

**Biological and molecular analysis of important
agricultural pest species in southern Australia:
Penthaleus species and *Halotydeus destructor***

Submitted by
Paul A. Umina, BSc. (Hons)

A thesis submitted in total fulfilment
of the requirements for the degree of
Doctor of Philosophy

Centre for Environmental Stress and Adaptation Research
School of Molecular Sciences
Faculty of Science, Technology and Engineering

La Trobe University
Bundoora, Victoria 3086
Australia

January 2004

TABLE OF CONTENTS

Table of Contents	ii
Summary	vii
Statement of Authorship	ix
Acknowledgements	x
Dedication	xi
Preface	xii
CHAPTER 1: General Introduction	1
1.1 Introduction	2
1.2 History	3
1.3 Systematic studies and morphology	4
1.4 Distribution	7
1.5 Life-cycle	9
1.6 Population genetics	11
1.7 Dispersal	14
1.8 Feeding and behaviour	16
1.9 Competition	18
1.10 Control	19
1.10.1 Chemical	19
1.10.2 Biological	21
1.10.3 Host plant resistance	23
1.10.4 Crop rotations	24
1.10.5 Other cultural controls	24
1.11 Outline of this study	25
1.12 References Cited	27
1.13 Table and Figures	44

CHAPTER 2: Diapause and implications for control of <i>Penthaleus</i> species and <i>Halotydeus destructor</i> in southeastern Australia	49
2.1 Abstract	50
2.2 Introduction	51
2.3 Materials and Methods	55
2.3.1 Field plots (2000-2001)	55
2.3.2 Field plots (2001-2002)	56
2.3.3 Shade-house experiment	58
2.3.4 Electrophoresis and comparisons of clones	59
2.4 Results	61
2.4.1 Field plots	61
2.4.1.1 2000-2001	61
2.4.1.2 2001-2002	62
2.4.2 Shade-house experiment	62
2.4.3 Clonal patterns	63
2.5 Discussion	65
2.6 References Cited	71
2.7 Table and Figures	77
 CHAPTER 3: Plant host associations of <i>Penthaleus</i> species and <i>Halotydeus destructor</i> and implications for integrated pest management	 83
3.1 Abstract	84
3.2 Introduction	85
3.3 Materials and Methods	88
3.3.1 Shade-house host plant experiment	88
3.3.2 Mite persistence on plants in the field	90
3.3.3 Host plant survey	92
3.3.4 Fitness measures	93
3.3.5 Data analysis	93

3.4	Results	95
3.4.1	Shade-house experiment - mite numbers	95
3.4.2	Shade-house experiment - plant damage	97
3.4.3	Field plots	99
3.4.4	Host plant survey	100
3.5	Discussion	101
3.6	References Cited	106
3.7	Tables and Figures	112

CHAPTER 4: Competitive interactions among four pest species of earth mites 122

4.1	Abstract	123
4.2	Introduction	124
4.3	Materials and Methods	127
4.3.1	Field competition experiment	127
4.3.2	Shade-house experiment	129
4.3.3	Data analysis	131
4.4	Results	133
4.4.1	Field competition experiment	133
4.4.2	Shade-house experiment	136
4.5	Discussion	138
4.6	References Cited	144
4.7	Tables and Figures	149

CHAPTER 5: Biochemical and molecular analysis of earth mite protein

extracts with the view of developing a field-based diagnostic kit 156

List of abbreviations 157

5.1	Abstract	159
5.2	Introduction	160
5.3	Materials and Methods	164

5.3.1	Preparation of crude mite protein extract for SDS-PAGE	164
5.3.2	Quantification of total mite protein	164
5.3.3	One-dimensional SDS-PAGE	165
5.3.4	Invitrogen gradient gels	165
5.3.5	Electroblotting	166
5.3.6	Two-dimensional (2-D) electrophoresis	166
5.3.6.1	First dimension	166
5.3.6.2	Second dimension	167
5.3.7	N-terminal amino acid sequencing	168
5.3.8	Internal amino acid sequencing	168
5.3.8.1	Digestion of proteins with trypsin	168
5.3.8.2	Cyanogen bromide cleavage	169
5.3.9	Reverse-phase HPLC peptide purification	170
5.3.10	Production of polyclonal antibodies against mite extracts	170
5.3.11	Serum preparation	171
5.3.12	Enzyme-linked immunosorbent assay to measure antibody titre	171
5.3.13	Characterisation of antiserum by immunoblotting	172
5.3.14	Affinity purification of antibodies	173
5.3.15	Inhibition ELISAs	174
5.3.16	Dialysis of protein samples	175
5.3.17	Coupling of protein to Amino-Link [®] gel	175
5.3.18	Immuno-depletion of antiserum using coupled protein	176
5.3.19	Production of monoclonal antibodies	177
5.3.20	Gel filtration (size exclusion) chromatography	178
5.3.21	Detection of protein glycosylation using periodic acid/Schiff's procedure	178
5.4	Results	180
5.4.1	Characterisation of mite proteins	180
5.4.2	Two-dimensional electrophoresis	181

5.4.3	N-terminal sequence analysis	182
5.4.4	Internal amino acid sequencing	184
5.4.4.1	Tryptic digestion of proteins	184
5.4.4.2	Cyanogen bromide cleavage of proteins	186
5.4.5	Analysis of polyclonal antibodies	187
5.4.6	Purification of antibodies immobilised on PVDF	189
5.4.7	Inhibition ELISAs	189
5.4.8	Immuno-depletion of antibodies	190
5.4.9	Monoclonal antibodies	192
5.4.10	Gel filtration chromatography	193
5.4.11	Analysis of glycosylation in mite protein extracts	194
5.4.12	Analysis of <i>H. destructor</i> extracts	195
5.5	Discussion	197
5.6	References Cited	206
5.7	Tables and Figures	214
5.8	Appendices	236
 CHAPTER 6: Overview and future directions		 239
6.1	Overview	240
6.2	Future directions	247
6.3	References Cited	253

SUMMARY

Blue oat mite species, *Penthaleus* spp. (Acari: Penthaleidae), and the redlegged earth mite, *Halotydeus destructor* (Tucker) (Acari: Penthaleidae), are some of the most serious and widespread agricultural pests in southern Australia. Management of these mites has been complicated by the recent discovery of three cryptic species of *Penthaleus*, whereas prior research had assumed a single species. The species differ markedly in their distribution, plant preferences and response to pesticides. This thesis looks at various aspects of the biology, ecology and molecular analyses of earth mites with particular emphasis on agricultural control.

I tested the initiation of diapause egg production in mites using field and shade-house experiments. *Halotydeus destructor* has a very different diapause response from *Penthaleus* species. Thus, carefully timed spring sprays are unlikely to be effective against *Penthaleus* spp., highlighting the importance of correctly distinguishing earth mite species before implementing control strategies. There is also variation in diapause induction among the three *Penthaleus* spp. that could contribute to their relative distributions and pest status.

I examined the effects of different plant hosts on the persistence and reproduction of earth mites. The results are consistent with field observations that mite species differ in their host plants. For all species, pasture is a suitable host and lentils are generally a poor host plant. Canola, ox-tongue, and a mixture of wheat and oats differ in their suitability as long-term hosts for each mite species. This information is important for

developing more sustainable management practices, such as weed management and crop rotations.

Competitive interactions between earth mites were also investigated in the field and the laboratory over a number of seasons. In order to make valid conclusions with an applied implication, competition was examined on four different plant treatments: pasture, canola, wheat/oats and ox-tongue. I showed that close competition exists between mite species and that competitive interactions differ temporally and on different plant types.

Finally, I undertook biochemical analysis of *Penthaleus* spp. for the development of a rapid and simple field test that could be used by agronomists and farmers to distinguish the different mite species on individual properties. This would allow appropriate control strategies to be devised, as well as reduce the ineffectual application of pesticides. Using mostly immunological techniques, I revealed important information about the complex nature of the *Penthaleus* group, the similarity between the three species, as well as develop methods for isolating and characterising species-specific proteins. Directions this work may take in future studies are also discussed.

STATEMENT OF AUTHORSHIP

Except where reference is made in the text of the thesis, this thesis contains no material published elsewhere or extracted in whole or in part from a thesis by which I have qualified for or been awarded another degree or diploma.

No other person's work has been used without due acknowledgement in the main text of the thesis.

This thesis has not been submitted for the award of any degree or diploma in any other tertiary institution.

Paul Umina

13th January 2004

ACKNOWLEDGMENTS

First and foremost, I would like to thank Ary Hoffmann who I am indebted to for his guidance and support. Ary provided me with an excellent learning environment and always supported me in developing my scientific skills. I would like to extend sincere gratitude to my co-supervisor, Leann Tilley, for her enthusiasm in the project and guidance throughout my candidature. A special thanks to Robin Anders, Andy Coley and Andrew Weeks for their input into this project and critical comments on various chapters of this thesis. I am grateful to James Ridsdill-Smith, Arne Janssen and Melina Miles for comments on this thesis. Additional thanks go to Colleen, and members of CESAR and the Biochemistry Department at La Trobe University, especially Andrea, Michelle, Chantelle, Kaylene, Akin, Melanie, Jo, Mitrovski and Philth. Your help and friendship has not gone unnoticed. I extend sincere gratitude to all my family for their continuing support and encouragement. I particularly wish to thank my father, Peter Umina. Dad, I'm sure you have collected more mites and constructed more field plots than you care to remember. For this my appreciation is immeasurable. I am grateful to those who provided field sites and helped with fieldwork, especially Andrew and Ken Wright. Thanks are also extended to the Grains Research and Development Corporation for providing financial assistance throughout this project. Finally, I wish to thank Sam for her constant support, understanding and encouragement.

DEDICATION

This thesis is entirely dedicated to my nonna,

Rina Barri.

**I love you. I miss you and will always remember the special way you made me
feel.**

PREFACE

The experimental results are divided into four self-contained chapters, some of which have also been prepared for publication in peer-reviewed journals. Chapter 2 is a paper with minor revisions and appeared in *Experimental and Applied Acarology* (2003) 31: 209-223. Chapter 3 has been accepted for publication in *Experimental and Applied Acarology* and Chapter 4 has been accepted for publication in *Journal of Economic Entomology*. A revised version of Chapter 1 has been submitted to *Experimental and Applied Acarology*.

CHAPTER 1.

General Introduction

1.1 INTRODUCTION

Blue oat mites (*Penthaleus* spp.) (Acari: Penthaleidae) are important agricultural pests in many temperate parts of the world, including America, Europe, Asia, South Africa, New Zealand and Australia (Chada 1956; Narayan 1962; Wallace and Mahon 1971). They attack a variety of crop and pasture plants, resulting in decreases in crop yields and available feed for livestock. Most damage is incurred at the establishment phase where mite feeding may necessitate entire crops being re-sown. The *Penthaleus* spp. are more serious pests in Australia than other regions and, as a result, much of the known literature comes from Australia. Most of this research has occurred in the last ten years, as their importance as agricultural pest species has become more apparent (Weeks and Hoffmann 1999; Robinson and Hoffmann 2001).

Previously, the actual damage caused by *Penthaleus* spp. in southern Australia was under-estimated owing to their frequent misidentification as another pest earth mite species, the redlegged earth mite, *Halotydeus destructor* (Tucker) (Acari: Penthaleidae). The *Penthaleus* spp. and *H. destructor* are similar in morphology, share similar life cycles, and occur sympatrically within Australia (Narayan 1962; Wallace and Mahon 1971; Qin and Halliday 1996b). The inability of farmers to distinguish between species has led to a common belief that *H. destructor* is the main agricultural pest species in southern Australia. As a consequence, most research has tended to focus on the biology and ecology of *H. destructor*, with less attention directed towards *Penthaleus* species (see Ridsdill-Smith 1997 for a review on the current knowledge of *H. destructor*). It is now known that the *Penthaleus* spp. are important pests causing substantial economic losses to the wool, meat, dairy and grain industries within

southern Australia. Here, I review the biology, ecology and population genetics of the *Penthaleus* spp. in southern Australia and throughout the world.

