It may be a challenge to produce *H. victoriensis* for biological control because of its predatory and cannibalistic characteristics. These predatory thrips not only feed on pest species but also eat each other, so when resources are limited, the population of *H. victoriensis* will decrease. This reduces its effectiveness as a biocontrol agent. While cannibalism was fairly frequent in laboratory cultures, it could be quite different in the field.

There are three reasons why *H. victoriensis* is less likely to be cannibalistic in the field. There is more space, a large number of alternative prey, and vulnerable stages can stay well hidden. This may be further explained by considering *H. victoriensis* as both a polyphagous and an opportunistic predator. During periods of food shortage it can become an aggressive predator in order to obtain protein. *Haplothrips victoriensis* can become either a predator or prey when there are more *H. victoriensis* than alternative prey. However, *H. victoriensis* has less opportunity

for cannibalism in the field where there are plenty of flowers, petals, stamens, pistils and many tiny hairs at the base of flowers to provide a complex habitat. *Haplothrips victoriensis* larvae use these plant structures as places to hide. Eggs are oviposited well into the hairs at the base of flowers so that larvae or adults of *H. victoriensis* have a reduced chance of finding the eggs and consuming them. When approaching the pupal stage, *H. victoriensis* tend to move to the soil or litter for metamorphosis into the pupal stages. Overall, I suggest that cannibalism is less likely to occur in *H. victoriensis* field populations.

Although the cannibalistic characteristic of *H. victoriensis* may be a disadvantage for culturing a population and for biological control, in other aspects, it may be an advantage for the ongoing existence of this species. In a natural environment, when *H. victoriensis* prey numbers dwindle, *H. victoriensis* can continue to exist because of its cannibalistic habit. This also has benefits for biological control when *H. victoriensis* is used in a crop area because it remains in the area that it was introduced. It may be always available in that area like "a dog guarding a garden" whereas other predators without cannibalistic characteristics would tend to move to other places because of lack of prey. Cannibalism could also help to explain why the *H. victoriensis* populations quickly fluctuate in the field as seen in this research and in research reported by Evans (1933; 1935), Steele (1935) and Andrewartha (1936).

As well as *H. victoriensis*, it was found that both *H. anceps* and WFT also have cannibalistic characteristics (see Section 4.3.2), but it is unknown if this occurs in the field and how wide-spread this habit may be amongst other thrips.

CHAPTER 5

LARVAL MORPHOLOGY, BARCODING, DISTRIBUTION AND HOSTS

5.1. Introduction

5.2. Materials and Methods

5.3. Results

5.4. Discussion

Chapter 5

Larval Morphology, Barcoding, Distribution and Hosts

5.1. Introduction

In Australia, there are 24 described *Haplothrips* species (Mound and Minaei, 2007). However, when this project was begun there were 11 *Haplothrips* species described by Pitkin (1973) and an additional species (*Haplothrips jarvisi* Kelly) was recorded by Mound (1996). The list of Australia species together with their distribution and ecology is shown in table 5.1. Three of them, *H. robustus* Bagnall, *H. varius* Hood and *H. victoriensis* Bagnall, are florivores and have been found in SA, whereas *H. anceps* Hood, *H. angustus* Hood, and *H. froggatti* Hood, are graminivores, and are only recorded in other Australian states (Table 5.1). The exotic *H. angustus* has already dispersed and is widespread in southern Australia (Pitkin, 1973).

Haplothrips species live in the flowers of many plants. *Haplothrips victoriensis* lives mainly in grass flowers (Bagnall, 1918; Pitkin, 1973; Mound and Minaei, 2007), but can be abundant in rose flowers (Evans, 1935), lucerne (Bailey and Caon, 1986), and on dahlia, *Olearia*, apple, peaches (Steele, 1935a), and clover (E. J. Martyn unpublished data). As part of this work into the distribution and host plants, an initial field survey was conducted in the main horticultural areas of SA.

Table 5.1. Ecology and distribution of *Haplothrips* species in Australia.

No.		Species	Ecology/hosts	Distribution
1		***H. anceps* Hood ***	Graminivore	NE coastal, QLD
2		***H. angustus* Hood ***	Graminivore	SE coastal, Murray-Darling basin, SW coastal, Lake Eyre basin, NSW, WA, NT
3		*H. bituberculatus* Girault	Predator	Coastal of N, NE, SE & SW, Murray-Darling basin, QLD, NSW, ACT, VIC, WA, NT
4		***H. froggatti* Hood**	Graminivore	Coastal of NE, SE & N, Murray-Darling basin, Lake Eyre basin, QLD, NSW, ACT, WA, NT
5		*H. gowdeyi* (Franlin)	Florivore	NE coastal, SE coastal, QLD, NSW
6		*H. jarvisi* Kelly ****	Unknown	Murray-Darling basin, VIC
7		*H. niger* (Osborn) **	Florivore	SE coastal, NSW, VIC; also widespread in temperate areas
8		*H. nigricoxa* (Girault) ***	Unknown	NE coastal, QLD
9		*H. pallescens* (Hood) *****	Graminivore	NE coastal, QLD
10		*H. robustus* Bagnall	Florivore	NE coastal, SE coastal, S Gulfs, Murray-Darling basin, Lake Eyre basin, QLD, NSW, ACT, VIC, SA
11		*H. varius* Hood	Florivore	NE coastal, SE coastal, S Gulfs, Murray-Darling basin, Lake Eyre basin, N coastal, QLD, NSW, VIC, SA, NT
12		***H. victoriensis* Bagnall ******	Florivore	NE coastal, SE coastal, S Gulfs, Murray-Darling basin, SW coastal, QLD, NSW, ACT, VIC, SA, WA
13		*H. acaciae* Mound & Minaei		SA, WA, NSW, Queensland
14		*H. angusi* Mound & Minaei		WA
15		*H. avius* Mound & Minaei	Cyperaceae	SA, Kangaroo Island, Queensland
16		*H. bellisi*	Darwin	North Territory
17		*H. collyerae* (Mound & Walker) comb. n.	Predator	Tasmania
18		*H. dicksoniae* Mound & Minaei	*Dicksonia* fronds with sori	ACT, NSW
19		*H. drisseni* Mound & Minaei		ACT, Tasmania
20		*H. fici* Mound & Minaei	*Ficus oppsita*	WA, NT
21		*H. gahniae* Mound & Minaei	*Gahnia* spp.	Adelaide hills, Kangaroo Island
22		*H. gomphrenae* Mound & Minaei	*Amaranthaceae*	WA, NT
23		*H. haideeae* Mound & Minaei	Grass flowers/ predator of T. *palmi*	NSW, Queensland, ACT
24		*H. howei* Mound & Minaei	possible predator	Lord Howe Island
25		*H. leucanthemi* (Schrank) **		Sydney, Wagga, Melbourne and Adelaide
26		*H. lyndi* Mound & Minaei ***		NT, Queensland
27		*H. ordi* Mound & Minaei	*Distichostemon hispidulus*	WA
28		*H. salicorniae* Mound & Walker	Florivore	NE coastal, QLD
29		*H. timori* Mound & Minaei	Mango (dead leaves)	NT, Humpty Doo, Lambell's Lagoon

The vertical label spanning rows 1–12 reads: Pre Mound and Minaei; 2007 (Pitkin, 1973 & Mound, 1996). The vertical label spanning rows 13–29 reads: Mound and Minaei, 2007.

(*), (**), (***) & (****): Synonymised by Mound and Minaei (2007)
(*****): Haplothrips pallescens (Hood) is synonymous with *Dyothrips* Kudo (Mound and Minaei, 2007)

Using Pitkin's key to Haplothripini (1973) it is difficult to distinguish the adult stage of *H. victoriensis* from other species, especially *H. froggatti*, *H. anceps* and *H. angustus,* all of which are similar to *H. victoriensis.* The key relies on colour features rather than morphological characters. For example, a very small area of the marginal part of the fore wing, which is difficult to observe even using a microscope, is either brown (*H. victoriensis*) or colourless (the three remaining species); antennal segment II and III are yellow or yellow tinged with brown and paler. The size of antennal segment three can also be used to differentiate between *H. anceps* and *H. angustus*. Obviously, *Haplothrips* spp. identification is complicated, especially as there are further undescribed *Haplothrips* species (Mound, 2006 pers. comm.), and Pitkin's key does not include the additional taxa described by Mound and Minaei (2007).

In an attempt to resolve the problems associated with larval identification, I generally examined larval morphology and manipulated COI barcoding of *H. victoriensis* and several of its similar taxa. Because of limited time, the main focus was on four morphologically similar species *H. victoriensis, H. froggatti, H anceps* and *H. angustus,* although where possible, other Australian *Haplothrips* larvae were included. As well, COI barcoding for these taxa was carried out to see if they could be more easily separated using molecular diagnostic sequences. COI barcoding also enabled me to construct a basic tree to examine preliminary phylogenetic relationships. It is also possible that these data will provide further information on the current distribution of these taxa in the horticultural areas of SA.

5.2. Materials and Methods

Collection:

Thrips are usually collected by sweep-netting (Steiner and Goodwin, 1998), sticky traps (Walsh et al., 2005), or beating flowers, plant branches or seed heads over a

white plastic tray (Mound and Kibby, 1998; Steiner and Goodwin, 1998). In this study, *Haplothrips* species were collected from flowers onto a while plastic tray by beating on the flowers several times. Individual thrips was collected from the tray by using either a fine brush or were sucked into a small jar using a hand held vacuum. The thrips were either placed in a prepared cage for laboratory rearing (see chapter 2) or into 99% alcohol for further analysis.

The thrips survey was conducted in late Autumn 2006 and from the beginning of spring 2006 to the end of Summer 2007 in the main horticultural areas of South Australia. Data collected included number of thrips, other common insects, time, location and host plants. Examples of each sample were mounted on slides using a technique from Mound (Appendix 8) and observed under a light microscope for identification (these slides have been placed in the Waite Insect and Nematode Collection). Keys in "Thysanoptera an Identification guide" (Mound and Kibby, 1998) were used for family classification and the Australian Haplothripini (Pitkin, 1973) and ThripsID by Moritz, Morris and Mound (2001) for species identification. Some specimens were also sent to CSIRO Entomology and identified by L. Mound.

A subsample of thrips adults from each site was reared separately in the laboratory. Similar looking larvae of the original parental thrips were divided in groups and reared to adults using the rearing techniques described in Chapter 2. This is important because some rearing cages were found to contain species two or three of larvae. The offspring of original thrips samples (including larvae, pupae and adults) were observed, recorded, collected and stored in 99% ethanol in a refrigerator. Samples from both the field and laboratory rearing were selected to conduct molecular analyses and morphological identification. Therefore, it critical to ensure

which larvae were from which adult and the next generational adult emerged from which larva. COI extractions were made from both larvae and adults.

DNA extraction

DNA extraction methods basically followed the Puregene® DNA Purification Kit (Gentra Systems Inc.) with minor modifications. The purpose of these modifications was to ensure that the exoskeletons of thrips adults were retained for later morphological examination (Morris and Mound, 2004). In other studies (Moritz *et al.*, 2000; Moritz *et al.*, 2002; Toda and Komazaki, 2002; Rugman-Jones *et al.*, 2006) the specimens were usually broken up during DNA extraction. This makes any subsequent morphological observations impossible. In brief, the modifications to the Puregene protocol were: rather than grinding an individual specimen, an entire *Haplothrips* specimen was incubated over night at $55^{\circ}C$ in a 1.5 mL centrifuge tube with 200 µL of Cell Lysis solution and 2 µL proteinase K. The supernatant was removed to a new labeled tube and the Puregene extraction protocol was undertaken on the supernatant. The extracted, intact thrips exoskeleton remaining in the first tube was washed twice in 60% ethanol and stored in 90-100% ethanol in a refrigerator for morphological examination.