1.2 HISTORY

The identity of *Penthaleus* spp. has been surrounded by confusion. Dugés (1834) first described the species in France as *Tetranychus majeur*, although this was later Latinised to *Tetranychus major* by Murray (1877). In 1835, Koch described the species under the name, *Penthaleus haematopus*, and then in 1838 as *Penthaleus erythropus* (Narayan 1962). In 1872, Thorell described it under the name *Penthaleus insulanus*. The first record outside Europe was by Banks (1902), taken from wheat in western North America. He initially described it under the name *Notophallus dorsalis*, then later in 1917 as a new species, *Notophallus viridis*. In 1908, Marchal, observing that this mite was damaging peas, gave it the common name, ‘pea mite’ (Narayan 1962). However in 1956, Chada coined what he thought was a more appropriate name, the ‘winter grain mite’, by which it is still known in America.

In Australia, *Penthaleus* was first recorded in New South Wales attacking oats by Froggatt (1921), who named the mite *Penthaleus bicolor*. He also used the common name ‘blue oat mite’, which is still in use in Australia, Africa and Europe. By 1934, the blue oat mite was recognised as an important pest throughout the southern agricultural areas of Australia (Swan 1934). Womersley (1935), recognising the synonymy among previously named specimens, gave this mite its current name, *Penthaleus major* (Dugés). Another *Penthaleus* species, *Penthaleus minor* (Canestrini), has been found in Australia and some parts of Europe and North America

(Thor and Willmann 1941; Narayan 1957; Qin and Halliday 1996a). This mite is uncommon, primarily found in native habitats such as moss and lichen, and is thought to be of little agricultural importance (Qin and Halliday 1995; Qin and Halliday 1996a).

Weeks *et al.* (1995) and Qin and Halliday (1996b) discovered a new blue oat mite species, *Penthaleus falcatus*. This pest species highlighted problems in previous studies because the morphological descriptions for *P. major* resembled those of *P. falcatus* (Qin and Halliday 1996a). Weeks and Hoffmann (1999) have also recently discovered another *Penthaleus* species of economic importance in Australia, adding to the uncertainty of species identification. This species, presently referred to as *Penthaleus* species *x*, has only been described from Australia and South Africa.

1.3 SYSTEMATIC STUDIES AND MORPHOLOGY

The superfamily Eupodoidea (Acari: Prostigmata), currently includes six families: Eriorhynchidae; Eupodidae; Penthaleidae; Penthalodidae; Rhagidiidae; and Strandtmanniidae, of which the first five are represented in Australia (Qin and Halliday 1997; Qin 1998). These families contain species that are predaceous, fungivorous or phytophagous and are found in the soil, humus, moss, lichen and litter (Krantz 1978; Qin 1996). The *Penthaleus* spp. (along with *H. destructor*) belong to the family Penthaleidae, which includes species that feed on lower plants as well as some higher plant-feeding species. They are soft-bodied mites, without external peritremes and with the tracheae originating at the base of the chelicerae (Jeppson *et al.* 1975). Recent work by Qin and Halliday (1997) suggests *Halotydeus* and *Penthaleus* would be better

grouped into separate families, although these authors only examined specimens from within Australia and New Zealand.

The genus *Penthaleus* is distinguished from other genera in the Penthaleidae family by the dorsal position of its anus. *Chromotydaeus quartus* also has its anus located dorsally but it is near the dorsal posterior margin (Qin and Halliday 1996a), while *H. destructor* has a posterior anal opening (Jeppson *et al.* 1975). Adult *Penthaleus* are between 700 - 1100 µm long and 550 - 920 µm wide, except *P. minor* which is considerably smaller (Qin and Halliday 1996a). The body itself is globular in shape, with a dark blue-black colour and an orange-red mark surrounding the anus (Pescott 1934; Narayan 1962). The four pairs of legs and gnathosoma are also red-orange in colour. There is some confusion over the number of immature stages in *Penthaleus* species. Narayan (1962) concluded *P. major* passes through three instars before the adult stage (egg, larva, protonymph, deutonymph and adults), whereas Chada (1956) showed one larval and three nymphal stages. Jeppson *et al.* (1975) states there are two nymphal stages in *P. major* (and a pre-larval - deutovum stage), but then later provides an incubation period for a third nymphal stage. Larvae are approximately 300 µm long, oval in shape, with three pairs of legs. Upon emergence, they are pink-orange in colour, which soon changes to brownish and then green (Narayan 1962). Nymphs are larger, having a body length between 450 - 540 µm. They also have four pairs of legs (usually red) and vary in colour from pale brown to green (Narayan 1962).

The three pest *Penthaleus* spp. can be identified both morphologically and via allozyme electrophoresis. Qin and Halliday (1996a) provide detailed morphological descriptions of *P. major* and *P. falcatus*. *Penthaleus* sp. *x* has not been formerly

described and named, although Weeks and Hoffmann (1999) provide a description. The main morphological difference between the species is the length and number of setae on the dorsal surface of the body (Figure 1.1). *Penthaleus major* has long setae arranged in four to five longitudinal rows, while *P. falcatus* has a higher number of short setae scattered in an irregular array. *Penthaleus* sp. *x* has dorsal setae of intermediate length and number. *Penthaleus minor* can be easily distinguished from the other *Penthaleus* spp. by its small, short movable digit of the chelicera and a shorter palp with a truncate apex to the tarsus (Jeppson *et al.* 1975; Qin and Halliday 1996a). This species has not been found in Australia since the original specimens were collected in 1962 (Weeks and Hoffmann 1999).

The *Penthaleus* spp. produce two physiologically different types of eggs: winter eggs; and summer/diapause eggs. These eggs are deposited either singly or in groups of three to six on the leaves, stems, and roots of food plants and/or on the soil surface (Chada 1956). Those on the leaves are usually fastened by a mucilaginous substance that is secreted next to and on the stem of plants (Jeppson *et al.* 1975). Narayan (1962) states single females lay between ten to 15 eggs in a lifetime although, in a laboratory experiment, Weeks and Hoffmann (1999) found an average of 42.6 eggs, with the highest number being 93 eggs. The eggs are ovoid and bright pink, and are easily distinguished from *H. destructor* eggs (Ridsdill-Smith 1991). Narayan (1962) found winter eggs took between 25 and 35 days to hatch in the laboratory. However, this is likely to be shorter in the field and dependent on moisture, temperature and humidity (Norris 1950; Wallace 1970a). The other type of eggs, diapause or 'aestivating eggs', have an extended incubation period between 110 and 140 days (Jeppson *et al.* 1975). These eggs are resistant to heat, drought and desiccating winds (Wallace 1970a). They

are slightly larger in diameter than winter eggs owing to a thicker membrane covering (Narayan 1962; Jeppson *et al.* 1975).

1.4 DISTRIBUTION

The *Penthaleus* spp. are found in circumpolar zones of the world, but absent in warm climates around the equator (Figure 1.2). In the north temperate zones, they have been recorded from numerous countries including France, Italy, England, Netherlands, Spain, Portugal, Germany, Greenland, Iceland, Norway, Russia, Japan, China, Taiwan, Canada, Morocco, and America (Middlekauff and Pritchard 1949; Narayan 1962; Wallace and Mahon 1971; Kanda *et al.* 1992; Gudleifsson *et al.* 2002). In southern temperate zones, they are found in Argentina, Mexico, Australia, New Zealand, and throughout southern Africa (Narayan 1962; Wallace and Mahon 1971). It is also likely that *Penthaleus* spp. exist in other parts of the world. Jeppson *et al.* (1975) suggest that *Penthaleus* spp. could probably survive in most areas in both hemispheres between the latitudes 25° and 50 to 55°, while unlikely to be found in the tropics except at high altitudes. In contrast, *H. destructor* is only known from Australia, New Zealand and South Africa (Ridsdill-Smith 1997).

Unfortunately, confusion over species identification casts doubt on the presently known distribution of the different *Penthaleus* spp. in some regions of Australia and throughout the world. For instance, Narayan (1962) identified ‘*P. major*’ from Kansas, but his illustration for this species resembles *P. falcatus* rather than *P. major*. Narayan (1962) also provided an illustration of a ‘*P. major*’ larva, which resembles *P. major* and not *P. falcatus*. André (1932) provided a somewhat different description of *P.*

major, raising doubt about distribution data. It is known that *P. falcatus* is found in New Zealand and Australia, while *P. sp. x* has been found in samples from Australia and South Africa (Weeks and Hoffmann 1999).

Within Australia, the distribution of *Penthaleus* species is fairly well known (Figure 1.2). Wallace and Mahon (1971) showed the inland distribution of *Penthaleus* was determined by a 190 mm isohyet for their growing season, although this research was undertaken prior to the three *Penthaleus* spp. being identified. Weeks and Hoffmann (1999) and Robinson and Hoffmann (2001) have mapped the species' distribution in southeastern Australia. They found *P. major* was the most common species, being distributed broadly over Victoria, New South Wales, the southern part of South Australia and parts of Tasmania. The distribution of *P. major* extended further inland into drier areas than previously found for blue oat mites (Weeks and Hoffmann 1999) suggesting their distribution had expanded. *Penthaleus falcatus* had a relatively patchy but similar distribution to *P. major*, while *P. sp. x* had a limited distribution in the northwestern part of Victoria and the northeastern part of New South Wales. Moisture and heat stress were the two factors implicated by a climatic matching program (CLIMEX: CRC for Tropical Pest Management, University of Queensland, Brisbane, Australia) as limiting the inland distribution of *P. major* and *P. falcatus*, however, it is not clear what limits their northern distribution (Robinson and Hoffmann 2001). For *P. sp. x*, CLIMEX was unable to identify any climatic variables restricting the distribution of this species (Robinson and Hoffmann 2001). The distribution of *H. destructor* does not extend as far inland or as far north as either *P. major* or *P. falcatus*.

The *Penthaleus* spp. are also known from southern Western Australia (along with *H. destructor*) although the distribution of each species has not been mapped. The presence of *P. major* has been confirmed by Qin and Halliday (1996a). As *P. falcatus* occupies a similar distribution to *P. major* in southeastern Australia and is therefore probably limited by the same factors, it is likely to also occur in Western Australia.

1.5 LIFE-CYCLE

Within Australia, the *Penthaleus* spp. are winter pests, having a similar life cycle to *H. destructor*. They are active from April to late October and diapause as eggs throughout the harsh summer months. *Penthaleus major* populations have been recorded at over 150 000 mites per square metre (Kanda and Hirai 1990), although they are usually found at densities of one magnitude below this level. In Australia, there are two or three generations a year, depending on the conditions, whilst in America only two generations occur per season (Narayan 1962; Jeppson *et al.* 1975). The generation time in the field is around eight to ten weeks, although species may differ from one another. In the laboratory, Weeks and Hoffmann (1999) found a generation time of less than seven weeks for *P. major* and around ten weeks for *P. falcatus*. *Halotydeus destructor* is thought to have a slightly shorter generation time than the *Penthaleus* spp. (Narayan 1962; Ridsdill-Smith and Gaull 1995; Ridsdill-Smith and Annells 1997), while that of *P. sp. x* has not been examined. *Penthaleus sp. x* emerges from diapause later, and therefore has a shorter active period. It is also generally larger than the other species (unpublished data), possibly reflecting a longer generation time. This may mean that *P. sp. x* completes no more than two generations a season.

The first generation of the *Penthaleus* spp. develops from diapause eggs following the onset of favourable temperature, moisture and relative humidity conditions (Jeppson *et al.* 1975). It takes approximately two weeks of exposure to favourable conditions for the diapausing eggs of *H. destructor* to hatch (Wallace 1970b). Unlike *H. destructor*, the duration of each developmental stage in the *Penthaleus* spp. is not accurately known, with several studies reporting conflicting results (Narayan 1962; Jeppson *et al.* 1975). The larval stage is thought to average seven to ten days, the nymphal stages may last between 17 and 24 days, while adults have been reported as living for up to 61 days (Narayan 1962; Jeppson *et al.* 1975). Directly preceding each life-stage is a pre-ecdysial resting stage that lasts approximately 24 hours (Narayan 1962).

Diapause is a highly evolved process that is induced well before the arrival of adversity and maintained for some time irrespective of environmental changes (Mansingh 1971; Tauber *et al.* 1984). Diapause varies markedly among different species in its incidence, maintenance, and termination, not only among different species but also among different populations within a species (Masaki and Wipking 1994). For *H. destructor*, the majority of diapause eggs are produced by the third generation of mites in mid-late spring, while the first two generations lay predominantly winter eggs (Wallace 1970b; James and O'Malley 1993; Ridsdill-Smith and Annells 1997). Climatic variables (in particular photoperiod) appear to be important regulatory factors of diapause induction in *H. destructor* (Ridsdill-Smith and Annells 1997), although Wallace (1970a) suggested increasing maturity of food plants in spring was also important. Recent findings suggest that the diapause strategies of *Penthaleus* spp. may differ from that of *H. destructor* (James and O'Malley 1993), although this remains largely unknown and requires further investigation.

The *Penthaleus* spp. were formerly known as winter pests of cereal, pasture and vegetable plants (Narayan 1962; Weeks and Hoffmann 1999). However, since 1979, *P. major* has also been observed as a summer pest of the cold temperate North Atlantic zone, usually attacking subarctic pastures and hayfields. The summer temperatures in this zone are comparable to winter temperatures in southern Australia. Few data on the life cycle of the *Penthaleus* spp. in the cold temperate zone have been published. Mite feeding damage has been reported from southwest Greenland (Nielsen 1984), the northern part of Iceland (Gudleifsson and Ólafsson 1987), and northern Norway (Johansen 1986). In Iceland, *P. major* is found at the onset of plant growth in spring when the snow cover melts, and plants are attacked in May, June and early July, while mites disappear in mid-summer (Gudleifsson *et al.* 2002). In some cases, large numbers are also seen in August and September. Unlike in other regions, two annual generations have been reported: a summer generation; and a winter generation (T.E. Hallas, personal communication). The generation time of the summer mites is around 64 days (not including egg development), which is similar to the time of winter development in Australia. It is believed that the winter generation of mites goes through a period of hibernation (rather than diapause) throughout the colder months (between October and May), predominantly as protonymphs (T.E. Hallas, personal communication).

1.6 POPULATION GENETICS

Prior to 1995, the reproductive modes of the *Penthaleus* spp. and *H. destructor* were unknown, with references in the literature to haplodiploid sexual reproduction (Ridsdill-Smith 1991), diplodiploid sexual reproduction (Ridsdill-Smith 1991) and

thelytokous parthenogenesis (Tucker 1925; Narayan 1962; Norton *et al.* 1993). Several authors have made reference to rare males in populations of *P. major*, with Narayan (1962) providing a formal description. However, as Qin and Halliday (1996a) pointed out, the illustrated male aedeagus is likely to be a female ovipositor. In over ten years of research into the *Penthaleus* spp. in Australia, males have never been found in the field (A.R. Weeks & M.T. Robinson, personal communication; unpublished data) and therefore, it is doubtful that they occur, at least in southern Australia.

Based on allelic patterns for a number of loci determined by allozyme electrophoresis, Weeks *et al.* (1995) demonstrated that both *P. major* and *P. falcatus* were diploid and reproduced by thelytokous parthenogenesis, with populations made up of female clones. In contrast, they demonstrated that *H. destructor* was also diploid, but reproduced sexually, with genotype frequencies in populations generally conforming to Hardy-Weinberg proportions. Weeks and Hoffmann (1999) showed that *P. sp. x* also reproduces by thelytokous parthenogenesis. Each *Penthaleus* species reproduces by a functionally apomictic mechanism (either apomictic parthenogenesis or premeiotic doubling), as all progeny have the same multilocus genotype as their mother, with heterozygosity being maintained (Weeks *et al.* 1995). The reproductive mode of *P. minor* is unknown.

Thelytokous organisms are assumed to be evolutionary deadends because they lack the genetic variation to adapt to a changing environment (Darlington 1939; White 1973; Maynard Smith 1978). While there are several putative examples of thelytokous taxa that have been evolutionarily successful (Judson and Normark 1996), most thelytokous organisms have arisen from close sexual relatives and are short lived on an

evolutionary time-scale (Weeks and Hoffmann 1998). Therefore, the *Penthaleus* species complex presents an intriguing system to look at the evolutionary implications of thelytokous parthenogenesis because all species are thelytokous and there are no known close sexual relatives (Weeks and Hoffmann 1999).

Weeks and Hoffmann (1998; 1999) and Robinson *et al.* (2002) found high levels of clonal variation within populations of *P. major* from southeastern Australia. Based on seven allozyme loci, they distinguished 30 multilocus genotypes, with populations consisting of up to 14 distinct clones. For *P. falcatus*, Weeks and Hoffmann (1999) identified six multilocus allozyme clones, while for *P. sp. x* only four allozyme clones were identified from populations in southeastern Australia. Two clones differed substantially in allozyme patterns (A.R. Weeks, personal communication) and may represent a cryptic species of *P. falcatus* with identical morphology. The reasons for the differences in clonal diversity among the species are not known, but could reflect the evolutionary history of these species (i.e. *P. major* is ancestral to *P. falcatus* and *P. sp. x*).

The high levels of genetic variation (clonal diversity) in *P. major* present a conundrum because it is expected that through time, the reproductively most efficient clone(s) should eliminate the less efficient ones (Vrijenhoek 1978; Vrijenhoek 1984; Fox *et al.* 1996). There is no evidence of sexual reproduction or recombination as a mechanism contributing to the maintenance of this clonal diversity, as has been found in other thelytokous systems (Vrijenhoek 1984; Jokela *et al.* 1997; Semlitsch *et al.* 1997). With a series of field experiments, Weeks and Hoffmann (1998) showed that *P. major* clones are ecologically distinct, vary both temporally and spatially in fitness, and that

these clones are under intense natural selection. The authors proposed that environmental heterogeneity was contributing to the maintenance of clonal diversity within populations. However, based on a model of Hoekstra (1978), the greater the difference in niche size, the less likely environmental heterogeneity could maintain genetic (clonal) diversity by itself.

Weeks and Hoffmann (in press), using a replicated reciprocal translocation field experiment among three geographically distant locations, found that allozyme-defined clones were under negative frequency-dependent selection: as a clone became relatively rare, its relative fitness increased (Figure 1.3). Regression models developed from these data predicted equilibrium frequencies for clones, which were corroborated by previously collected field data. The authors concluded that negative frequency-dependent selection is the mechanism that maintains clonal diversity in natural populations of *P. major*. In support of this, Robinson *et al.* (2002) found no geographic pattern to the frequencies of allozyme-defined clones, although clonal diversity decreased from the centre to the peripheral areas of the distribution of *P. major*, presumably because of reduced niche availability at a species margins. Under negative frequency-dependent selection, niche variability/size will dictate clonal diversity.

1.7 DISPERSAL

Dispersal is an aspect of population structure that has not been studied extensively in earth mites, despite its importance for control strategies. Passive dispersal (wind) by adult mites is possible, but seems unlikely since the mites spend most of their time on the soil surface (Ridsdill-Smith 1997). Long-range dispersal is thought to occur during

the summer via the movement of diapause eggs (Ridsdill-Smith 1997), although this has never been quantified. Eggs may also be dispersed long distances on soil adhering to livestock and farm machinery and through transportation of plant material. Information on long distance dispersal in other phytophagous mites is also scarce. Some species of spider mites are thought to exhibit a type of aerial dispersal at the adult stage (Sabelis and Dicke 1985), where adults adopt a specific stance and are subsequently 'blown' by the wind.

Dispersal in earth mites can also involve locomotory movement (walking). Weeks *et al.* (2000) used a mark-release-recapture technique to estimate dispersal in mites. They found that adult *P. major* did not move further than ten metres in their lifetime in any one direction within a pasture environment. Distances moved by *H. destructor* and *P. major* were similar, and therefore movement rates in *P. falcatus* or *P. sp. x* are also likely to be similar, although this has not been tested. Weeks *et al.* (2000) also examined movement between adjacent cropping environments and showed that mites can exhibit some directional movement, perhaps involving an olfactory response to favourable/unfavourable host plants.

The estimate of adult dispersal suggests outbreaks of earth mites will remain relatively localised within a season, and field observations support this finding. Dispersal rates are, however, insufficient to explain the rapid expansion of the *Penthaleus* spp. throughout Australia (Wallace and Mahon 1971). Therefore, it is likely that long-distance dispersal occurs by other means, such as the movement of summer (diapause) eggs by wind or human assistance.

1.8 FEEDING AND BEHAVIOUR

Recently, numerous studies have examined the feeding mechanisms and behaviour of *H. destructor* (Gaull and Ridsdill-Smith 1996; Gaull and Ridsdill-Smith 1997; Jiang *et al.* 1997; Thackray *et al.* 1997; Ridsdill-Smith and Pavri 2000a). In contrast, few studies have examined the feeding biology of the *Penthaleus* spp. with only one study actually distinguishing between species (Weeks and Hoffmann 1999). The feeding biology of the *Penthaleus* spp. is therefore not well understood.

Typically, *Penthaleus* spp. penetrate the epidermal cells of plants and remove the cellular contents in a similar fashion to *H. destructor*. *Halotydeus destructor* makes a hole approximately 3 μm in diameter, which is probably smaller than that of the *Penthaleus* spp., which have larger mouthparts (Qin and Halliday 1996a; Qin and Halliday 1996b). The paired movable digits of the *Penthaleus* spp. can be moved separately or together, but do not form a channel for transporting fluid. The subcapitulum is applied to the plant surface and the cell contents are sucked out using a pharyngeal pump (Nuzzaci and de Lillo 1991). The removal of cell contents by the *Penthaleus* spp. typically results in silvery to grey patches on plant foliage. In the paddock, this is often mistaken for frost damage. Mites feeding on plants cause the release of plant volatiles, which at low levels attract *H. destructor* (Jiang *et al.* 1997). This results in feeding aggregations of between three and 36 individuals of *H. destructor* (Ridsdill-Smith 1997). The *Penthaleus* spp. are probably not attracted by these compounds, as they are found feeding singly or occasionally in small groups of five to ten (André 1932; Narayan 1962).

Young mites prefer to feed on the sheath leaves or tender shoots near the soil surface, while adults feed on the more mature plant tissues (Narayan 1962), probably because they are more robust and have larger mouthparts. The early life-stages of the *Penthaleus* spp. are also likely to feed on lower plants and microflora found on the soil surface (McDonald *et al.* 1995). Maclellan *et al.* (1998) suggested the presence of microflora may permit populations to survive, develop and reproduce when higher plants are unavailable or are unsuitable as hosts. The *Penthaleus* spp. are most active during the cooler parts of the day, tending to feed at night and in cloudy weather (Gudleifsson *et al.* 2002). They seek protection during the warmer part of the day on the moist soil surface or under foliage and may even dig into the moist, cool soil under extreme conditions (Jeppson *et al.* 1975).