PCR amplification and Sequencing

In this investigation, the target fragment which was amplified was the mitochondrial gene cytochrome oxidase 1 (COI). The primers used to amplify COI in the polymerase chain reaction (PCR) were LCO1490 and HCO2198 (Folmer *et al.*, 1994). Other components for PCR performance per specimen are 16 µL Nuclease-free H_2O, 2.5 µL 10X Buffer 25 mM $MgCl_2$, 2.5 µL 10 mM dNPT, 0.1 µL enzyme Hot Master taq, 1 µL of each primer and 2 µL extracted DNA. The PCR solutions of thrips specimens were run in Agarose Gel Electrophoresis at 100 volt, 70 ampere for about

20-30 minutes. The PCR gel piece was stained using Ethidium Bromide and photographed under UV light. Figure 5.1 is an example of an agarose gel. The DNA extraction and PCR work were performed in the laboratory of Australian Centre for Evolutionary Biology and Biodiversity at the University of Adelaide. PCR products were purified using the Ultraclean PCR Clean-up Kit (MOBIO Laboratories Inc.). Sequencing reactions were performed using ABI Big Dye Terminator Chemistry. DNA sequences were resolved on an ABI 3700 sequencer at the Sequencing Center of Institute of Medical and Veterinary Science.   All sequences were edited with reference to chromatograms using BioEdit version 7.0.1 (Hall, 1999). A neighbor-joining tree of the resultant sequence alignment was constructed using the Kimura-2-parameter model in MEGA 3.0 (Kumar *et al.*, 2004).
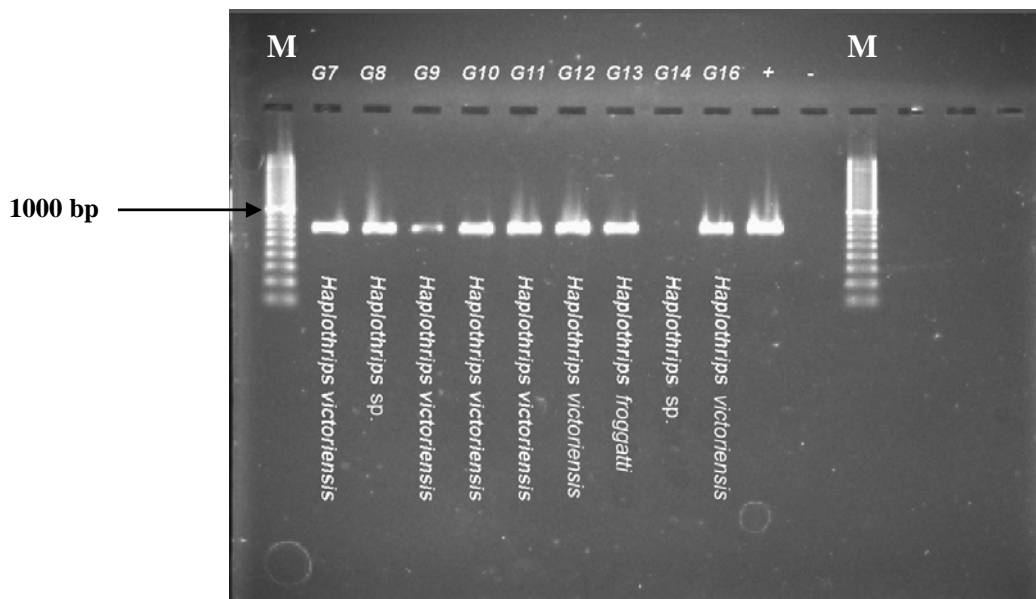
Figure 5.1. Bands of COI genes of *Haplothrips* species on the agarose gel amplified by the primers LCO1490 and HCO2198.

5.3. Results

5.3.1. Plant hosts for *H. victoriensis* in South Australia

Fifty five thrips samples were collected at 22 sites in the main horticultural regions of South Australia. *Haplothrips victoriensis* were found on several different plant families and areas in SA (see Table 5.2 and Fig. 5.2). These thrips generally live in flowers which have a complex structure allowing them to hide from predators. This includes, for example, roses, gazania, eucalypt and sow thistle that have many soft hairs on stamens and pistils, or flowers of lucerne, canola and coastal bearded heath that have many small flowers on a small branch. *Haplothrips victoriensis* was not found in flowers having a simple structure such as day lily (*Hemerocalli*s sp.). I could not find *H. victoriensis* on day lily flowers even if the day lily grew around roses where *H. victoriensis* was abundant. *Haplothrips victoriensis* were often found in flowers that hosted other insect species such as *Thrips imaginis, Thrips tabaci*, WFT, other thrips and aphids. Populations of *H. victoriensis* were found easily in spring, summer and early autumn and the thrips were abundant in the field from late spring to summer. It was also observed that *H. victoriensis* populations seemed to have large fluctuations in the field. For example, up to 30-40 thrips per rose flower (15 February 2007) could be found in the Waite rose garden but in the following 2-3 weeks (the first week of March 2007) only 0-5 thrips.

Figure 5.2.
Collection sites for
H. victoriensis in SA.

Table 5.2. Plant host for *H. victoriensis* in South Australia.

No.	Common name	Scientific name	Location
01	rose	*Rosa* spp (Rosales: Rosaceae)	Clarence Park, Waite Campus, Regency Park, Lyndoch, Strathalbyn, Meadows, Willunga, Victor Harbor, Waikerie, Barmera
02	lucerne	*Medicago sativa* (Fabales: Fabaceae)	Murray Bridge, Glen Osmond
03	salvation jane	*Echium plantagineum* (Lamiales: Boraginaceae)	Virginia, Barossa Valley
04	yellow gazania	*Gazania uniflora* (Asterales: Asteracae)	Renmark
05	india mustard	*Brassica juncea* (Capparales: Brassicaceae)	Virginia, Murray Bridge
06	canola	*Brassica napus* (Capparales: Brassicaceae)	Barossa
07	burr medic	*Medicago polymorpha* (Fabales: Fabaceae)	Loxton North, Loxton
08	sow thistle	*Sonchus oleraceus* (Asterales: Asteracae)	Loxton
09	valencia oranges	*Citrus sinensis* (Sapindales: Rutaceae)	Loxton
10	red flower eucalypt	*Eucalyptus cladocalyx*	Delamere
11		*Cytisus* sp.	Aldgate
12	coastal bearded heath	*Leucopogon parviflorus* (Ericales: Epacridaceae)	Kangaroo Island

5.3.2. Immature morphology

In this part of the project, I attempted to examine the larval morphology of *H. victoriensis* and its similar taxa including *H. anceps, H. angustus and H. froggatti. Haplothrips anceps* and its larvae were found on flowers of spiny flat-sedge (*Cyperus gymnocaulos*) at Waite Campus. The morphological characteristics of these larvae were recorded from laboratory reared thrips. Mound recently examined the morphology of *H. anceps* larvae and concluded that *H. anceps* is synonymous with *H. angustus* (Mound, 2006 pers. comm.). The synonymy was also confirmed in Mound and Minaei (2007). Therefore, it was planned to include three remaining close similar taxa in the investigations. However, despite many attempts, the larvae of *H. froggatti* were not able to be collected. The larvae of three *Haplothrips* species (*H. victoriensis, H. anceps* and *H. robustus*) were able to be reared and observed in the laboratory. The larval morphology of these three species is shown in Figs 5.3. and 5.4.

400 µm
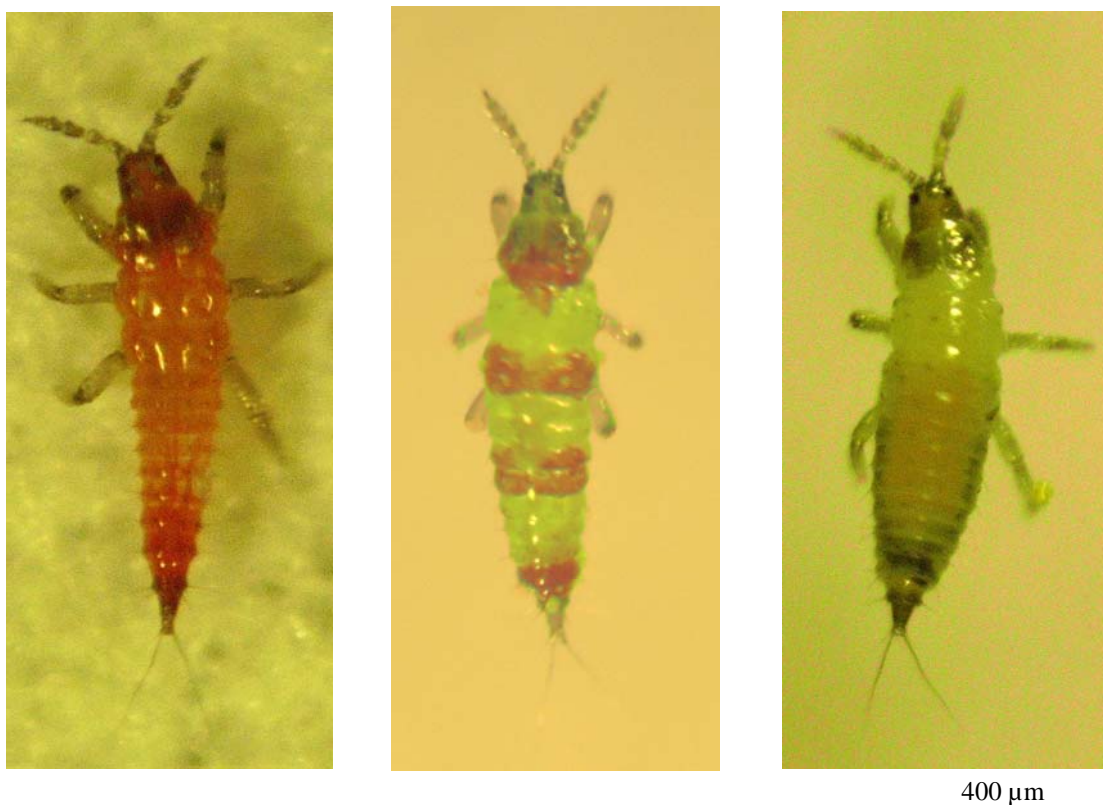
Figure 5.3. The first instar larvae of *H. victoriensis* (left), *H. anceps* (middle) and *H. robustus* (right).

400 µm

Figure 5.4. The second instar larvae of *H. victoriensis* (left), *H. anceps* (middle) and *H. robustus* (right).

The size of the first and second instar larvae of all three species is similar. The legs and antennae of both larval stages have a similar colour. However, they can be separated from each other based on their different body colour. In the first instar (Fig. 5.3), the three species have the same dark brown colour at the tube (abdominal segment 10), posterior half of abdominal segment 9 and pronotum. However, *H. victoriensis* has bright red body which is different from the basically yellowish body of *H. angustus*. *Haplothrips anceps* is also distinctive with its light yellow body with three horizontally dorsal red stripes. In the second instar (Fig. 5.4), the body colour of these three species is more contrasted. The head, pronotum, abdominal segments 9 and 10 and two side small stripes on abdominal segment 8 are dark brown for both *H. victoriensis* and *H. anceps* whereas the head, pronotum, abdominal segments 9 and 10 and two side small stripes on abdominal segment 8 of *H. robustus* are black. The basic body colour of these three *Haplothrips* species is totally different, bright red for *victoriensis*, yellowish for *robustus*, and light yellow with two horizontally red stripes for *anceps.*

When developing to prepupae and pupae, the whole of the body of the three thrips changes to a similar pink to red (Fig. 5.5 and 5.6). The prepupae and pupae of these thrips look similar so their morphologies are very difficult to distinguish separately.

The colour change of *H. robustus* from instar II to instar V was also recorded in Fig. 5.6. The second instar larva changes its body colour from yellow to light red at the end of the second larval stage. The prepupa (instar III) and the first pupa (instar IV) are light colour and the second pupa (instar V) is red colour.

400 µm

Figure 5.5. The prepupae of *H. victoriensis* (left), *H. anceps* (middle) and *H. robustus* (right).