Contrary to popular belief, the *Penthaleus* spp. attack a variety of agriculturally important plants, including small grains and grasses, legumes, vegetables, ornamental flowers, cotton, peanuts and various weeds (Jeppson *et al.* 1975). Within Australia, they most commonly feed on pasture plants and small grain crops (Table 1.1). Weeks and Hoffmann (1999) were the first to show clear differences in plant preferences for the different *Penthaleus* species. *Penthaleus major* primarily feeds on wild oats and thick-bladed grasses within pastures (Weeks and Hoffmann 1999; Robinson and Hoffmann 2001). *Penthaleus falcatus* predominantly attacks canola and broad-leaved weeds, while *P. sp. x* prefers wheat and thick-bladed grasses (Weeks and Hoffmann 1999; Robinson and Hoffmann 2001). Between 1998 and 2000, Robinson and Hoffmann (2001) conducted a survey to assess the pest status of earth mites within southeastern Australia. *Penthaleus major*, *P. falcatus*, *P. sp. x* and *H. destructor* were all confirmed as pests of economic significance, with damage most commonly found

on oats, wheat, canola, lucerne and pastures. Out of 119 reported mite outbreaks, 21% were caused by *H. destructor*, and the remainder attributed to the *Penthaleus* spp. (Robinson and Hoffmann 2001), clearly showing that in southeastern Australia the *Penthaleus* spp. may be of equivalent or even greater economic importance than *H. destructor*. Further work is required to examine the effects of different plant hosts on each species, as this information is important for developing sustainable management practices such as crop rotations.

1.9 COMPETITION

Competition is an important factor affecting the population dynamics and performance of various animal and plant communities (Connell 1983, Underwood 1986). Despite the applied implications, there have been few experiments investigating the direct role of inter- and intraspecific competition in earth mite populations. The ability of each species to reach high densities makes competition likely when resources, such as food and space, are limiting. Ridsdill-Smith and Annells (1997) suggested that intraspecific competition was responsible for the reduced abundance of *H. destructor* at a pasture site in Western Australia. Weeks and Hoffmann (2000) were the first to directly examine competitive interactions in earth mites within Australia. Using field mesocosms set up on pasture, they manipulated starting densities of *P. major* and *H. destructor* and demonstrated the importance of inter- and intraspecific competition both during the active mite period and over the diapause generation. In field trials in southeastern Australia, Ridsdill-Smith and Pavri (2000b) found effective control of *H. destructor* in spring led to an increase in *Penthaleus* numbers the following autumn, perhaps owing to a reduction in competitive pest pressure. Therefore, suppression or

eradication of one species may result in its displacement by another earth mite species. Weeks and Hoffmann (2000) postulated that *Penthaleus* spp. are less likely to compete with one another than with *H. destructor* because of differences in host plant preferences. However, competitive interactions within the *Penthaleus* spp. complex have not been investigated, despite the implications for pest management.

1.10 CONTROL

1.10.1 Chemical

Chemical control has been, and continues to be, the most common management option for the control of the *Penthaleus* species. Unfortunately, all pesticides in use are only effective against the active stages of mites. The pesticides will not kill aestivating or winter eggs (James 1996). Although recent improvements in application timing have improved the efficacy of pesticides (James and O'Malley 1992; Ridsdill-Smith 1997), there are still sporadic reports of chemical control problems. Chemical sprays are most commonly applied at the time of infestation, when mites are detected at high levels and crops have already shown signs of damage. The majority of pesticides recommended for the *Penthaleus* spp. are organophosphates, such as omethoate and methidathion. Other registered chemicals include pyrethroids, such as bifenthrin and alpha-cypermethrin, which are recommended in many instances because of their increased specificity to pest earth mites and their reduced effect on several acarine predators (James *et al.* 1995).

Pesticides are also used in the prevention of mite infestation and feeding damage. Several alternative methods are available. Pesticides with persistent residual effects can be used as bare earth treatments, before or at the time of sowing to kill emerging

mites and protect the plants throughout their seedling stage where most damage occurs (James and O'Malley 1992). Pesticides with a long residual effect can also be applied as border sprays to prevent mites moving into a crop or pasture. Systemic pesticides are often applied as seed dressings to maintain the pesticide at toxic levels within the plants as they grow. This strategy can be effective in minimising damage to plants during the sensitive establishment phase. However, if mite numbers are high, a substantial amount of damage can still occur before the pesticide has much effect (James 1996). Chemical seed dressings can also be toxic to *Rhizobia* spp. and reduce nodulation and nitrogen fixation of some legume varieties (Evans *et al.* 1991).

Umina and Hoffmann (1999) found in laboratory tests that *P. falcatus* had a much higher tolerance to omethoate, methidathion, bifenthrin and endosulfan than *P. major*, *P. sp. x* and *H. destructor*. This suggests that control strategies based on one species may not be adequate for other earth mite species, and this may help to explain control failures that are not due to poor application. Species also responded differently to alpha-cypermethrin, phosmet, chlorpyrifos and lambda-cyhalothrin (Robinson and Hoffmann 2000), with *P. falcatus* generally the most tolerant and *H. destructor* the least tolerant species. These results allow preliminary recommendations for the control of the different species with pesticides. It appears that bifenthrin, methidathion, endosulfan and phosmet will be the most effective against *P. major* and *P. sp. x*, while it may be difficult to control *P. falcatus* with any of the pesticides, particularly omethoate, unless relatively high concentrations are used. Since chemical control is the only short-term management option available, pesticide resistance in earth mites could pose an emerging problem. Recently, Hoffmann *et al.* (1997) reported tolerance to

omethoate in Victorian populations of *H. destructor*. Resistance to pesticides has also been reported in other species of phytophagous mites (Flexner *et al.* 1995)

Improvements to earth mite management practices that rely solely on chemicals can be made with careful consideration of earth mite ecology and biology. Research has shown a cost-effective means of controlling *H. destructor* is to spray adults just before diapause eggs are produced in spring (Ridsdill-Smith and Pavri 1998). Although this approach (known as Timerite[®]) appears successful against *H. destructor*, spring spraying may not be effective in the control of the *Penthaleus* spp. if the species differ in their timing of diapause egg production. Information regarding the diapause induction in each mite species is critical as Timerite[®] control becomes more common and widespread.

1.10.2 Biological

Differing pesticide tolerance levels in *Penthaleus* spp., evidence of resistance in some *H. destructor* populations (Umina and Hoffmann 1999; Hoffmann *et al.* 1997), and public concerns about the environmental impact and safety of chemical applications, are driving research into alternative sustainable methods for control, including biological control. Wallace introduced *Anystis wallacei*, a known predator of *Penthaleus* spp. in France, into Western Australia in 1965 (Michael *et al.* 1991). *Anystis wallacei* established successfully and reduced pest numbers. Because of a very low migration rate, subsequent attempts have been made to introduce *A. wallacei* into new areas (Gardner and Gardner 1994). The predicted benefits of *A. wallacei* are yet to be demonstrated.

James (1995) identified a complex of natural enemies that operate against the *Penthaleus* species. Although no systematic survey has been conducted, at least 19 predators and one pathogen are known to attack mites in southern New South Wales (James 1995). A detailed understanding of this complex of species would facilitate effective pest management programs that are not based solely on chemical control. The most important predators appear to be from the mite families Anystidae, Bdellidae, Erythraeidae, Parasitidae and Cunaxidae (James 1995). Weiss and McDonald (1998) have also postulated that the European earwig, *Forficula auricularia*, may contribute to regulating populations of earth mites and other micro-invertebrate pests in pastures. Preserving natural enemies when using chemicals may prevent population explosions in established pastures, although this is difficult since the pesticides generally used to control the *Penthaleus* spp. and related pests are broad spectrum (James 1996). Microbial pesticides, using viral, bacterial or fungal pathogens, are a relatively new strategy also being investigated (Say *et al.* 1994). Several fungi have been identified as pathogenic to earth mites and considered for biological control (Hastings 1994; Say *et al.* 1994). Entomopathogenic fungi usually infect their hosts through specialised spores, which attach to, germinate on, and penetrate the integument (Chandler *et al.* 2000). The *Penthaleus* spp. make good hosts for fungal pathogens because they are soft bodied and inhabit environments with humid microclimates (Ferro and Southwick 1984; Evans 1992). The pathogen, *Neozygites acaracida*, is prevalent amongst the *Penthaleus* spp. during wet winters and could be responsible for ‘population crashes’ (James 1995), although no data are available to support this conjecture.

1.10.3 Host plant resistance

The development of host plant resistance is an important part of the current approach to reducing losses caused by earth mites. Several screening programs have been completed in Australia to identify resistant plant varieties, mainly against *H. destructor* (Ridsdill-Smith 1997). Sub clover varieties, legume species, annual medics and canola accessions have been screened for damage resistance to *H. destructor* feeding (Gillespie 1993; Gillespie 1994; Lake and Howie 1995; Ridsdill-Smith *et al.* 1995; Moritz and McDonald 1995). Chemical and physical mechanisms of resistance to *H. destructor* have been identified for sub clover, which contains antifeedant properties, with the basis of resistance being antixenosis (Jiang and Ridsdill-Smith 1996). Increased strength of the cotyledon results in reduced damage because mites cannot penetrate the epidermis of the resistant varieties (Jiang and Ridsdill-Smith 1996). Levels of the volatile compound 1-octen-3-one have also been correlated with reduced mite feeding (Jiang *et al.* 1996). It is unknown whether these mechanisms also reduce feeding damage by the *Penthaleus* species. With gene transfer technology now available, work has also started to identify plant proteins that may confer resistance to mite attack (Higgins and Ridsdill-Smith 1994; Weinman *et al.* 1995). These studies are yet to reach the field-testing stage. The identification and use of resistant plant varieties is an attractive option for minimising earth mite damage because it is cost effective and willingly adopted by farmers (Gillespie 1994). However, the *Penthaleus* spp. have different plant hosts and therefore need to be considered separately from *H. destructor*. Resistance to *H. destructor* may not necessarily confer resistance to all three (or any) *Penthaleus* species.

1.10.4 Crop rotations

An effective way of controlling any agricultural pest is to rotate crops or pastures with non-host crops (Pimentel 1993). McDonald *et al.* (1995) suggested wheat, lupins and oats could act as rotation crops to minimise in-paddock infestations of earth mites, however, they did not consider the *Penthaleus* species. Potted plant experiments indicated that *P. major* and *P. sp. x* could reproduce on oats and wheat, while *P. falcatus* was unable to successfully reproduce on oats, wheat, barley or canola (Robinson and Hoffmann 2001). While this provides some information to make control recommendations, conflicting results (McDonald *et al.* 1995; Robinson and Hoffmann 2001) highlight the need for further studies.

1.10.5 Other cultural controls

Cultural control techniques including tillage practices, trap and border crops, and mixed cropping can reduce overall infestation levels to below economic damage thresholds, particularly when employed in conjunction with other means of control (Pimentel 1993). The aims of cultural control include reduced pest colonisation, reduced reproduction and survival, an increase in pest dispersal from the system, or a combination of these (Dent 1991). The effectiveness of border trials preventing earth mite reinvasion has been assessed. Merton *et al.* (1995) found that a 5-metre wide wheat border around a canola crop was unsuccessful in preventing the movement of mites into the crop, although recent dispersal data suggests that the border should have been wider (Weeks *et al.* 2000). Appropriate grazing management can be used to significantly reduce *Penthaleus* populations to below damaging thresholds (Grimm *et al.*, 1995). This is likely owing to shorter pasture having reduced relative humidity, which increases mite mortality and limits food resources (Ridsdill-Smith 1997).