400 µm

Figure 5.6. The first pupae of *H. victoriensis* (left), *H. anceps* (middle) and *H. robustus* (right).
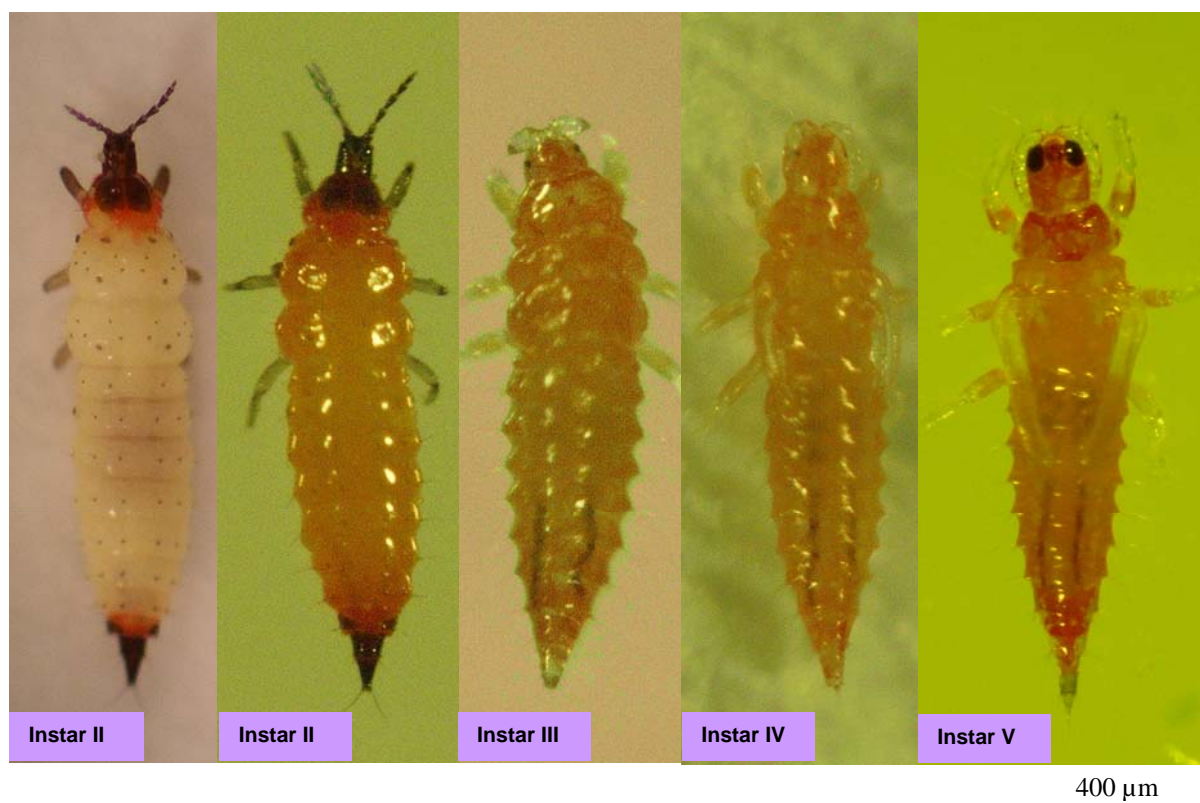
Figure 5.7. Colour change of *H. robustus* from second instar larva to pupa.

5.3.3. Phylogenetic tree of *H. victoriensis* and several of its similar taxa

Forty specimens were trialed in this molecular investigation. Thirty-one specimens produced reliable COI sequences which produced an alignment of approximately 630 nucleotides, and were used to construct a phylogenetic tree (Fig. 5.8). Three additional COI sequences of *H. froggatti* (EF634241), *Haplothrips reuteri* (EF634240) and *Apterygothrips australis* (EF634239) from Genbank were included in the phylogenetic tree. Figure 5.8 shows that *H. victoriensis* and its similar taxa can be readily separated using COI. This demonstrates that there is potential to develop a species specific primer for identifying *H. victoriensis, H. anceps, H. froggatti* and probably other *Haplothrips* taxa in Australia. Interestingly, there is no genetic variation and therefore no cryptic speciation within *H. victoriensis* in SA. However, *H. robustus* does show some small variation (Fig. 5.8). However, what is not yet known is whether there is any phenotypic variation.
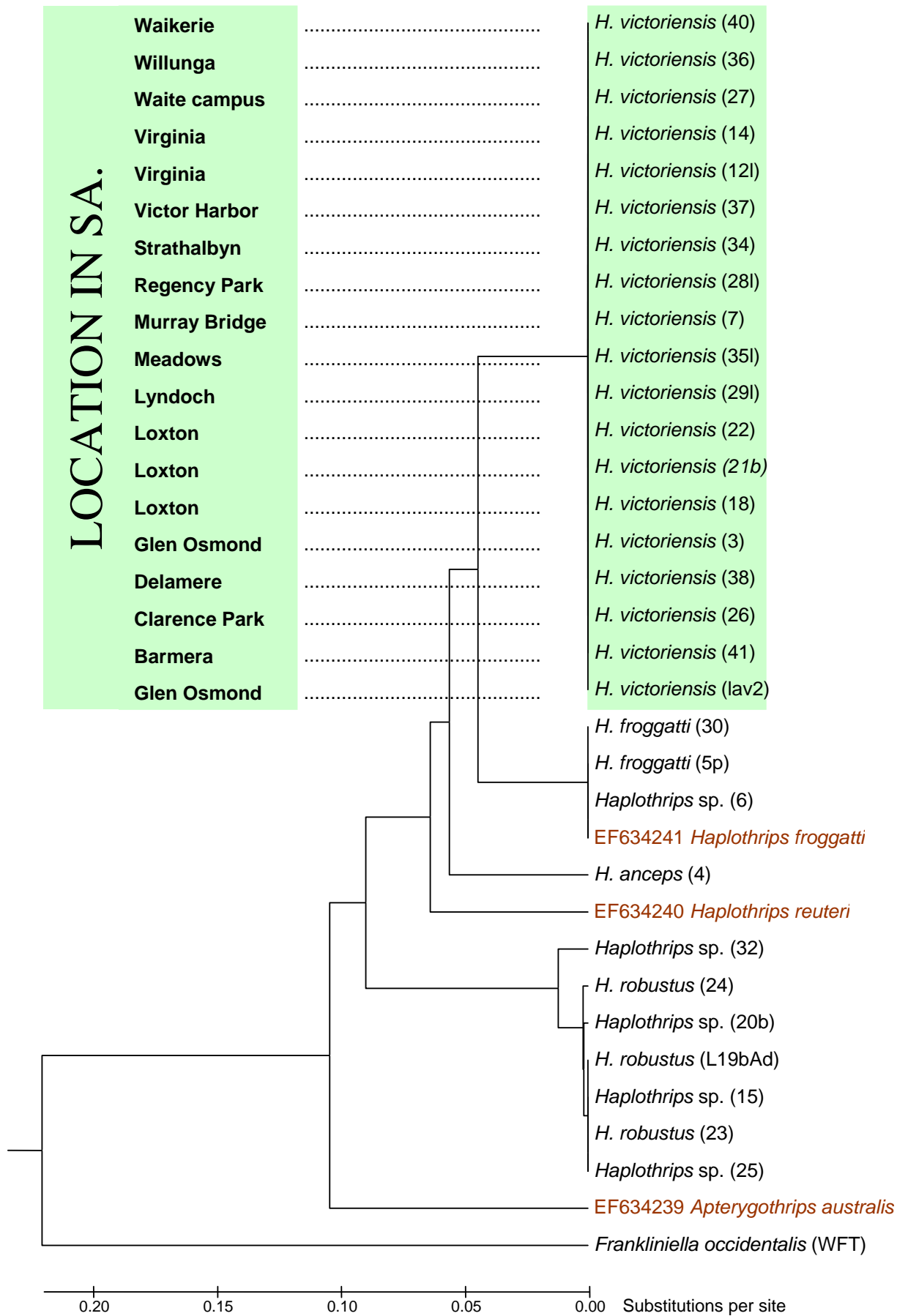
Figure 5.8. Preliminary neighbour joining tree of *H. victoriensis* and some of its similar taxa in SA.

5.4. Discussion

This research contributes additional information about the distribution and host plants of *H. victoriensis* in South Australia. According to previous work, *H. victoriensis* was not only abundant in Adelaide on roses (Evans, 1933, 1935), in southern Australia (Steele, 1935b; CSIRO, 2006), but also, according to Pitkin (1973), the thrips was found in NSW, ACT and WA on *Chrysanthemum moniliferum* [Compositae], in Tasmania on *Olearia,* hazel and clover (E. J. Martyn, 1948, 1952, unpublished data from slide specimens). Given *H. victoriensis* is found on such a wide range of host plants (Table 5.2) it would suggest that these thrips occur in many parts of Australia.

Obviously, larvae of *H. victoriensis*, *H. anceps* and *H. robustus* can be readily separated from each other (see above). Depending on their morphology, distribution and host plants, we can initially separate the larvae of these three thrips species in the field. However, are the three known larvae similar to the larvae of other *Haplothrips* species? This is possibly the case but needs for more work to be certain, for example, *H. tritici* larvae appear similar to *H. victoriensis* larva (Fig. 5.9). *Haplothrips tritici* is a pest on wheat and very common in Serbia (Ljiljana *et al.*, 2008) and has not been recorded in Australia (Mound and Minaei, 2007). Abundant red thrips larvae similar to *H. victoriensis* have been observed on a wheat crop on the Mount Gambier (Judy Bellati, 2007 person. comm.), so it may be prudent to investigate these larvae to rule out an incursion of *H. tritici.*

In addition, there is a conflicting point in a key for common flower thrips larvae by Kirk (1987) and my obsevations on *Haplothrips* larval morphology. In the step 1 and 2 of the key to thrips larvae of Kirk (1987), thrips larvae of Phlaeothripidae have only red body in general. If thrips larval bodies appear from white to yellowish orange,

they are not Phlaeothripidae species and belong to other Thysanoptera families whereas I have consistently observed that larvae of *H. robustus* (Thysanoptera: Phlaeothripidae) appears in yellow colour. Therefore, it appears that to build a key to *Haplothrips* larvae is complicated since about 250 species have been recorded in this genus worldwide (Mound and Minaei, 2007). It may, however, be possible to construct a key to separate *Haplothrips* larvae for a particular geographic region, for example, South Australia.

NOTE:
This figure is included on page 92
of the print copy of the thesis held in
the University of Adelaide Library.

Figure 5.9. *Haplothrips tritici* on wheat grain. a: photo of P. Bielza (Lewis, 1997); b: photo from Agro Web Ukraine (2008).

From the phenomena of variable colour forms observed of larvae, pupae and adults of the three *Haplothrips* species (see Figs 3.3, 5.3, 5.4, 5.5, 5.6 and 5.7, for example), colour change of Terebrantia larva in my observations (yellow larvae, pink pupae and dark brown adult), and the yellow colour of larvae, pupae and adults of WFT (Fig. 5.10), two questions arise: why are WFT pupae and adults only yellow? and why do yellow parts on larval bodies of *H. anceps* and *H. robustus* change to

pink, light red or red colours in pupal stages before becoming dark brown or black

adults?. It may be that if adult thrips are dark brown or black, their prepupal and

pupal bodies usually contain pigments in a range of colour from pink to red.

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Figure 5.10. Life stages of WFT, from left to right: egg, instar I, instar II, instar III, instar IV and adults. (source from "Thrips as crop pests" Lewis. T. in 1997, photos by Jack K. Clark, Davis, California).

Having a complete exoskeleton remaining after DNA extraction can be an

advantage compared with other techniques. The Puregene protocol is similar to the

"salting-out" protocol in Rugman-Jones et al. (2006), where individual specimens

were pierced on one side of the abdomen by a sterilized minute pin before

performing the DNA extraction. The exoskeleton was kept intact and is suitable for

morphological examination, allowing taxonomic comparison of phenotypically similar

species.

The molecular results support the hypothesis that *H. victoriensis* and its similar

taxa can be separated by COI barcoding. With the results of molecular analysis (Fig.

5.8), possible primers, including about 20 nucleotides, can be designed in order to

separate *H. victoriensis, H. froggatti, H. anceps,* and *H. robustus. Haplothrips*

victoriensis, *H. anceps* and *H. robustus* are very difficult to distinguish from each other in the key of Pitkin (1973). *Haplothrips victoriensis* and *H. anceps* have remain difficult to identify taxonomically, especially, since the male genitalia are similar (Mound and Minaei, 2007), whereas these thrips clearly separated into three clades in the phylogenetic tree (Fig. 5.8). It would be worldwide obtaining COI sequences of all 24 Australian *Haplothrips* species (Mound and Minaei, 2007) as the final result may be different. It would appear possible to create primers for separating Australian *Haplothrips* based on COI to quickly identify the taxa.

The phylogenetic tree (Fig. 5.8) shows that three clades of *H. victoriensis*, *H. froggatti* and *H. anceps* appear to be closely related but *H. reuteri* and *H. robustus* are more basal. This is also the case with the morphology of these species with *victoriensis, froggatti* and *anceps* key very similar (Pitkin, 1973; Mound and Minaei, 2007). Therefore, genotype can partly support phenotype in terms of explaining relationships within this group. However, in close examination, *H. victoriensis* is more similar to *H. anceps* than *H. froggatti* in terms of morphology (Mound and Minaei, 2007), but *H. victoriensis* appears to have a closer relationship with *H. froggatti* than *H. anceps* in the COI tree.

Nineteen COI sequences of *H. victoriensis* were arranged in the same clade in the phylogenetic tree (Fig. 5.8). These specimens were collected at many sites in the agricultural areas from Victor Harbor, Delamere and Strathalbyn (Fleurieu Peninsula), Clarence Park, Glen Osmond and Regency Park (metropolitan Adelaide), Virginia (north of Adelaide), and Lyndoch (Barossa Valley), Murray Bridge, Barmera, Waikerie and Loxton (Murray basin, Riverland/ Murraylands) (Figs 5.2 & 5.8). The lack of any genotypic variation in *H. victoriensis* in SA may be due to cultural activities of humans. *Haplothrips victoriensis* is usually abundant on a range of plants such as

rose and lucerne, and these plants are commonly grown in SA. Growers may have unintentionally moved and dispersed *H. victoriensis* to the various horticultural areas.

In addition, *H. victoriensis* is a predator of a range of species belonging to different orders (see Chapter 4) so it appears well adapted to settle in a range of different areas where prey is probably already present. However, only a preliminary survey of some horticultural areas of SA was conducted, so whether *H. victoriensis* also occurs in other areas in SA, and whether this lack of genetic variation occurs in other regions such as in Victoria, Western Australia or Tasmania, needs to be investigated.

In contrast with *H. victoriensis*, *H. robustus* shows some genetic variation (Fig. 5.8) although it must be stressed that only several specimens were analysed. This apparent variation of *H. robustus* needs to be further examined across many sites in Australia to see whether it is a species complex or not, but as the number of substitutions per site is quite small, this probably indicates a small amount of genetic variation with *H. robustus* rather than a species complex.

Some *Haplothrips* specimens such as species 6, 32, 20b, 15 and 25 (Fig. 5.8) were not able to be determined on a morphological basis. However, they fall out within defined clades in the tree. *Haplothrips* sp. 6 is clearly *H. froggatti* because it shows no difference from the rest of the *H. froggatti*. Although the *H. robustus* clade is variable, *Haplothrips* sp. 20b, 15 and 25 appear likely to be *H. robustus*. *Haplothrips* sp. 32 falls out basally to the *robustus* clade so this species may be either *H. robustus*, an already described species closely related to *H. robustus*, or could be a new species. However, although testing would be necessary to determine this, the relatively small difference in substitutions per site would suggest it is *H. robustus.*

The phylogenetic tree is essentially still extremely preliminary. Therefore, further studies need to be conducted to examine phylogenetic relationship among Australian *Haplothrips* taxa. These could use genes such as COII (Avise *et al.*, 1987; Bernasconi *et al.*, 2000), ITS1, ITS2 (Rugman-Jones *et al.*, 2006), 16S (Dowton *et al.*, 1998), 18S (Gimeno *et al.*, 1997; Inoue and Sakurai, 2007), and CAD (Mukae *et al.*, 2002; Moulton and Wiegmann, 2004; Danforth *et al.*, 2006; Scheffer *et al.*, 2007).

CHAPTER 6

GENERAL CONCLUSIONS AND

FUTURE RESEARCH

CHAPTER 6

General Conclusions and Future Research

The project attempted to investigate biological aspects of *H. victoriensis* in SA, with the view to examine its potential as a biological control agent and the results are presented in the four main chapters (2-5) which addressed several main objectives:

- Can *H. victoriensis* be reared in the laboratory for biological observations and experiments? (Chapter 2)

- What are the life history and developmental stages of *H. victoriensis*? (Chapter 3)

- What is the potential of *H. victoriensis* as a biological control agent (predator) on thrips and other common horticultural pests in SA? (Chapter 4)

- What are the distribution and host plants of *H. victoriensis* in SA (Chapter 5)?

- Can *H. victoriensis* and its morphologically close taxa be easily separated from each other based on larval morphology and/or DNA barcoding? (Chapter 5)

Haplothrips victoriensis was reared in several different types of cages and its prey was also cultured in the laboratory for use as a food source. Rearing cages were designed and applied specifically for this project. The Petri dish cage was designed to rear *H. victoriensis* larvae for biological observations. Western flower thrips (WFT) adults were also reared to collect a large number of WFT eggs. These designs could readily be applied to future studies on this and other thrips species.

Haplothrips victoriensis takes 16-22 days to develop from egg to adult when fed with honey, mixed pollen and *Tyrophagus* mites at 25°C and 16L:8D. Sixteen developmental patterns of *H. victoriensis* were recorded in clearly striking images. Its development and longevity are significantly different when fed with different food

sources. It was also found that *H. victoriensis* may be an obligate predator, although further experimentation is required to confirm this.

Haplothrips victoriensis can feed on a range of different prey, most of which are serious horticultural pests in SA. It is a predator of TSM, *Tyrophagus* mites, WFT, plague thrips, alfalfa aphid, greenhouse whitefly and diamondback moth but not on ash whitefly. *Haplothrips victoriensis* shows high potential for biological control not only of thrips pests but also of other horticultural pests. Another species, *Haplothrips brevitubus* has also been suggested as having good potential as a predator of the mulberry thrips, *Pseudodendrothrips mori* in Japan (Kakimoto *et al.*, 2006).

The cannibalistic behaviour of *H. victoriensis* needs to be considered when it commonly appears in *H. victoriensis* populations because it can be become a predator of other pests or also preys on each other. This is a challenge for both its use as a biological control agent and for the population itself. However, it could be that the cannibalistic behaviour occurs less in the field where flowers and leaves can provide hiding places for *H. victoriensis* larvae. The cannibalistic behaviour may also help to explain why *H. victoriensis* populations dramatically fluctuate in the field. When its population increases to a very high level, the number of *H. victoriensis* may decrease by cannibalism.

The preliminary field survey, although confined essentially to the horticultural areas of South Australia, contributes to further knowledge of the distribution and range of plant hosts of *H. victoriensis*. The thrips mainly live in flowers in which their structure is usually complex with many petals, stamens and hairs at the base of the flowers (e.g. roses, gazania, and eucalypt) or many small flowers on one branch (e.g. lucerne, canola and coastal bearded heath). The thrips can be easily found on those flowers in Spring and Summer.

Haplothrips victoriensis, H. anceps, and *H. robustus* can be separated from each other based on the morphology of the first and second instar larvae but their prepupae and pupae are very similar. Moreover, on a worldwide basis, larval morphologies of other *Haplothrips* species probably also show little variation. It would be worthwhile to attempt to construct a key for larval *Haplothrips* species worldwide.

COI barcoding of 24 *H. victoriensis* species was undertaken and it was found there was no variation in specimens from the major horticultural areas in South Australia. This situation may be similar in other areas of SA or other states where the thrips are found, however further studies need to be undertaken to further explore this (see in future research Section below). The three thrips species were clearly separated into three separate clades in the phylogenetic tree (Fig. 5.8), so there is potential to be able to readily identify them based on COI barcoding with specifically designed primers.

However, there are further issues arising from this research which need to be considered in any future studies.

➢ *Biological aspects:*

- Although the developmental time and rate of immature of *H. victoriensis* were investigated when the thrips was reared with snapdragon stamens at a range of temperatures (12, 16.5, 18.7, 20.8, 22.5 and 26°C) by Andrewartha (1936) and with different food sources (see Chapter 3), in this research, the observations and experiments were conducted at a constant 25°C, 65% RH and 16L:8D. The effect of a wider range of temperatures, different humidities and lighting regimes on the development, fecundity and longevity of *H. victoriensis* should be investigated. These results will contribute further to the biology and the potential for mass rearing of *H. victoriensis.*

- Although suitable in these experiments, further investigation of the "correct" pollen and honey diet could be explored, especially as it relates to any further work on mass rearing this thrips.

- Further food selection trials of *H. victoriensis* on a wider range of potential prey need to be undertaken in order to determine appropriate prey for rearing *H. victoriensis* and possibly the potential for biological control of a wider range of pest species. It would also be useful to determine the impact of this thrips on other insects, especially beneficial insects, which, of course, will be essential before any biological control program could be implemented.

- Food sources including *Tyrophagus* mites, WFT eggs, two-spotted mite eggs, lucerne flowers, lucerne anthers, peach fruits, and snapdragon stamens have been found to be variously effective in the past. However, are there other food sources that may be applicable?

- At this stage, I have not attempted to mass rear *H. victoriensis* in the sorts of numbers that would be required for biological control experiments in either glasshouses or the field. Further research on mass rearing is therefore essential.

- The capabilities of *H. victoriensis* for pest control in greenhouses and in the field need to be evaluated for potential biological control. The pest species would include WFT, TSM, greenhouse whitefly or diamondback moth, but should be broadened to include other common pests.

- The cannibalistic capabilities of *H. victoriensis* need to be further explored. We would more likely have a better understanding of this characteristic of *H. victoriensis* in a more natural environment.

- Cannibalism of *H. anceps* was also found in this study, so it too may be a predator of other thrips species. Therefore, the predatory characteristics of *H. anceps* should be further explored. The results of this project suggest that the cannibalism may occur (from common or rare) with thrips which are recorded as predators, including *T. imaginis, T. tabaci* and *Frankliniella schultzei* (Wilson *et al.*, 1996). That is, there are other predatory thrips that could be examined for cannibalism. Furthermore, these results may also contribute to an explanation for the dramatic fluctuations in the populations of thrips in the field which has been remained a big question (Funderburk, 2002).

- The fecundity of *H. victoriensis* has not yet been investigated. The impact of sex ratios in the laboratory, based on what occurs in the field, could also be investigated, with a series of experiments to determine the appropriate ratio for the parental generation. However, *Haplothrips* males and females are very similar in appearance so distinguishing them easily in the laboratory will be essential.

➢ *Larval morphologies and COI barcoding:*

- Although there is no variation in *H. victoriensis* populations in SA, the variation of *H. victoriensis* and its morphological similar taxa in other areas in SA and in Victoria, WA and Tasmania. Other than COI, other that could used to examine variation include COII, ITS1, ITS2, 16S, 18S and CAD.

- A molecular phylogeny of the 24 *Haplothrips* species in Australia should be attempted using COI and other genes such as CAD, and primers designed for rapid taxonomic identification. This would, in conjunction with a morphological phylogeny, allow for confirmation or otherwise of the status of the taxa involved, determine if there are as yet unidentified species and perhaps

answer various biogeographic and taxonomic questions. This approach could also be expanded to examine *Haplothrips* on a worldwide basis.

CHAPTER 7

REFERENCES

References

Andrewartha, H. G. and Kilpatrick, D. T. (1951) The apple thrips. *Journal of Department Agriculture, South Australia* 54 (12): 586-592; 595.

Andrewartha, H. V. (1936) Thrips Investigation. 8. The influence of temperature on the rate of development of the immature stages of *Thrips imaginis* Bagnall and *Haplothrips victoriensis* Bagnall. *Journal of Council for Scientific Industrial Research, Australia* 9 (1): 57-64 pp.