Pesticide border sprays may prevent movement of *Penthaleus* spp. into crops from adjacent paddocks harbouring large populations. In two experiments conducted in Victoria, Australia, using wheat and canola crops, borders sprayed with omethoate and repeated over two field seasons were effective at widths greater than 15 metres (unpublished data).

Weed management, in particular management of broad-leaved weeds, has been shown to reduce mite populations (Swan 1934). Weeds and/or other non-vascular flora can provide an alternative food source, particularly for juvenile stages, and a more suitable environment for mites within a crop or pasture (McDonald *et al.*, 1995; Maclellan *et al.*, 1998). Cultivation (Tucker 1925; Merton *et al.* 1995), summer pasture burning (Swan 1934; Wallace 1961), maintaining fallow land (Newman 1923; Swan 1934), and flooding of summer pastures (James and O'Malley 1991) can also reduce mite numbers, however, these are not usually considered practical for current farming methods.

1.11 OUTLINE OF THIS STUDY

The overall aim of this study was to look at various aspects of the biology and ecology of *Penthaleus* spp. and *H. destructor*, with particular emphasis on improving pest management.

Chapter 2 examines the timing of diapause egg production in *H. destructor* and the three pest *Penthaleus* species. Field experiments were conducted in two consecutive years in a pasture paddock, and a shade-house experiment was used to examine

diapause strategies from several geographically different sites. The effectiveness of a ‘carefully timed’ spring spray against each mite species was also examined. Chapter 3 considers the effects of different plant hosts on the persistence and reproduction of earth mites using shade-house and field experiments. Five plant treatments were examined. Feeding damage to plants was assessed as well as mite numbers. The results of an extensive survey looking at field associations of mites with pasture, weed and crop plants, are also described. The implications of the findings are discussed with reference to the direction of future research and control options.

Chapter 4 examines the effects of inter- and intraspecific competition on the population dynamics of earth mites. This extends the findings of Weeks and Hoffmann (2000) to include all pest species. Competitive interactions were examined both in the shade-house and in the field, using four different plant treatments. The field experiment was conducted across several seasons to investigate the effects of annual environmental variation. Chapter 5 describes experiments designed to identify mite proteins for use in the development of a field-based species identification kit. Proteomic and immunological techniques were used to characterise mite extracts, compare species’ profiles and isolate species-specific proteins and antibodies. Directions that this research should take are considered.

Chapter 6 overviews the results of the four experimental chapters and provides recommendations for future work.

1.12 REFERENCES CITED

André M. 1932. Note sur un acarien (*Penthaleus major* Dugés) nuisible aux plantes potagères. Bulletin du Musée National d'Histoire Naturelle (Paris) 4: 284-291.

Banks N. 1902. New genera and species of acarians. Canadian Entomologist 34: 172.

Banks N. 1917. New mites, mostly economic (Arach. Acar.). Entomological News 28: 193-199.

Chada H. L. 1956. Biology of the winter grain mite and its control on small grains. Journal of Economic Entomology 49: 515-520.

Chandler D., Davidson G., Pell J. K., Ball B. V., Shaw K. and Sunderland K. D. 2000. Fungal biocontrol of Acari. Biocontrol Science and Technology 10: 357-384.

Connell J. H. 1983. On the prevalence and relative importance of interspecific competition: evidence from field experiments. American Naturalist 122: 661-696.

Darlington C. D. 1939. The evolution of genetic systems. Cambridge University Press, Cambridge, UK.

Dent. D. 1991. Insect pest management. CAB International, Wallingford, UK.

Dugés A. 1834. Recherches sur l'ordre des Acariens. Annales des Sciences Naturelles, Zoologie 2: 18-63.

Evans G. O. 1992. Principles of Acarology. CAB International, Wallingford, UK.

Evans J., Seidel J., O'Connor G. E., Watt J. and Sutherland M. 1991. Using omethoate insecticide and legume inoculant on seed. Australian Journal of Experimental Agriculture 31: 71-76.

Ferro D. N. and Southwick E. E. 1984. Microclimates of small arthropods: estimating humidity within the leaf boundary layer. Environmental Entomology 13: 926-929.

Flexner J. L., Westigard P. H., Hilton R. and Croft B. A. 1995. Experimental evaluation of resistance management for twospotted mite (Acari: Tetranychidae) on southern Oregon pear: 1987-1993. Journal of Economic Entomology 88: 1517-1524.

Fox J. A., Dybdahl M. F., Jokela J. and Lively C. M. 1996. Genetic structure of coexisting sexual and clonal subpopulations in a freshwater snail (*Potamopygrus antipodarum*). Evolution 50: 1541-1548.

Froggatt W. W. 1921. Blue oat mite (*Notophallus bicolor*, n. sp.). Agricultural Gazette of New South Wales 32: 33-34.

Gardner W. K. and Gardner J. A. 1994. Introduction and spread of *Anystis* and *Neomolgus* in Victoria. In: McDonald G. and A.A. Hoffmann (eds.), Proceedings of

the Second National Workshop on Redlegged Earth Mite, Lucerne Flea and Blue Oat Mite. Department of Agriculture, Rutherglen, Australia, pp. 68.

Gaull K. R. and Ridsdill-Smith T. J. 1996. The foraging behaviour of redlegged earth mite, *Halotydeus destructor* (Acarina: Pentheleidae), in an annual subterranean clover pasture. Bulletin of Entomological Research 86: 247-252.

Gaull K. R. and Ridsdill-Smith T. J. 1997. Host plant acceptance by redlegged earth mite, *Halotydeus destructor* (Tucker) (Acarina: Pentheleidae). Journal of Insect Behavior 10: 859-869.

Gillespie D. J. 1993. Redlegged earth mite (*Halotydeus destructor*) resistance in annual pasture legumes. In: Delfosse E. (ed.), Pests of pastures: weed, invertebrate and disease pests of Australian sheep pastures. CSIRO Australia, Melbourne, Australia, pp. 211-213.

Gillespie D. J. 1994. Identification and development of annual legume varieties with resistance to redlegged earth mite. In: McDonald G. and A.A. Hoffmann (eds.), Proceedings of the Second National Workshop on Redlegged Earth Mite, Lucerne Flea and Blue Oat Mite. Department of Agriculture, Rutherglen, Australia, pp. 1-4.

Grimm M., Michael P., Hyder M. and Doyle P. 1995. Effects of pasture pest damage and grazing management on efficiency of animal production. Plant Protection Quarterly 10: 62-64.

Gudleifsson B. E. and Ólafsson S. 1987. Grasmaurar. Freyr 83: 356-358.

Gudleifsson B. E., Hallas T. E., Ólafsson S. and Sveinsson T. 2002. Chemical control of *Penthaleus major* (Acari: Prostigmata) in hayfields in Iceland. Journal of Economic Entomology 95: 307-312.

Hastings C. 1994. *Penthaleus major*. An occasional little-known pest. Phytoma 465: 43-44.

Higgins T. J. and Ridsdill-Smith T. J. 1994. Preliminary testing of proteins with potential for the design of genetically engineered subterranean clovers with protection against redlegged earth mite. In: McDonald G. and A.A. Hoffmann (eds.), Proceedings of the Second National Workshop on Redlegged Earth Mite, Lucerne Flea and Blue Oat Mite. Department of Agriculture, Rutherglen, Australia, pp. 8.

Hoekstra R. F. 1978. Sufficient conditions for polymorphism with cyclical selection in a subdivided population. Genetical Research 31: 67-73.

Hoffmann A. A., Porter S. and Kovacs I. 1997. The response of the major crop and pasture pest, the red-legged earth mite (*Halotydeus destructor*) to pesticides: dose-response curves and evidence for tolerance. Experimental and Applied Acarology 21: 151-162.

James D. G. 1995. Biological control of earth mites in pasture using endemic natural enemies. Plant Protection Quarterly 10: 58-59.

James D. G. 1996. Integrated management of redlegged earth mite in pasture. *In*: Virgona J. and D. Michalk (eds.), Proceedings of the Eleventh Annual Conference of the Grassland Society of New South Wales. The Grassland Society of New South Wales, Orange, Australia, pp. 76-83.

James D. G. and O'Malley K. J. 1991. Oversummering of eggs of *Halotydeus destructor* (Tucker) (Acarina: Penthaleidae): Diapause termination and mortality. Australian Entomological Magazine 18: 35-41.

James D. G. and O'Malley K. J. 1992. Control of redlegged earth mite *Halotydeus destructor* on bare earth. Plant Protection Quarterly 7: 10-11.

James D. G. and O'Malley K. J. 1993. Phenology of egg production and diapause in *Halotydeus destructor* Tucker and *Penthaleus major* Dugés (Acari: Penthaleidae) in southern New South Wales during 1988/89. General and Applied Entomology 24: 33-38.

James D. G., O'Malley K. and Rayner M. 1995. Effect of alphacypermethrin and bifenthrin on the survival of five acarine predators of *Halotydeus destructor* (Acari: Penthaleidae). Experimental and Applied Acarology 19: 647-654.

Jeppson L. R., Keifer H. H. and Baker E. W. 1975. Mites Injurious to Economic Plants. University of California Press, Berkeley, America.

Jiang Y. and Ridsdill-Smith T. J. 1996. Examination of the involvement of mechanical strength in antixenotic resistance of subterranean clover cotyledons to the redlegged earth mite *Halotydeus destructor* (Acarina: Pentheleidae). Bulletin of Entomological Research 86: 263-270.

Jiang Y., Ridsdill-Smith T. J. and Ghisalberti E. L. 1996. Assays for the effects of volatile compounds from artificially damaged cotyledons of subterranean clover on the redlegged earth mite, *Halotydeus destructor*. Experimental and Applied Acarology 20: 61-72.

Jiang Y., Ridsdill-Smith T. J. and Ghisalberti E. L. 1997. The effect of volatile metabolites of lipid peroxidation on the aggregation of redlegged earth mites *Halotydeus destructor* (Acarina: Pentheleidae) on damaged cotyledons of subterranean clover. Journal of Chemical Ecology 23: 163-174.

Jokela J., Lively C. M., Fox J. A. and Dybdahl M. F. 1997. Flat reaction norms and “frozen” phenotypic variation in clonal snails (*Potamopyrgus antipodarum*). Evolution 51: 1120-1129.

Johansen T. J. 1986. Middskader I eng I Nord-Norge. Norden 90: 17, 34.

Judson O. P. and Normark B. B. 1996. Ancient asexual scandals. Trends in Ecology and Evolution 11: 41- 46.

Kanda K. and Hirai Y. 1990. Outbreak of the winter grain mite, *Penthaleus major* (Dugés) in forage grasses in Nasu district. Japanese Journal of Applied Entomology and Zoology 34: 79-81.

Kanda K., Hirai Y., Takahashi K., Kobayashi J. and Ohtomo K. 1992. Cultural control of the winter grain mite, *Penthaleus major* (Dugés) in cool season grass. Japanese Journal of Applied Entomology and Zoology 36: 153-157.

Krantz G. W. 1978. A manual of Acarology, 2nd edition. Oregon State University Book Stores, Corvallis, Oregon, America.

Lake A. W. H. and Howie J. H. 1995. Selection for redlegged earth mite resistance in annual *Medicago* species. Plant Protection Quarterly 10: 45-46.

MacLennan K. E., McDonald G. and Ward S. A. 1998. Soil microflora as hosts of redlegged earth mite (*Halotydeus destructor*). Entomologia Experimentalis et Applicata 86: 319-323.