Avise, J. C., Arnold, J., Ball, R. M., Bermingham, E., Lamb, T., Neigel, J. E., Reeb, C. A. and Saunders, N. C. (1987) Intraspecific phylogeography: the mitochondrial DNA bridge between population genetics and systematics. *Annual Review of Ecology and Systematics* 18: 489-522.

Bagnall, R. S. (1918) Brief descriptions of new Thysanoptera. -IX. *The Annals and Magazine of Natural History - ninth series* 1 (3): 201-221.

Bailey, P. and Caon, G. (1986) Predation on two-spotted mite, *Tetranychus urticae* Koch (Acarina, Tetranychidae) by *Haplothrips victoriensis* Bagnall (Thysanoptera, Phlaeothripidae) and *Stethorus nigripes* Kapur (Coleoptera, Coccinellidae) on seed lucerne crops in South-Australia. *Australian Journal of Zoology* 34 (4): 515-525.

Baker, G., Keller, M., MacGregor, A., Jackman, D. and Purvis, S. (2000) *Development of an integrated pest management system for thrips in citrus: Summary, conclusions and recommendations of the 1997-2000 Kelly's citrus thrips research project.* SARDI, Adelaide University & Victorian DNRE, [accessed 27 July 2006]. Available from http://www.sardi.sa.gov.au/pages/ento/hort_pests/kct_report97_00.htm:sectID=960&tempID=1.

Bellows, T. S. (2001) Restoring population balance through natural enemy introductions. *Biological Control* 21 (3): 199-205.

Bernasconi, M. V., Valsangiacomo, C., Piffaretti, J. C. and Ward, P. I. (2000) Phylogenetic relationships among Muscoidea (Diptera : Calyptratae) based on mitochondrial DNA sequences. *Insect Molecular Biology* 9 (1): 67-74.

Beshear, R. J. (1983) New records of thrips in Georgia (Thysanoptera, Terebrantia, Tubulifera). *Journal of the Georgia Entomological Society* 18 (3): 342-344.

Cate, J. R. and Hinkle, M. K. (1994) *Integrated pest management: the path of a paradigm*. Washington D.C.: The National Audubon Society.

Childers, C. C. (1997) Feeding and oviposition injuries to plants. In *Thrips as crop pests*, ed. by Lewis, T. pp 505-537. U.K.: CAB International.

CSIRO. (2006a) *Common names* CSIRO, [accessed 12 March 2006]. Available from http://www.ento.csiro.au/aicn/name_c/a_4466.htm.

CSIRO. (2006b) *Haplothrips victoriensis Bagnall* CSIRO, 19 September 2004 [accessed 28 Feb 2008]. Available from http://www.ento.csiro.au/aicn/name_s/b_1849.htm.

Danforth, B. N., Fang, J. and Sipes, S. (2006) Analysis of family-level relationships in bees (Hymenoptera : Apiformes) using 28S and two previously unexplored nuclear genes: CAD and RNA polymerase II. *Molecular Phylogenetics and Evolution* 39 (2): 358-372.

Daughtrey, M. L., Wick, R. L. and Peterson, J. L. (1995) *Compendium of Flowering Plant Diseases*. St. Paul, MN: APS Press.

Davidson, J. and Andrewartha, H. G. (1948) Annual trends in a natural population of *Thrips imaginis* (Thysanoptera). *The Journal of Animal Ecology* 17 (2): 193-199.

Davis, D. W. and McMurtry, J. A. (1979) Introduction. p. I. In *Biological control and insect pest management*, ed. by Davis, D. W., Hoyt, S. C., McMurtry, J. A. and AliNiazee, M. T. pp 102. Berkeley: Agricultural Experiment Station, University of California, Division of Agriculture and Natural Resources

Dowton, M., Austin, A. D. and Antolin, M. F. (1998) Evolutionary relationships among the Braconidae (Hymenoptera: Ichneumonoidea) inferred from partial 16S rDNA gene sequences. *Insect Molecular Biology* 7 (2): 129-150.

Driesche, R. V. (1998) *Western flower thrips in greenhouses: A review of its biological control and other methods* Department of Entomology, University of Massachusetts, Amherst, MA 01003 USA, [accessed April 20 2006]. Available from http://www.biocontrol.ucr.edu/WFT.html.

Evans, J. W. (1933) Thrips investigation. I. the seasonal fluctuations in numbers of *Thrips imaginis* Bagnall and associated blossom thrips. *Journal of Council for Scientific Industrial Research Australia* 6 (3): 145-159.

Evans, J. W. (1935) Thrips investigation. 6. Further observations on the seasonal fluctuations in numbers of *Thrips imaginis* Bagnall and associated blossom thrips. *Journal of Council for Scientific Industrial Research Australia* 8 (2): 86-92.

Flint, M. L. and Van Den Bosch, W. (1981) *Introduction to integrated pest management*. New York: Plenum Press.

Folmer, O., Black, M., Hoeh, W., Lutz, R. and Vrijenhoek, R. (1994) DNA primers for amplification of mitochondrial cytochrome c oxidase subunit I from diverse metazoan invertebrates. *Molecular Marine Biology and Biotechnology* 3 (5): 294-299.

Funderburk, J. E. (2002) Ecology of thrips. In *Thrips and Tospoviruses: Proceedings of the 7th International Congress on Thysanoptera*, ed. by Marullo, R. and Mound, L. A. pp 121-128. Canberra, Australia: CSIRO Entomology.

Biological aspects of Haplothrips victoriensis in SA

Gimeno, C., Belshaw, R. and Quicke, D. L. J. (1997) Phylogenetic relationships of the Alysiinae/Opiinae (Hymenoptera: Braconidae) and the utility of cytochrome b, 16S and 28S D2 rRNA. *Insect Molecular Biology* 6 (3): 273-284.

Goodwin, S. and Steiner, M. Y. (1996) Survey of Australian native natural enemies for control of thrips. *Bulletin IOBC/WPRS* 19 (1): 47-50.

Hajek, A. E. (2004) *Natural enemies: an introduction to biological control.* UK: Cambridge University Press.

Hall, T. A. (1999) BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. *Nucleic Acids Symposium Series* 41: 95-98.

Harrison, R. G. (1989) Animal mitochondrial DNA as a genetic marker in population and evolutionary biology. *Trends in Ecology and Evolution* 4 (1): 6-11.

Hulshof, J., Ketoja, E. and Vanninen, I. (2003) Life history characteristics of *Frankliniella occidentalis* on cucumber leaves with and without supplemental food. *Entomologia Experimentalis et Applicata* 108 (1): 19-32.

Inoue, T. and Sakurai, T. (2007) The phylogeny of thrips (Thysanoptera : Thripidae) based on partial sequences of cytochrome oxidase I, 28S ribosomal DNA and elongation factor-1 alpha and the association with vector competence of tospoviruses. *Applied Entomology and Zoology* 42 (1): 71-81.

Jenser, G., Gaborjanyi, R., Szenasi, A., Almasi, A. and Grasselli, M. (2003) Significance of hibernated *Thrips tabaci* Lindeman (Thysanoptera, Thripidae) adults in the epidemic of tomato spotted wilt virus. *Zeitschrift Fur Angewandte Entomologie* 127 (1): 7-11.

Kakimoto, K., Inoue, H., Hinomoto, N., Noda, T., Hirano, K., Kashio, T., Kusigemati, K. and Okajima, S. (2006) Potential of *Haplothrips brevitubus* (Karny) (Thysanoptera: Phlaeothripidae) as a predator of mulberry thrips *Pseudodendrothrips mori* (Niwa) (Thysanoptera: Thripidae). *Biological Control* 37 (3): 314-319.

Kelly, R. and Mayne, R. J. B. (1934) *The Australian thrips.* Sydney, N.S.W.: Australian Medical Publishing Company Limited.

Kirk, W. D. J. (1984) Ecological studies on *Thrips imaginis* Bagnall (Thysanoptera) in flowers of *Echium plantagineum* L. in Australia. *Australian Journal of Ecology* 9 (1): 9-18.

Kirk, W. D. J. (1985) Aggregation and mating of thrips in flowers of *Calystegia sepium. Ecological Entomology* 10 (4): 433-440.

Kirk, W. D. J. (1987a) A Key to the larvae of some common Australian flower thrips (Insecta, Thysanoptera), with a host plant survey. *Australian Journal of Zoology* 35 (2): 173-185.

Kirk, W. D. J. (1987b) Effects of Trap size and scent on satches of *Thrips imaginis* Bagnall (Thysanoptera, Thripidae). *Journal of the Australian Entomological Society* 26: 299-302.

Kirk, W. D. J. (1997) Feeding. In *Thrips as crop pests*, ed. by Lewis, T. pp 119-174. U.K.: CAB International.

Kirk, W. D. J. and Terry, L. I. (2003) The spread of the western flower thrips *Frankliniella occidentalis* (Pergande). *Agricultural and Forest Entomology* 5 (4): 301-310.

Kumar, S., Tamura, K. and Nei, M. (2004) MEGA3: Integrated software for molecular evolutionary genetics analysis and sequence alignment. *Briefings in Bioinformatics* 5 (2): 150-163.

Lewis, T. (1973) *Thrips: their biology, ecology and economic importance*. London: Academic Press.

Lewis, T. (1997a) Pest thrips in perspective. In *Thrips as crop pests*, ed. by Lewis, T. pp 1-13. U.K.: CAB International.

Lewis, T. (1997b) Chemical control. In *Thrips as crop pests*, ed. by Lewis, T. pp 567-593. U.K.: CAB International.

Ljiljana, A., Radoslava, S. and Milenko, D. (2008) *Thrips from coloured water traps in Serbian wheat fields* [accessed 22 March 2008]. Available from http://www.ento.csiro.au/thysanoptera/Symposium/Section9/51-Andjus-et-al.pdf.

Loomans, A. J. M. and Murai, T. (1997) Culturing thrips and parasitoids. In *Thrips as crop pests*, ed. by Lewis, T. pp 477-503. U.K.: CAB International.

Loomans, A. J. M., Murai, T. and Greene, I. D. (1997) Interactions with hymenopterous parasitoids and parasitic nematodes. In *Thrips as crop pests*, ed. by Lewis, T. pp 355-397. U.K.: CAB International.

Lunt, D. H., Zhang, D. X., Szymura, J. M. and Hewitt, G. M. (1996) The insect cytochrome oxidase I gene: evolutionary patterns and conserved primers for phylogenetic studies. *Insect Molecular Biology* 5 (3): 153-165.

Macphee, A. W. (1953) The influence of spray programs on the fauna of apple orchards in Nova Scotia: V. The predacious thrips *Haplothrips faurei* Hood. *Canadian Entomologist* 85: 33-40.

Malipatil, M. B., Postle, A. C., Osmelak, J. A., Hill, M. and Moran, J. (1993) First record of *Frankliniella occidentalis* (Pergande) in Australia (Thysaoptera: Thripidae). *Journal of Australian Entomological Society* 32: 378.

Milne, M. and Walter, G. H. (1997) The significance of prey in the diet of the phytophagous thrips, *Frankliniella schultzei*. *Ecological Entomology* 22 (1): 74-81.

Moritz, G. (1997) Structure, growth and development. In *Thrips as crop pests*, ed. by Lewis, T. pp 15-63. U.K.: CAB International.

Moritz, G., Morris, D. C. and Mound, L. A. (2001) (ThripsID - Pest thrips of the world) [An interative identification and information system]. Cd-rom published for ACIAR by CSIRO Publishing, [accessed 2006]. Available from http://www.publish.csiro.au/pid/3024.htm.

Moritz, G., Delker, C., Paulsen, M., Mound, L. A. and Burgermeister, W. (2000) Modern methods for identification of Thysanoptera. *Bulletin OEPP* 30 (3-4): 591-593.

Moritz, G., Paulsen, M., Delker, C., Picl, S. and Kumm, S. (2002) Identification of thrips using ITS-RFLP analysis. *Thrips and Tospoviruses: Proceedings of the 7th international symposium on Thysanoptera*: 365-367.

Morris, D. C. and Mound, L. A. (2004) Molecular relationships between populations of South African citrus thrips (*Scirtothrips aurantii* Faure) in South Africa and Queensland, Australia. *Australian Journal of Entomology* 43: 353-358.