Mansingh A. 1971. Physiological classification of dormancies in insects. The Canadian Entomologist 103: 983-1009.

Masaki S. and Wipking W. 1994. Insect life-cycle polymorphism: Introduction. *In*: Danks H.V. (ed.), Insect life-cycle polymorphism: theory, evolution and ecological consequences for seasonality and diapause control. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp. 1-3.

Maynard Smith J. 1978. The evolution of sex. Cambridge University Press, Cambridge, UK.

McDonald G., Moritz K., Merton E. and Hoffmann A. A. 1995. The biology and behaviour of redlegged earth mite and blue oat mite on crop plants. Plant Protection Quarterly 10: 52-55.

Merton E., McDonald G. and Hoffmann A. 1995. Cultural control of redlegged earth mite, blue oat mite and lucerne flea in canola. Plant Protection Quarterly 10: 65-66.

Michael P. J., Dutch M. E. and Pekin C. J. 1991. A review of the predators of redlegged earth mite, blue oat mite and lucerne flea. *In*: Ridsdill-Smith T.J. (ed.), Proceedings of a National Workshop on Redlegged Earth Mite, Lucerne Flea and Blue Oat Mite. Department of Agriculture, South Perth, Australia, pp. 115-120.

Middlekauff W. W. and Pritchard A. E. 1949. Field tests to control the mite *Penthaleus major*. Journal of Economic Entomology 42: 852.

Moritz K. and McDonald G. 1995. Developing redlegged earth mite resistance in canola. *In*: Potter T.D. (ed.), Proceedings of the Australian Research Assembly on Brassicas. South Australian Research and Development Institute, Adelaide, Australia, pp. 30-35.

Murray A. 1877. Economic Entomology, Aptera. Chapman and Hall, Piccadilly, UK.

- Narayan D. S. 1957. A new species of *Penthaleus* from Mexico (Acarina: Penthaleidae). Journal of Kansas Entomological Society 30: 111-113.
- Narayan D. S. 1962. Morphological, biological and ecological studies on the winter grain mite, *Penthaleus major* (Dugés), Penthaleidae; Acarina Part 1. Journal of Zoological Society of India 14: 45-63.
- Neilsen P. 1984. Entomologiske undersøgelser I og omkring fåreholderområdet I Sydrönland. Arbejdsgruppen Vedrørende Miljø og Fåreavl. Rapport nr. 2. 28 p.
- Newman L. J. 1923. Red-legged velvet earth mite. Western Australian Department of Agriculture Bulletin 106: 1-4.
- Norris K. R. 1950. The aestivating eggs of the red-legged earth mite, *Halotydeus destructor* (Tucker). Bulletin Commonwealth Scientific and Industrial Research Organisation, No. 253.
- Norton R. A., Kethley J. B., Johnston D. E. and O'Connor B. M. 1993. Phylogenetic perspectives on genetic systems of reproductive modes of mites. In: Wrensch D.L. and M.A. Ebbert (eds.), Evolution and diversity of sex ratio in mites and insects. Chapman & Hall, New York, America, pp. 8-99.
- Nuzzaci G. and de Lillo E. 1991. Contributo alla conoscenza delle parti boccali di *Penthaleus major* (Duges) (Acari: Eupodoidea Penthaleidae). Atti XVI Congresso Nazionale Italiano di Entomologia. Bari-Martina, Franca (Ta): 265-277.

Pescott R. T. M. 1934. The Pea Mite and Red Legged Earth Mite. Journal of Agriculture Victoria, June: 295-297.

Pimentel D. 1993. Cultural controls for insect pest management. *In*: Corey S.A, Dall D.J. and W.M. Milne (eds.), Pest control and sustainable agriculture. CSIRO Australia, Canberra, Australia, pp. 35-38.

Qin T. K. 1996. A review and cladistic analysis of the Eupodoidea (Acari: Acariformes). Systematic and Applied Acarology 1: 77-105.

Qin T. K. 1998. A checklist and key to species of Eupodoidea (Acari: Prostigmata) from Australia and New Zealand and their subantarctic islands. Journal of The Royal Society of New Zealand 28: 295-307.

Qin T. K. and Halliday R. B. 1995. Systematic studies of redlegged earth mite *Halotydeus destructor* (Tucker) and related species (Acarina: Penthaleidae). Plant Protection Quarterly 10: 50-52.

Qin T. K. and Halliday R. B. 1996a. The Australian species of *Chromotydeus* Berlese and *Penthaleus* Koch, C.L. (Acarina, Penthaleidae). Journal of Natural History 30: 1833-1848.

Qin T. K. and Halliday R. B. 1996b. Revision of the Australian and South African species of *Halotydeus* (Acarina: Penthaleidae). Bulletin of Entomological Research 86: 441-450.

Qin T. K. and Halliday R. B. 1997. Eriorhynchidae, a new family of Prostigmata (Acarina), with a cladistic analysis of eupodoid species of Australia and New Zealand. Systematic Entomology 22: 151-171.

Ridsdill-Smith T. J. 1991. Biology and ecology of redlegged earth mite, blue oat mite and lucerne flea. *In*: Ridsdill-Smith T.J. (ed.), Proceedings of a National Workshop on Redlegged Earth Mite, Lucerne Flea and Blue Oat Mite. Department of Agriculture, Perth, Australia, pp. 36-41.

Ridsdill-Smith T. J. 1997. Biology and control of *Halotydeus destructor* (Tucker) (Acarina: Penthaleidae) – a review. Experimental and Applied Acarology 21: 195-224.

Ridsdill-Smith T. J. and Annells A. J. 1997. Seasonal occurrence and abundance of redlegged earth mite *Halotydeus destructor* (Acari, Penthaleidae) in annual pastures of south western Australia. Bulletin of Entomological Research 87: 413-423.

Ridsdill-Smith T. J. and Gaul K. R. 1995. An improved method for rearing *Halotydeus destructor* (Acari: Penthaleidae) in the laboratory. Experimental and Applied Acarology 19: 337-345.

Ridsdill-Smith T. J. and Pavri C. 1998. Spring spraying for redlegged earth mites. Western Focus (Australian Grain), October-November: 1-4.

- Ridsdill-Smith T. J. and Pavri C. C. 2000a. Feeding life style of redlegged earth mite, *Halotydeus destructor* (Acari: Penthaleidae), in pastures and the role of broad-leafed weeds. *Experimental and Applied Acarology* 24: 397-414.
- Ridsdill-Smith T. J. and Pavri C. 2000b. Single spring spray protects pastures. *Farming Ahead* 103: 60-63.
- Ridsdill-Smith T. J., Jiang Y. and Ghisalberti E. L. 1995. A method to test compounds for feeding deterrence towards redlegged earth mites (Acarina: Penthaleidae). *Annals Applied Biology* 127: 593-600.
- Robinson M. T. and Hoffmann A. A. 2000. Additional tests on the effects of pesticides on cryptic species of blue oat mite (*Penthaleus* spp.) and the redlegged earth mite (*Halotydeus destructor*). *Australian Journal of Experimental Agriculture* 40: 671-678.
- Robinson M. T. and Hoffmann A. A. 2001. The pest status and distribution of three cryptic blue oat mite species (*Penthaleus* spp.) and the redlegged earth mite (*Halotydeus destructor*) in southeastern Australia. *Experimental and Applied Acarology* 25: 699-716.
- Robinson M. T., Weeks A. R. and Hoffmann A. A. 2002. Geographic patterns of clonal diversity in the earth mite species *Penthaleus major* with particular emphasis on species margins. *Evolution* 56: 1160-1167.

Sabelis M. W. and Dickie M. 1985. Long-range dispersal and searching behaviour. *In*: Helle W. and M.W. Sabelis (eds.), Spider mites, their biology, natural enemies and control. Elsevier, Amsterdam, The Netherlands, pp. 141-160.

Say M. M., Rath A. C. and Carr C. 1994. Progress in the development of entomopathogenic fungi for control of redlegged earth mites, *Halotydeus destructor* (Tucker) and lucerne flea, *Sminthurus viridis* (L.). *In*: McDonald G. and A.A. Hoffmann (eds.), Proceedings of the Second National Workshop on Redlegged Earth Mite, Lucerne Flea and Blue Oat Mite. Department of Agriculture, Rutherglen, Australia, pp. 63-66.

Semlitsch R. D., Hotz H. and Guex G. D. 1997. Competition among tadpoles of coexisting hemiclones of hybridogenetic *Rana esculenta*: support for the frozen niche variation model. *Evolution* 51: 1249-1261.

Swan D. C. 1934. The red-legged earth mite, *Halotydeus destructor* (Tucker) in South Australia: with remarks upon *Penthaleus major* (Dugés). *Journal of Agriculture South Australia* 38: 353-367.

Tauber M. J., Tauber C. A. and Masaki S. 1984. Adaptations to hazardous seasonal conditions: dormancy, migration, and polyphenism. *In*: Huffaker C.B. and R.L. Rabb (eds.), *Ecological Entomology*. John Wiley and Sons, New York, America, pp. 149-183.

Thackray D. J., Ridsdill-Smith T. J. and Gillespie D. J. 1997. Susceptibility of grain legume species to redlegged earth mite (*Halotydeus destructor*) damage at the seedling stage. Plant Protection Quarterly 12: 141-144.

Thor S. and Willmann C. 1941. Acarina: Eupodidae, Penthaleidae, Penthaleidae, Rhagidiidae, Pachygnathidae, Cunaxidae. Das Tierreich 71a: 1-186.

Thorell T. 1872. Ofversigt of Kongliga Vetenskaps. Akademiens Forhandlingar 8: 702.

Tucker R. W. E. 1925. The black sand mite: *Penthaleus destructor* n. sp. Union South Africa Department of Agriculture, Entomological Memoirs No. 3: 23-26.

Umina P. A. and Hoffmann A. A. 1999. Tolerance of cryptic species of blue oat mites (*Penthaleus* spp.) and the redlegged earth mite (*Halotydeus destructor*) to pesticides. Australian Journal of Experimental Agriculture 39: 621-628.

Underwood T. 1986. The analysis of competition by field experiments. In: Kikkawa J. and D.J. Anderson (eds.), Community ecology: pattern and process. Blackwell Scientific, Oxford, UK, pp. 29-31.

Vrijenhoek R. C. 1978. Coexistence of clones in a heterogeneous environment. Science 199: 549-552.

Vrijenhoek R. C. 1984. Ecological differentiation among clones: the frozen niche variation model. *In*: Wohrmann K. and V. Loeschcke (eds.), Population biology and evolution. Springer-Verlag, Berlin, Germany, pp. 217-231.

Wallace M. M. H. 1961. Pasture burning and its effect on the aestivating eggs of *Halotydeus destructor* (Tucker). Australian Journal of Experimental Agriculture and Animal Husbandry 1: 109-111.

Wallace M. M. H. 1970a. Diapause in the aestivating egg of *Halotydeus destructor* (Acari: Eupodidae). Australian Journal of Zoology 18: 295-313.

Wallace M. M. H. 1970b. The influence of temperature on post-diapause development and survival in the aestivating eggs of *Halotydeus destructor* (Acari: Eupodidae). Australian Journal of Zoology 18: 315-329.

Wallace M. M. H. and Mahon J. A. 1971. The distribution of *Halotydeus destructor* and *Penthaleus major* (Acari: Eupodidae) in Australia in relation to climate and land use. Australian Journal of Zoology 19: 65-76.

Weeks A. R. and Hoffmann A. A. 1998. Intense selection of mite clones in a heterogeneous environment. Evolution 52: 1325-1333.

Weeks A. R. and Hoffmann A. A. 1999. The biology of *Penthaleus* species in southeastern Australia. Entomologia Experimentalis et Applicata 92: 179-189.