Moulton, J. K. and Wiegmann, B. M. (2004) Evolution and phylogenetic utility of CAD (rudimentary) among Mesozoic-aged Eremoneuran Diptera (Insecta). *Molecular Phylogenetics and Evolution* 31 (1): 363-378.

Mound LA (1968) A review of R.S. Bagnall's Thysanoptera collections. *Bulletin of the British Museum (Natural History)* Entomology. 11 : 1–181.

Mound, L. A. (1971) Gall-forming thrips and allied species (Thysanoptera: Phlaeothripinae) from Acacia trees in Australia. *Bulletin of the British Museum (Natural History) Entomology* 25: 387-460.

Mound, L. A. (1972) Species complexes and the generic classification of leaf-litter thrips of the Tribe Urothripini (Phlaeothripidae). *Australian Journal of Zoology* 20: 83-103.

Mound, L. A. (1996) Thysanoptera. In *Zoological Catalogue of Australia*, ed. by Wells, A. pp 249–336, 397–414 (Index). Melbourne, Australia: CSIRO Australia.

Mound, L.A. (2002) So many thrips–so few tospoviruses? In: Marullo, R., Mound, L.A. (Eds.), *Thrips and Tospoviruses. Proceedings of the 7th International Symposium on Thysanoptera. Australian National Insect Collection*, Canberra 15–18.

Mound, L. A. (2004a) Australian long-tailed gall thrips (Thysanoptera : Phlaeothripinae, Leeuweniini), with comments on related Old World taxa. *Australian Journal of Entomology* 43: 28-37.

Mound, L. A. (2004b) Australian Thysanoptera - biological diversity and a diversity of studies. *Australian Journal of Entomology* 43 (3): 248-257.

Mound, L. A. (2006) *CSIRO entomology - Thysanoptera (Thrips) of the world checklist* 10/07/2006 [accessed 08/10/2007]. Available from http://www.ento.csiro.au/thysanoptera/worldthrips.html.

Mound, L. A. and Marullo, R. (1996) *The thrips of Central and South America: an introduction.* Vol. 6: Memoirs on Entomology, International.

Mound, L. A. and Kibby, G. (1998) *Thysanoptera: An identification guide.* 2nd ed. Wallingford, Oxon UK.

Mound, L. A. and Jackman, D. (1998) Thrips in the ecomomy and ecology of Australia. *Proceedings of the Sixth Australian Applied Entomological Research Conference* 1: 472-478.

Mound, L. A. and Marullo, R. (1998) Biology and identification of Aeolothripidae (Thysanoptera) in Australia. *Invertebrate Taxonomy* 12 (6): 929-950.

Mound, L. A. and Morris, D. C. (2001) Domicile constructing phlaeothripine Thysanoptera from Acacia phyllodes in Australia: *Dunatothrips* Moulton and *Sartrithrips* gen.n., with a key to associated genera. *Systematic Entomology* 26 (4): 401-419.

Mound L. A. & Masumoto M. (2005) The genus Thrips (Thysanoptera, Thripidae) in Australia, New Caledonia and New Zealand. Zt. 1020 : 1–64.

Mound, L. A. and Tree, D. J. (2007) Oriental and Pacific Thripidae (Thysanoptera) new to Australia, with a new species of *Pseudodendrothrips schmutz*. *Australian Entomologist* 34 (1): 7-14.

Mound, L. A. and Morris, D. C. (2007) The insect Order Thysanoptera: classification versus systematics. *Zootaxa* 1668: 395-411.

Mound, L. A. and Minaei, K. (2007) Australian insects of the *Haplothrips* lineage (Thysanoptera – Phlaeothripinae). *Journal of Natural History* 41: 2919-2978.

Mound, L. A., Heming, B. S. and Palmer, J. M. (1980) Phylogenetic relationships between the families of recent Thysanoptera (Insecta). *Zoological Journal of the Linnean Society* 69 (2): 111-141.

Mukae, N., Yokoyama, H., Yokokura, T., Sakoyama, Y. and Nagata, S. (2002) Activation of the innate immunity in *Drosophila* by endogenous chromosomal DNA that escaped apoptotic degradation. *Genes & Development* 16 (20): 2662-2671.

Murai, T. (2001) Development and reproductive capacity of *Thrips hawaiiensis* (Thysanoptera : Thripidae) and its potential as a major pest. *Bulletin of Entomological Research* 91 (3): 193-198.

Murai, T. and Loomans, A. J. M. (2001) Evaluation of an improved method for mass-rearing of thrips and a thrips parasitoid. *Entomologia Experimentalis et Applicata* 101 (3): 281-289.

Nakahara S. (1994) The genus Thrips Linnaeus (Thysanoptera: Thripidae) of the New World. *Technical Bulletin.* United States Department of Agriculture 1822: 1–183.

Paliwal, Y. C. (1976) Some characteristics of Thrip vector relationship of tomato spotted wilt virus in Canada. *Canadian Journal of Botany-Revue Canadienne De Botanique* 54 (5-6): 402-405.

Pallant, J. (2007) *SPSS Survival Manual: A step-by-step guide to data analysis using SPSS for Windows (Version 15).* 3rd ed. NSW: Allen & Unwin.

Palmer, J. M., Mound, L. A. and du Heaume, G. J. (1989) *CIE guides to insects of importance to man. 2. Thysanoptera.* Edited by Betts, C. R. London: CAB International Institute of Entomology and British Museum (Natural History).

Parrella, M. P. (1996) Introduction: western flower thrips - identification, biology and research on the development of control strategies. Paper read at Proceedings for the Twelfth Conference on Insect Management on Ornamentals, 17-19 Feb, at San Francisco.

Penton, E. H. and Crease, T. J. (2003) *DNA barcoding animal life COI* Barcodes of life, [accessed 8 June 2006]. Available from http://www.barcodeoflife.org/primer/Lab_Protocols.html.

Pitkin, B. R. (1973) A revision of the Australia Haplothripini, with descriptions of three new species (Thysanoptera: Phlaeothripidae). *Journal of the Australian Entomological Society* 12 (4): 315-339.

Rugman-Jones, P. F., Hoddle, M. S., Mound, L. A. and Stouthamer, R. (2006) Molecular identification key for pest species of Scirtothrips (Thysanoptera : Thripidae). *Journal of Economic Entomology* 99 (5): 1813-1819.

Sabelis, M. W. and Rijn, P. C. J. v. (1997) Predation by insects and mites. In *Thrips as crop pests,* ed. by Lewis, T. pp U.K.: CAB International.

Sakimura, K. (1963) *Frankliniella fusca,* an additional vector for the Tomato Spotted Wilt Virus, with notes on *Thrips tabaci* another vector. *Phytopathology* 53: 412–415.

Scheffer, S. J., Winkler, I. S. and Wiegmann, B. M. (2007) Phylogenetic relationships within the leaf-mining flies (Diptera : Agromyzidae) inferred from sequence data from multiple genes. *Molecular Phylogenetics and Evolution* 42 (3): 756-775.

Steele, H. V. (1935) *Thrips investigation: Some common Thysanoptera in Australia.* Melbourne: Council for scientific and industrial research.

Steiner, M. Y. and Goodwin, S. (1998) Methods for collecting and rearing thrips (Thysanoptera) and their natural enemies. *Australian Journal of Entomology* 37 (2): 101-106.

Steiner, M. Y. and Goodwin, S. (2005) Management of thrips (Thysanoptera : Thripidae) in Australian strawberry crops: within-plant distribution characteristics and action thresholds. *Australian Journal of Entomology* 44: 175-185.

Ullman, D. E., Cho, J. J., Mau, R. F. L., Westcot, D. M. and Custer, D. M. (1992) A midgut barrier to tomato spotted wilt virus acquisition by adult western flower thrips. *Phytopathology* 82 (11): 1333-1342.

Ullman, D.E. (1996) Thrips and Tospoviruses: advances and future directions. Acta Hortic 431: 310–324.

University of Massachusetts. (2003) *Pest management: Western flower thrips and Tospoviruses* University of Massachusetts, [accessed Feb 23 2006]. Available from http://www.umass.edu/umext/floriculture/fact_sheets/pest_management/wft_03.html.

Vassiliou, V. A. (2007) Chemical control of *Pezothrips kellyanus* (Thysanoptera : Thripidae) in citrus plantations in Cyprus. *Crop Protection* 26 (10): 1579-1584.

Walsh, K., Boonham, N., Barker, I. and Collins, D. W. (2005) Development of a sequence-specific real-time PCR to the melon thrips *Thrips palmi* (Thysanoptera: Thripidae). *Journal of Applied Entomology* 129 (5): 272-279.

Watson, D. M., Du, T. Y., Li, M., Xiong, J. J., Liu, D. G., Huang, M. D., Rae, D. J. and Beattie, G. A. C. (2000) The effect of two prey species, *Chrysomphalus aonidum* and *Corcyra cephalonica,* on the quality of the predatory thrips, *Aleurodothrips fasciapennis*, reared in the laboratory. *BioControl* 45 (1): 45-61.

Wikipedia-contributors. (2006) *DNA barcoding* Wikipedia, The Free Encyclopedia, 26 April 2006 [accessed 8 June 2006]. Available from http://en.wikipedia.org/w/index.php?title=DNA_barcoding&oldid=50182763.

Wilson, L. J., Bauer, L. R. and Walter, G. H. (1996) 'Phytophagous' thrips are facultative predators of twospotted spider mites (Acari: Tetranychidae) on cotton in Australia. *Bulletin of Entomological Research* 86 (3): 297-305.

Young, G. R. and Zhang, L. (2001) IPM of melon thrips, *Thrips palmi* Karny (Thysanoptera: Thripidae), on eggplant in the top end of the Northern Territory. *Technical Bulletin - Department of Primary Industry and Fisheries, Northern Territory of Australia* (No. 288): 101-111.

Yudin, L. S., Cho, J. J. and Mitchell, W. C. (1986) Host range of western flower thrips, *Frankliniella occidentalis* (Thysanoptera: Thripidae), with special references to *Leucaena glauca. Environmental Entomology* (15): 1292-1295.

Appendix 1

Statistical analyses for developmental rate of first instar larvae of
Haplothrips victoriensis

Table A. Descriptives

	N= no. of observations	Mean	Std. Deviation	Std. Error	Minimum	Maximum
A. Honey, pollen	10	0.1040	0.03658	0.01157	0.06	0.20
B. Honey, pollen & *Tyrophagus* mites	12	0.2575	0.04864	0.01404	0.20	0.33
C. Honey, pollen & WFT eggs	11	0.2773	0.05368	0.01619	0.20	0.33
Total	33	0.2176	0.08913	0.01552	0.06	0.33

Table B. Test of Homogeneity of Variances

Levene Statistic	df1	df2	Sig.
2.758	2	30	0.079

(P=0.079 > 0.05 so the variances of three treatments are not significantly different.)

Table C. Analysis of developmental rate variances of first instar larvae in three different food sources

ANOVA

	Sum of Squares	Df	Mean Square	F	Sig.
Between Groups	0.187	2	0.094	42.011	0.000
Within Groups	0.067	30	0.002		
Total	0.254	32			

(They are 99% significantly different (P=0.000))

Table D. Homogeneous Subset comparisons using Tukey HSD test indicate the significant differences of each pair groups at p=0.05 level.

Food sources		Subset for alpha = 0.05	
	N	2	1
A. Honey, pollen	10	0.1040	
B. Honey, pollen & *Tyrophagus* mites	12		0.2575
C. Honey, pollen & WFT eggs	11		0.2773
Sig.		1.000	0.595

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 10.939.
b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
(Developmental rate of first instar larvae in group A is significantly lower than that in group B and C at 0.05 levels.)

Appendix 2

Statistical analyses for developmental rate of second instar larvae of
Haplothrips victoriensis

Table A. Descriptives

	N = no. of observations	Mean	Std. Deviation	Std. Error	Minimum	Maximum
A. Honey, pollen	9	0.0589	0.01537	0.00512	0.04	0.09
B. Honey, pollen & *Tyrophagus* mites	9	0.1533	0.02179	0.00726	0.14	0.20
C. Honey, pollen & WFT eggs	11	0.1755	0.01809	0.00545	0.14	0.20
Total	29	0.1324	0.05409	0.01004	0.04	0.20

Table B. Test of Homogeneity of Variances

Levene Statistic	df1	df2	Sig.
0.743	2	26	0.486

(The variances of three treatments are not significantly different with P=0.486 > 0.05.)