Weeks A. R. and Hoffmann A. A. 2000. Competitive interactions between two pest species of earth mites, *Halotydeus destructor* and *Penthaleus major* (Acarina: Penthaleidae). Journal of Economic Entomology 93: 1183-1191.

Weeks A. R. and Hoffman, A. A. in press. Frequency-dependent selection maintains clonal diversity in asexual organisms: evidence from natural populations of the earth mites species *Penthaleus major*. Proceedings of the National Academy of Sciences America.

Weeks A. R., Fripp Y. J. and Hoffmann A. A. 1995. Genetic structure of *Halotydeus destructor* and *Penthaleus major* populations in Victoria (Acari: Penthaleidae). Experimental and Applied Acarology 19: 633-646.

Weeks A. R., Turelli M. and Hoffmann A. A. 2000. Dispersal patterns of pest earth mites (Acari: Penthaleidae) in pastures and crops. Journal of Economic Entomology 93: 1415-1423.

Weinman J. J., Djordjevic M. A., Creaser E. H., Lawson C. G. R., Broderick K., Mathesius U., Gärtner E., Pittock C., de Majnik J. and Rolfe B. G. 1995. Preparing subterranean clovers for future biotechnology: molecular analysis of genes and proteins involved in stress and defence reactions and the construction of transgenic plants. Plant protection Quarterly 10: 47-49.

Weiss M. J. and McDonald G. 1998. European earwig, *Forficula auricularia* L. (Dermaptera: Forficulidae), as a predator of the redlegged earth mite, *Halotydeus*

destructor (Tucker) (Acarina: Pentheleidae). Australian Journal of Entomology 37: 183-185.

White M. J. D. 1973. Animal cytology and evolution. Cambridge University Press, Cambridge, UK.

Womersley H. 1935. On the name of the 'blue oat mite' of Australia. Bulletin of Entomological Research 26: 163.

1.13 TABLE AND FIGURES

Table 1.1: List of agriculturally important plants that are commonly attacked by *Penthaleus* spp. and *H. destructor* within Australia.

Species	<i>P. major</i>	<i>P. falcatus</i>	<i>P. sp. x</i>	<i>H. destructor</i>
Thick-bladed grasses (<i>Poaceae</i> spp.)	+	+	+	+
Rye grasses (<i>Lolium</i> spp.)	+	-	+	+
Phalaris (<i>Phalaris aquatica</i> L.)	+	-	-	+
Clovers (<i>Trifolium</i> spp.)	-	-	-	+
Oats (<i>Avena sativa</i> L.)	+	-	+	+
Wheat (<i>Triticum aestivum</i> L.)	+	+	+	+
Lucerne (<i>Medicago sativa</i> L.)	+	?	+	+
Peas (<i>Pisum</i> spp.)	+	+	?	+
Poppies (<i>Papaver</i> spp.)	-	+	-	+
Canola (<i>Brassica napus</i> L.)	-	+	-	+
Bristly ox-tongue (<i>Picris echioides</i> L.)	-	+	-	+
Cats ear (<i>Hypochoeris</i> spp.)	-	+	-	+
Cape weed (<i>Arctotheca calendula</i> L.)	-	-	-	+

+ = commonly attacked; - = not commonly attacked; ? = uncertain.

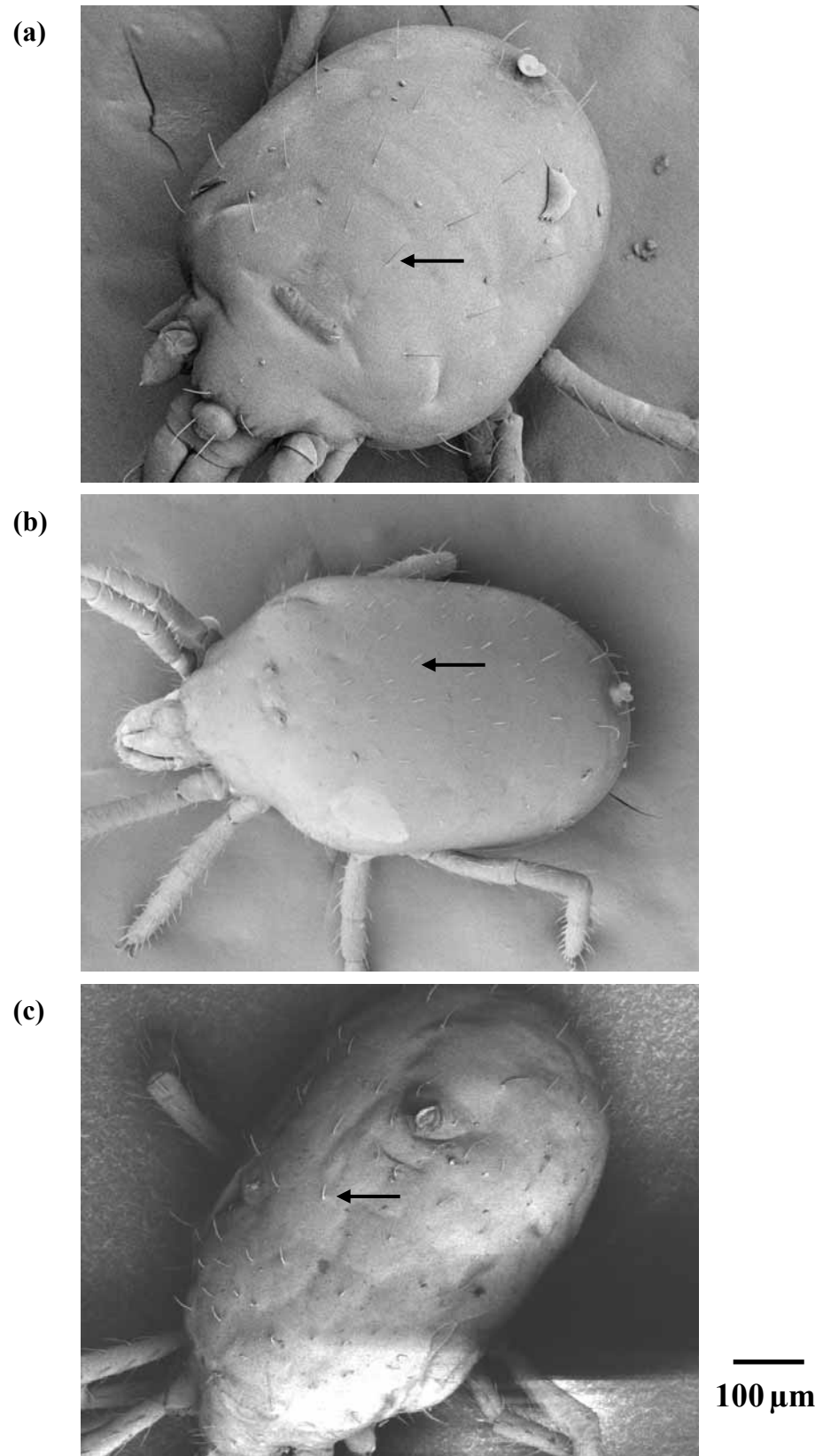


Figure 1.1: Scanning electron micrographs of (a) *P. major*, (b) *P. falcatus* and (c) *P. sp. x*, showing differences in the morphology and arrangement of dorsal setae. Arrows indicate dorsal setae.

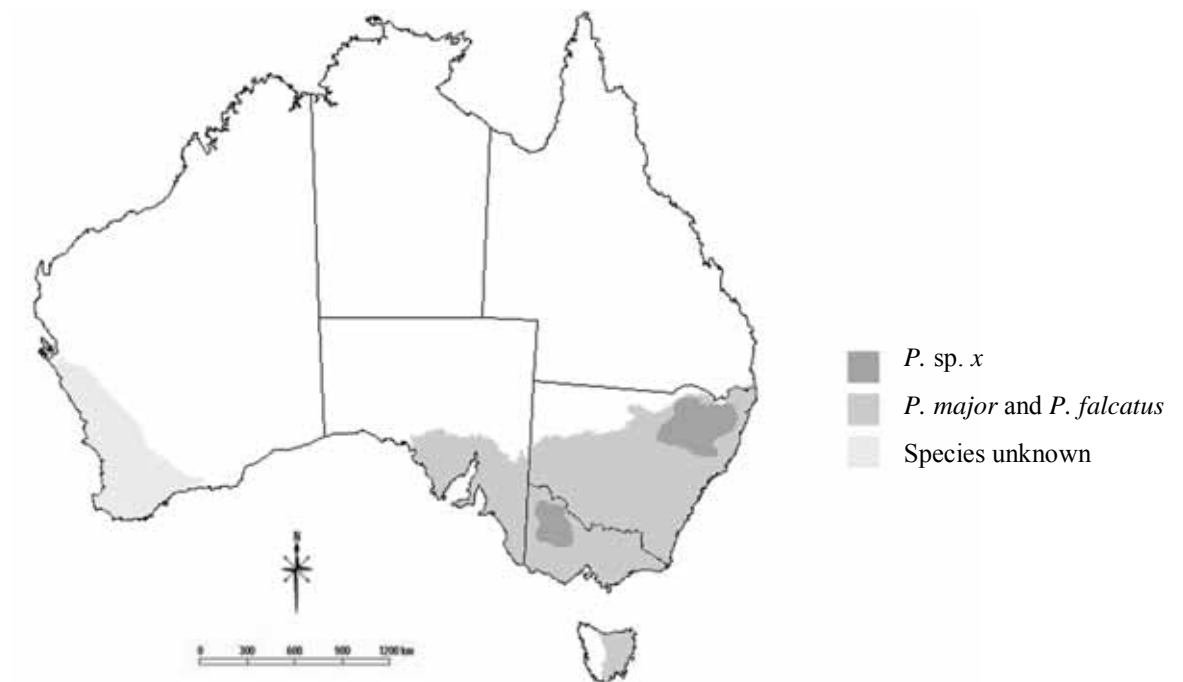
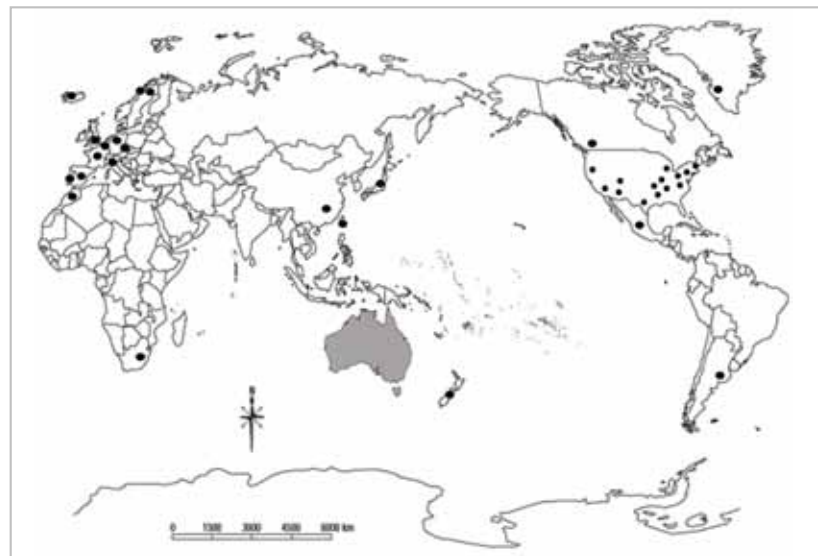


Figure 1.2: Map of Australia and the world showing the distribution of the pest *Penthaleus* species. Closed circles indicate the known presence throughout the world.