Table C. Analysis of developmental rate variances of second instar larvae in three different food sources

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.073	2	0.036	105.852	0.000
Within Groups	0.009	26	0.000		
Total	0.082	28			

(They are 99% significantly different (P=0.000))

Table D. Homogeneous Subset comparisons using Tukey HSD test indicate the significant differences of each pair groups at p=0.05 level.

		Subset for alpha = 0.05		
Food sources	N	2	3	1
A. Honey, pollen	9	0.0589		
B. Honey, pollen & *Tyrophagus* mites	9		0.1533	
C. Honey, pollen & WFT eggs	11			0.1755
Sig.		1.000	1.000	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 9.581.
b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
(These three groups are 95% significantly different in each pair. Developmental rate of second instar larvae is fastest for group C and lowest for group A.)

Table D. Homogeneous Subset comparisons using Tukey HSD test indicate the significant differences of each pair groups at p=0.01 level.

Food sources	N	Subset for alpha = 0.01		
		1	2	1
A. Honey, pollen	9	0.0589		
B. Honey, pollen & *Tyrophagus* mites	9			0.1533
C. Honey, pollen & WFT eggs	11			0.1755
Sig.			1.000	0.038

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 9.581.
b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
(Developmental rate of second instar larvae in group A is significantly lower than that in group B and C at 0.05 levels.)

Appendix 3

Statistical analyses for developmental rate of *Haplothrips victoriensis* in the pupal stage

Table A. Descriptives

	N = no. of observations	Mean	Std. Deviation	Std. Error	Minimum	Maximum
A. Honey, pollen	9	0.1800	0.02598	0.00866	0.14	0.20
B. Honey, pollen & *Tyrophagus* mites	11	0.2318	0.02523	0.00761	0.20	0.25
C. Honey, pollen & WFT eggs	11	0.2227	0.02611	0.00787	0.20	0.25
Total	31	0.2135	0.03332	0.00598	0.14	0.25

Table B. Test of Homogeneity of Variances

Levene Statistic	df1	df2	Sig.
0.325	2	28	0.725

(The variances of three treatments are not significantly different with P=0.725 > 0.05.)

Table C. Analysis of developmental rate variances of pupae in three different food sources
ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	0.015	2	0.007	11.096	0.000
Within Groups	0.019	28	0.001		
Total	0.033	30			

(They are 99% significantly different (P=0.000))

Table D. Homogeneous Subset comparisons using Tukey HSD test indicate the significant differences of each pair groups at p=0.05 level.

Food sources		Subset for alpha = 0.05	
	N	2	1
A. Honey, pollen	9	0.1800	
B. Honey, pollen & *Tyrophagus* mites	11		0.2227
C. Honey, pollen & WFT eggs	11		0.2318
Sig.		1.000	0.707

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 10.241.
b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
(Developmental rate of pupae in group A is significantly lower than that in group B and C at 0.05 levels.)

Appendix 4

Statistical analyses for developmental time of *Haplothrips victoriensis* in the immature stage

Table A. Descriptives

	N = no. of observations	Mean	Std. Deviation	Std. Error	Minimum	Maximum
A. Honey, pollen	9	35.3333	5.93717	1.97906	27	46
B. Honey, pollen & *Tyrophagus* mites	9	16.0000	1.11803	0.37268	14	17
C. Honey, pollen & WFT eggs	11	15.0909	1.30035	0.39207	14	18
Total	29	21.6552	9.91851	1.84182	14	46

Table B. Test of Homogeneity of Variances

Levene Statistic	df1	df2	Sig.
10.732	2	26	**0.000**

(The variances of three treatments are significantly different at P=0.000 < 0.05 so Non-parametric methods were used to statistically analyse this result.)

NON-PARAMETRIC METHODS

Kruskal-Wallis Test to indicate significant differences of ranks of three or more groups

Table C. Ranks of three different food sources

	Food sources	N	Mean Rank
Developmental time of immature thrips	**A.** Honey, pollen	9	25.00
	B. Honey, pollen & *Tyrophagus* mites	9	12.89
	C. Honey, pollen & WFT eggs	11	8.55
	Total	29	

Table D. Test Statistics(a,b): Analysis of ranks between three independent treatments of food sources

	Developmental time of immature thrips
Chi-Square	19.760
df	2
Asymp. Sig.	0.000

a Kruskal Wallis Test
b Grouping Variable: Food sources
(They are 99% significantly different (P=0.000))

Mann-Whitney Test to indicate significant differences of ranks of two groups

Table E. Ranks of treatment A and B

	Food sources	N	Mean Rank	Sum of Ranks
Developmental time of immature thrips	**A.** Honey, pollen	9	14.00	126.00
	B. Honey, pollen & *Tyrophagus* mites	9	5.00	45.00
	Total	18		

Table F. Test Statistics(b): Analysis of ranks between treatment A and B of food sources

	Developmental time of immature thrips
Mann-Whitney U	0.000
Wilcoxon W	45.000
Z	-3.599
Asymp. Sig. (2-tailed)	0.000
Exact Sig. [2*(1-tailed Sig.)]	0.000(a)

a Not corrected for ties.
b Grouping Variable: Food sources
(Haplothrips victoriensis larvae of the group A develop significantly longer than the larvae of group B at 99% confidence.)

Table G. Ranks of treatment B and C

	Food sources	N	Mean Rank	Sum of Ranks
Developmental time of immature thrips	**B.** Honey, pollen & *Tyrophagus* mites	9	12.89	116.00
	C. Honey, pollen & WFT eggs	11	8.55	94.00
	Total	20		

Table H: Test Statistics(b): Analysis of ranks between treatment B and C of food sources

	Developmental time of immature thrips
Mann-Whitney U	28.000
Wilcoxon W	94.000
Z	-1.696
Asymp. Sig. (2-tailed)	0.090
Exact Sig. [2*(1-tailed Sig.)]	0.112(a)

a Not corrected for ties.
b Grouping Variable: Food sources
(Developmental time of H. victoriensis larvae of both groups B and C is not significant difference with P=0.09>0.05.)

Appendix 5

Statistical analyses for effect of the addition of WFT to a basic diet on longevity of *Haplothrips victoriensis*

Table A. Statistical parameters of two experimental treatments

	Diets	N	Mean	Std. Deviation	Std. Error Mean
Longevity (days)	**A.** Honey & pollen	10	59.7000	13.93676	4.40719
	B. Honey, pollen & WFT larvae	6	109.6667	11.27239	4.60193

Table B. Test of Homogeneity of Variances for two independent treatments

		Levene's Test for Equality of Variances	
		F	Sig.
Longevity (days)	Equal variances assumed	0.072	0.792
	Equal variances not assumed		

(Their variances are not significant difference with P=0.792>0.05)

Table C. Indicating a statistically significant difference of two diet treatments by independent-samples t-test

t-test for Equality of Means						
t	df	Sig. (2-tailed)	Mean Difference	Std. Error Difference	99% Confidence Interval of the Difference	
Lower	Upper	Lower	Upper	Lower	Upper	Lower
-7.416	14	0.000	-49.96667	6.73786	-70.02421	-29.90912
-7.842	12.524	0.000	-49.96667	6.37190	-69.28248	-30.65085

(Haplothrips victoriensis feeding on honey and pollen has longevity significantly longer than the thrips feeding on the additional WFT with p=0.000)

Biological aspects of Haplothrips victoriensis in SA

Appendix 6

Statistical analyses for the consumption of predatory *H. victoriensis* on *Tyrophagus* mites

Table A. Descriptives

Treatment	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
T1. First instar larvae	11	2.55	0.820	0.247	1	4
T2. Second instar larvae	10	7.10	2.025	0.640	4	10
T3. Adult	9	5.22	2.539	0.846	2	9
Total	30	4.87	2.662	0.486	1	10

Table B. Test of Homogeneity of Variances

Levene Statistic	df1	df2	Sig.
4.748	2	27	0.017

(The variances of three treatments are significantly different at P=0.017 < 0.05 so Non-parametric methods were used to statistically analyse this result.)

NON-PARAMETRIC METHODS
Kruskal-Wallis Test to indicate significant differences of ranks of three or more groups

Table C. Ranks of three differently feeding stages of *H. victoriensis* on *Tyrophagus* mites

	Treatment	N	Mean Rank
Feeding on *Tyrophagus* mites	**T1**. First instar larvae	11	7.23
	T2. Second instar larvae	10	23.05
	T3. Adults	9	17.22
	Total	30	

Table D. Test Statistics(a,b): Analysis of ranks between three differently feeding stages on *Tyrophagus* mites

	Feeding on *Tyrophagus* mites
Chi-Square	17.777
df	2
Asymp. Sig.	0.000

a Kruskal Wallis Test
b Grouping Variable: Stages
(They are 99% significantly different between three treatments with P=0.000)

Mann-Whitney Test was used to indicate significant differences of ranks of first instar larval and adult consumption on *Tyrophagus* mites.

Table E. Ranks of treatment T1 and T3

	Stages	N	Mean Rank	Sum of Ranks
Feeding on *Tyrophagus* mites	**T1**. First instar	11	7.18	79.00
	T3. Adult	9	14.56	131.00
	Total	20		

Table F. Test Statistics(b): Analysis of ranks between treatment T1 and T3 of consumption experiment on *Tyrophagus* mites.

	Feeding on *Tyrophagus* mites
Mann-Whitney U	13.000
Wilcoxon W	79.000
Z	-2.845
Asymp. Sig. (2-tailed)	0.004
Exact Sig. [2*(1-tailed Sig.)]	0.004(a)

a Not corrected for ties.
b Grouping Variable: Stages
(Adult consumption on Tyrophagus mite is significantly higher than first instar larval consumption at 99% confidence.)

Mann-Whitney Test was used to indicate significant differences of ranks of second instar larval and adult consumption on *Tyrophagus* mites.

Table G. Ranks of treatment T2 and T3

	Stages	N	Mean Rank	Sum of Ranks
Feeding on *Tyrophagus* mites	**T2.** Second instar	10	12.10	121.00
	T3. Adult	9	7.67	69.00
	Total	19		

Table H. Test Statistics(b): Analysis of ranks between treatment T2 and T3 of consumption experiment on *Tyrophagus* mite

	Feeding on *Tyrophagus* mites
Mann-Whitney U	24.000
Wilcoxon W	69.000
Z	-1.735
Asymp. Sig. (2-tailed)	0.083
Exact Sig. [2*(1-tailed Sig.)]	0.095(a)

a Not corrected for ties.
b Grouping Variable: Stages
(Consumption of second instar larva and adult on Tyrophagus mite is not significantly different.)

Appendix 7

Statistical analyses for consumption of predatory *H. victoriensis* on WFT eggs

Table A. Descriptives

Treatment	N	Mean	Std. Deviation	Std. Error	Minimum	Maximum
W1. First instar	10	12.80	2.251	0.712	9	17
W2. Second instar	11	24.09	5.069	1.528	14	30
W3. Adult	11	8.91	4.805	1.449	0	15
Total	32	15.34	7.819	1.382	0	30

Table B. Test of Homogeneity of Variances

Levene Statistic	df1	df2	Sig.
2.967	2	29	0.067

(The variances of three treatments are not significantly different with P=0.067 > 0.05.)

Table C. Analysis of variances of three differently feeding stages of *H. victoriensis* on WFT eggs

ANOVA

	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	1361.801	2	680.900	37.018	0.000
Within Groups	533.418	29	18.394		
Total	1895.219	31			

(They are 99% significantly different between three treatments with P=0.000)

Table D. Homogeneous Subset comparisons using Tukey HSD test indicate the significant differences of each pair groups at p=0.01 level.

Stages	N	Subset for alpha =0.01		
		1	2	1
W3. Adult	11		8.91	
W1. First instar	10		12.80	
W2. Second instar	11			24.09
Sig.			0.109	1.000

Means for groups in homogeneous subsets are displayed.
a Uses Harmonic Mean Sample Size = 10.645.
b The group sizes are unequal. The harmonic mean of the group sizes is used. Type I error levels are not guaranteed.
(WFT egg consumptions of adult and first instar larva are in the same level but these are significantly lower than WFT egg consumption of second instar larva at 0.01 level.)

Biological aspects of Haplothrips victoriensis in SA

Appendix 8

TECHNIQUES FOR PREPARING MICRO-SLIDES
[As used at Canberra for thrips by Laurence Mound — laurence.mound@csiro.au]

Microscope slide mounts need to be made in order to study either small parts of large insects, or whole mounts of minute insects. Techniques are best considered under two headings: those appropriate for routine identifications, and those required for archival and taxonomic reference purposes. Remember, however, that preparation techniques are largely dependent on the care with which specimens were collected and preserved in the first place.

COLLECTING
Aphids and many flies are best collected into 80% alcohol. In contrast, thrips are best collected into a fluid known as AGA, a mixture of 10 parts of 60% ethyl alcohol with 1 part of glycerine and 1 part of acetic acid. This mixture helps to distend the body of most thrips and keeps the body parts supple. *However, 80 - 95% alcohol followed by storage in a freezer may still yield good microscope slides, and the unmounted specimens are useful for DNA studies. In 2006 this is the collecting fluid that I am using generally, BUT I DO NOT YET KNOW HOW LONG SPECIMENS CAN BE PRESERVED THIS WAY – CERTAINLY THEY MUST BE KEPT IN A FREEZER.*

Thrips may be beaten from flowers, leaves and dead twigs. A small but heavy garden trowel is a convenient beating implement. The most convenient beating tray for thrips is a plastic Australian Barbeque tray. The feet of thrips adhere to this surface, and they can then be picked off with a small brush or grass stem into the collecting fluid in tubes.

The best tubes for field use are plastic Ependorf Tubes, as used in molecular biology laboratories for centrifuging, because these have screw tops that include a sealing ring, and do not leak or break. Ensure that each tube contains a label, written in pencil.

Specimens of most small insects that are to be stored in alcohol should be kept in the dark, preferably at temperatures well below 0ºC, to prevent loss of colour.

A — SLIDE PREPARATION FOR ROUTINE IDENTIFICATIONS
The following method, using a water-soluble mountant, such as Hoyers or CMC10 (from Masters Co. Inc. 890 Lively Blvd. Wood Dale, IL 60191, (630) 238-9292, masterscoinc@aol.com). This method is rapid and thus relatively inexpensive. Slides prepared in such media are not 'permanent', but can remain useable for several, or even many, years. This method is recommended for all routine identification work, and is particularly appropriate for larvae and for small pale adults. Note the importance of placing newly prepared slides onto a hot-plate or in an oven for several hours, because specimens often collapse initially but then recover slowly when heated.

1. Remove the specimens from the collecting fluid into clean 70% alcohol.
2. If the specimens are reasonably flexible attempt to open the wings and straighten the antennae using micro-pins (see below).
3. Place a drop of Hoyers Mountant or CMC10 Mountant onto a cover slip (13mm circle, No. 0 or 1). Place a thrips into this drop, ventral side uppermost, and gently lower a slide onto the drop. Invert the slide as soon as the mountant has spread sufficiently.
4. Place immediately into an oven, or onto a hot-plate, at about 40-50°C. Leave for 6 hours before attempting to study.
5. Leave in the oven for about 3 weeks to dry the mountant, then ring with nail varnish, and label appropriately (see below).
COMMENTS - The cover slip used should be small — large cover slips crush specimens and need more mountant. The traditional method of mounting a specimen onto a slide and then placing the cover slip onto this with forceps is more difficult and often introduces bubbles of air.

B — SLIDE PREPARATION FOR TAXONOMIC RESEARCH

Biological aspects of Haplothrips victoriensis in SA

The objective is to prepare specimens onto slides with their shape and colour retained in a condition as close as possible to the natural, living state but with the body cleared so that surface detail is visible. This ideal is difficult to achieve, and a compromise must be adopted.

Most specimens should be macerated gently to reveal fine details of body sculpture and minute setae. A few specimens should be prepared for study without maceration in order to preserve their natural colouration.

TOOLS: Specimens can be manipulated with fine micro-pins, mounted in sealing wax on match sticks. Use a pair of such pins, one straight the other with the apex bent. A simple lifting tool to move specimens from one dish to another can be made from a small loop of fine wire. Alternatively, alcohols can be changed in dishes using an hypodermic needle or fine glass pipette. The most appropriate dishes to use are 'excavated blocks' — glass blocks 15mm high and 40mm square with a median excavation of about 5ml volume, and with a glass lid to prevent evaporation.

MACERATION

The objective of maceration is to remove the body contents. This is done by soaking the specimens in a weak NaOH solution for an appropriate period — NaOH solution seems to cause less damage to the body surface than KOH solution. The length of the period of treatment must be determined by experiment.

Students are recommended to experiment with **very weak** NaOH solution (2%) for longer periods, such as overnight (for black specimens even longer), because a long, slow process is more easily controlled than attempting to get results quickly. Maceration should always be carried out at room temperature; heating causes damage to setae and the body surface. **Note that this contrasts with the techniques used for preparation of aphid and coccid specimens**.

1. Place up to 20 thrips into clean water in an excavated block, but fewer specimens if they are very large; it is best if the specimens float with their wings on the surface. Leave for 1 to 3 hours.
2. Add to the water an equal volume of 5% NaOH solution. Pale specimens may require only one hour, but I usually leave specimens overnight in this weak solution. Black specimens may be left for 2 or more days. This period can be determined only by experience, but should always be as short as possible to minimise damage.
3. Transfer the specimens from NaOH solution to **water** for a few hours, using a needle or wire loop. Gently massage each specimen to expel most of the body contents, and spread the legs and antennae.
4. Store the specimens in 60% alcohol for 12 to 24 hours.

DEHYDRATION

Alcohols and clove oil will absorb water from the atmosphere if not protected, particularly under warm humid conditions. The objective of the dehydration schedule is to remove water, then to render the specimens translucent with clove oil. Clearing can be improved by massaging each specimen gently with the back of the bent needle.

1. Replace the 60% alcohol with 70% alcohol and leave for about 1 hour; unmacerated specimens must be punctured to speed the entry of alcohols, and the legs, antennae and wings should be spread.
2. Replace with 80% alcohol and leave for 20 minutes.
3. Replace with 95% alcohol and leave for 10 minutes.
4. Replace with absolute alcohol and leave for 5 minutes.
5. Replace with fresh absolute alcohol and leave for another 5 minutes.
6. Transfer to clove oil and leave for about half an hour before mounting.

MOUNTING: To facilitate this process it is best first to prepare a small mounting block. This is done by fixing to the centre of a microscope slide a 2mm deep layer of 1 inch square white card. Mark the centre of this with crossed lines, and then cover it securely with plastic tape to provide a clean, shiny surface.

1. Place a clean 13 mm diameter cover slip onto the mounting block; put a drop of Canada Balsam onto the centre of the cover slip and into this place one thrips specimen <u>ventral side uppermost</u>.
2. Spread the legs and wings, and straighten the antennae by pressing on the basal segments with a fine needle.

3. Invert a clean microscope slide and lower it firmly but gently onto the specimen in balsam on the cover slip. As soon as the surfaces touch, re-invert the slide with the coverslip adhering; this technique usually avoids the inadvertent introduction of bubbles which ruin so many students' slides. [Sometimes it helps to place a small drop of balsam (or xylene if the drop placed on the cover slip was found to be too large) in the centre of the slide before touching the balsam of the cover slip.]
5. Place the slide onto a hot-plate at once, at about 45°C, to drive off the xylene as quickly as possible. Then dry the slides until they are hard in an oven at about 45°C for several weeks. The quantity of balsam must be sufficient - <u>after it has dried</u> - to support the coverslip without distorting the specimen.

LABELLING: An insect specimen is of limited value if it is not labelled with its original data.
1. With the head of the thrips directed toward you, the right hand label should indicate the host plant, followed by the country (in capital letters) and then the locality and date, with collector's name (and code number).
2. The left hand label should indicate the sex, morph and genus and species names with author, with sufficient room left for any special notes to be added about that particular specimen e.g. measurements, number of wing setae etc.

Appendix 9

Extraction Protocol

1. Add a single specimen to a 1.5 centrifuge tube. Be careful to avoid any cross-contamination. Make that no ethanol is in the tubes.

2. Add 200 µL of CELL LYSIS solution.

3. Add 2 µL of PROTEINASE K to each tube.

4. Incubate at 55°C over night.

5. Remove supernatant to a new labelled tube. An exoskeleton of the specimen in empty tubes will be washed twice in 60% ethanol and kept in 90-100% ethanol for further morphological identification.

6. Add 50 µL of PROTEIN PRECIPITATION solution (Ammonium acetate (AMA)) to each tube.

7. Vortex for 5-10 seconds.

8. Place in freezer for 30 mins.

9. Spin in centrifuge for 4-5 minutes at 11-12000 rpm. A tight white pellet will appear in the bottom of tubes. (If the solution of tubes is spun for too long, the white pellet will turn clear. In this case, vortex the solution again and place back in the freezer for another 30 mins). Note: when the tubes are put in the centrifuge, turn the back of the tubes at the highest position.

10. Add 300 µL Isopropanol to new labelled tubes (O-ring centrifuge tube).

11. Add the supernatant to Isopropanol.

12. Place the in the freezer for 2h – overnight.

13. Place the tubes in a centrifuge and spin at 11-12000 rpm for 7 minutes.

14. Use a 1000 µL pipette to gently remove supernatant one at a time (this needs to be done carefully as the DNA pellet may move)

15. Add 500 µL 75% ethanol and gently mix.

16. Spin at high speed 11-12000rpm for 7 minutes.

17. Remove supernatant (this needs to be done carefully as the DNA pellet may move), ensure no ethanol remains. (remove supernatant one time by a 1000 µL pipette first, after that use a 200 µL pipette to remove the remaining supernatant as many times as possible.

18. Allow to air dry for 30 minutes.

19. Add 50 µL TLE (or water)

20. Allow to reconstitute overnight, or place at 65°C for an hour.

21. Store in refrigerator. DNA are ready to use.

PCR Running, Gel Running and Photograph

COI amplification
- Master mixture preparation:
    + Add Nuclease free H_2O to a 1.5 mL microfuge tube (16 µL/a sample)
    + Add hot master buffer (2.5 µL/sample)
    + Add dNPT (2.5 µL/sample)
    + Add two primers (LCO1490 and HCO2198) (1 µL a primer/sample)
    + Add Hot master enzyme into the solution (0.1 µL/sample)
    + Mix and centrifuge lightly
- Divide the solution to smaller white marked tubes, each containing 23 µL mixed solution.
- Using plugged tips to avoid contamination and add 2 µL DNA solution of samples to the correct marked tubes.
- Light centrifuge.
- Place all samples in PCR machine.
- Amplify a COI fragment in PCR: denature at 94^oC for 30 sec; anneal at 47^oC for 30 sec; extension 70^oC for 45 sec; number of cycles: 35.

Agarose Gel Electrophoresis
- Prepare a tray for agarose gel.
- Pour agarose gel liquid into the tray placed on a flat bench about 5-7mm thick and leave the tray with gel for 8-10 mins for solidifying.
- Cut a piece of paraffin paper and pin it on a plate of soft material for keeping.
- Take 8 µL Dye glycerol and drop 8 small drops on paraffin paper (1 µL Dye glycerol/sample)
- Add 3 µL of each PCR solution to each drop.
- Place the gel tray in a electrophoresis chamber in the correct way (remember: run to red)
- Pour 1TBE running buffer to the chamber so that the buffer covers the gel above to a thickness of 1-4mm.
- Load 3 µL ID solution to the first well of the gel.
- Continuously load all the solution (4 µL) of prepared drops to each well in order and record the order.
- Turn on power and set at 100 volts, 70 ampere and leave on for 20-30 mins.

Biological aspects of Haplothrips victoriensis in SA