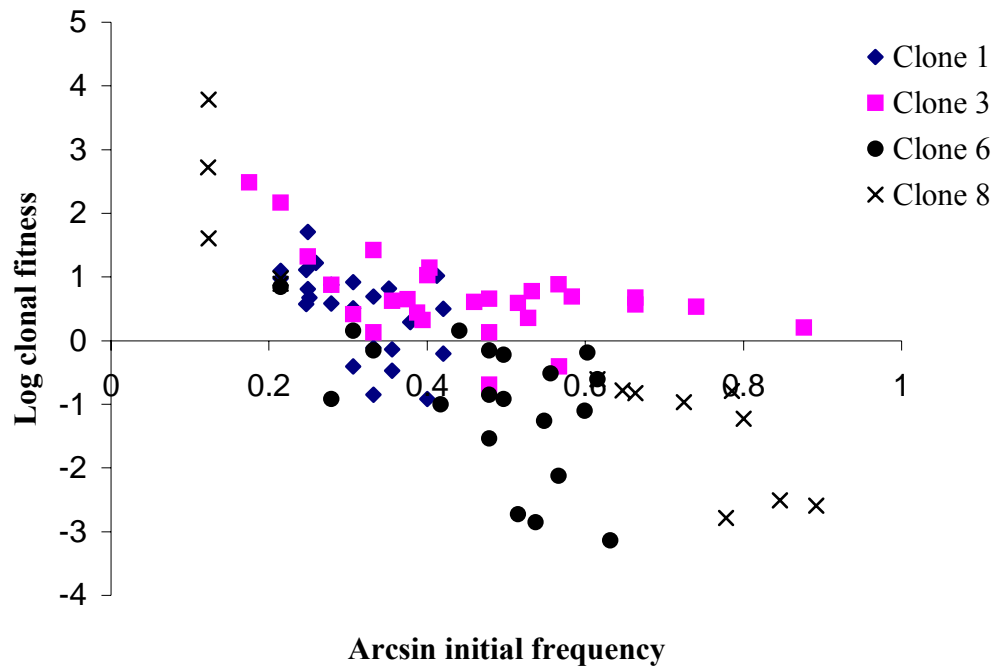


Figure 1.3: Linear regression showing the negative relationship between clonal fitness and initial frequency of four common clones reciprocally translocated among three geographically distant sites. The data are taken from Weeks and Hoffmann (in press) and regressions for each clone have been combined. Fitness estimates were natural log transformed (y axis) and initial frequencies were arcsine transformed (x axis). All regressions (for each clone) are significant. See Weeks and Hoffmann (in press) for further explanation.

CHAPTER 2.

Diapause and implications for control of *Penthaleus* species and *Halotydeus destructor* in southeastern Australia

2.1 ABSTRACT

Blue oat mites, *Penthaleus* spp., and redlegged earth mites, *Halotydeus destructor* (Tucker) are major winter pests of a variety of crops and pastures. In southern Australia these earth mites exhibit a facultative egg diapause to survive unfavourable summer conditions. The initiation of diapause egg production in earth mites was investigated using field and shade-house experiments. Species differed in their timing of diapause. *Halotydeus destructor* mainly produced diapausing eggs towards the end of the active mite season in spring, although small numbers were also produced in winter. In contrast, *Penthaleus major* (Dugés) produced diapause eggs almost immediately after emergence in autumn and continued producing these eggs throughout the season. *Penthaleus falcatus* (Qin and Halliday) also produced diapause eggs in early winter, although the first appearance of these eggs was slightly later in the season than for *P. major*. The diapause response of an undescribed species was also somewhat later than in *P. major* and *P. falcatus*, but earlier than in *H. destructor*. Electrophoresis of *P. major* samples indicated that clones of this parthenogenic species may differ in the timing of diapause egg production, providing another potential selective factor contributing to the maintenance of clonal diversity within this group. These results highlight the importance of determining species composition when devising control strategies for earth mite outbreaks.

2.2 INTRODUCTION

Redlegged earth mites, *Halotydeus destructor* (Tucker) (Acari: Penthaleidae), and blue oat mites, *Penthaleus* spp. (Acari: Penthaleidae), are important agricultural pests in southern Australia. They attack a variety of winter crops and pastures, causing reduced crop yields and reduced feed for livestock. Earth mites are restricted to regions with a Mediterranean-type climate, characterised by hot dry summers and cool moist winters (Wallace and Mahon 1971; Ridsdill-Smith and Annells 1997). During spring and summer the annual vegetation dies off, humidity levels drop and temperatures rise. Earth mites overcome these adverse conditions by producing aestivating or diapausing eggs (Wallace 1970a).

Earth mites are active from April to November, passing through two or three generations in the field, with each generation lasting six to eight weeks (Halliday 1991; Ridsdill-Smith 1997; Weeks and Hoffmann 1999). It is thought that the first two generations lay non-diapausing eggs on foliage, and then, in early spring, oviposition ceases and diapause eggs are produced (Wallace 1970b). Diapause eggs are either retained in adult bodies (*H. destructor*), or are laid on the soil surface and/or base of plants (*Penthaleus* spp.). For *H. destructor*, diapause eggs are thought to be produced when the weekly maximum temperature reaches 20°C (Ridsdill-Smith and Annells 1997). Diapause development is completed after exposure to high temperatures (> 50°C) and high relative humidity (Wallace 1970a). The aestivating eggs, which are resistant to heat, drought and desiccating winds (Wallace 1970a), hatch in autumn following rainfall and a drop in temperature. In Western Australia, the first appearance

of *H. destructor* in pasture can be predicted by the first week at which the weekly maximum temperature falls below 21.5°C (Ridsdill-Smith and Annells 1997).

Currently, chemicals are the most commonly used control option against earth mites. However, pesticides are often applied in an ad hoc fashion with farmers sometimes applying four or five pesticide sprays in a single season. Control failures following pesticide application are becoming prevalent and may be explained by the differing tolerance of earth mite species or the development of resistance (Hoffmann *et al.* 1997; Umina and Hoffmann 1999). Several alternative control practices have been investigated for earth mite management, including cultural control (Merton *et al.* 1995; Grimm *et al.*, 1995), biological control (James 1995; Michael 1995) and host plant resistance (Gillespie 1991; Moritz and McDonald 1995; Lake and Howie 1995; Ridsdill-Smith *et al.* 1995). However, no single method is effective in all situations, and chemicals are likely to remain a major weapon for the control of earth mites in the foreseeable future.

Improvements to earth mite management practices that rely solely on chemicals can be made with careful consideration of earth mite ecology and biology. A single spring spray at the right time has been found to reduce the number of over-summering diapause eggs produced by *H. destructor* (Ridsdill-Smith and Pavri 1998; Ridsdill-Smith and Pavri 2000). Recent research has shown that a cost-effective means of controlling *H. destructor* is to spray just before diapause eggs are produced (Ridsdill-Smith and Pavri 1998). This optimum date can be predicted using climatic variables. Timerite[®] (developed by the Woolmark Company and Commonwealth Scientific and

Industrial Research Organisation) is a product now available which provides farmers with the optimum date for spraying mites on their property. The spray date is constant from year to year, but varies between farms as little as 10 km apart. Across 40 field trials, the reduction in autumn mite populations averaged 95% using this approach (Ridsdill-Smith and Pavri 2000).

Most research on earth mites has focused on *H. destructor*, with little attention directed towards *Penthaleus* spp. This is due to a belief that *H. destructor* is responsible for most of the economic damage. However, pest surveys throughout southeastern Australia indicate that *Penthaleus* spp. are important pests in many instances and are of equal or greater importance than *H. destructor* (Robinson and Hoffmann 2001; Chapter 3). Moreover, *Penthaleus* spp. are entirely responsible for mite damage to crops and pastures in northern New South Wales where *H. destructor* is absent.

There are three cryptic pest species of *Penthaleus* in southeastern Australia, complicating the management of earth mites. These are *Penthaleus major* (Dugés) (Acari: Penthaleidae), *Penthaleus falcatus* (Qin and Halliday), and a recently discovered species that has not been formally described, *Penthaleus* species *x* (Weeks and Hoffmann 1999). The three *Penthaleus* spp. and *H. destructor* differ in their distribution, plant host preferences and response to pesticides (Weeks and Hoffmann 1999; Umina and Hoffmann 1999; Robinson and Hoffmann 2001). Despite these differences, all species are currently treated identically in terms of control. This is of concern, particularly as suppression or eradication of one species of earth mite may result in another species increasing in relative abundance (Weeks and Hoffmann 2000; Chapter 4).

While Timerite[®] is currently very successful against *H. destructor*, its effectiveness in controlling *Penthaleus* spp. remains unclear. James and O'Malley (1993) provided limited evidence suggesting the incidence of diapause in *Penthaleus* and *H. destructor* differs, although the *Penthaleus* species were not distinguished and sample sizes were small in their study.

Here I examine the diapause characteristics of *Penthaleus* spp. and consider three questions. Do *Penthaleus* spp. have a similar diapause pattern to *H. destructor* in southeastern Australia? How do the diapause characteristics of *P. major*, *P. falcatus* and *P. sp. x* compare with one another? Finally, can this diapause information be used to devise carefully timed sprays for the three *Penthaleus* species?

2.3 MATERIALS AND METHODS

2.3.1 Field plots (2000-2001)

In 2000, a field experiment was set up in a pasture paddock near Whittlesea (37° 33' 14 S, 145° 06' 27 E). Twenty-four plots (1 m²) were constructed at this site using white corflute sheeting (95 cm high, 0.5 cm thick), following Weeks and Hoffmann (2000). Briefly, the sheets were placed in the ground approximately 15 cm and fixed to wooden stakes that provided a frame for the sheeting. To prevent mite movement, Tac-Gel[®] (Rentokil, active ingredient polybutene) was applied to the upper edge of the corflute. When mites emerged from diapause (late May), the plots were sprayed twice, two weeks apart, with the organophosphate Imidan[®] (Crop Care, active ingredient 150 g/L Phosmet) at a rate of 0.5 ml/L. The plots were then left for an additional two weeks before introducing mites (the residual effect of Imidan[®] is approximately one week).

Penthaleus major and *H. destructor* were collected from adjacent paddocks, mostly containing thick-bladed grasses (*Poaceae* spp.) and subterranean clover (*Trifolium subterraneum*), by suction using a Ryobi Sweepervac 1100A. Mites were then placed in plastic containers with vegetation and paper towelling to absorb excess moisture. They were brought back to the laboratory, sorted, counted and transferred to the plots. One thousand adult *P. major* were added to each of 11 plots, and 1000 adult *H. destructor* added to another 11. Two plots acted as controls (no mites added). At regular intervals throughout the mite season, two plots, one containing *P. major* and one *H. destructor*, were sampled once and then sprayed with Imidan[®] applied at the rate of 0.5 ml/L. Sampling of individual plots involved nine random suctions, each with a diameter of 12 cm and lasting 5-10 seconds. As Imidan[®] is only effective

against the active mite stages and does not kill diapausing or non-diapausing eggs, plots were re-sprayed two weeks later to ensure all resident mites were eliminated.

At the time of first sampling, it became apparent that mite survival inside the plots was poor. Therefore, on each proceeding spray date, *H. destructor* and *P. major* were collected from the surrounding paddock, sorted and approximately 1000 adult mites were released into each of the remaining unsprayed plots. The poor survival rates of the mites may have been due to moist conditions inside the plots (James 1991), caused by the limited sunlight and air movement. Two remaining plots, one containing *P. major* and one containing *H. destructor*, were left unsprayed and acted as additional controls. Sprayed and control plots were sampled throughout the season to assess the effectiveness of chemical applications. The period over which the plots were sprayed encompassed the Timerite[®] date for the field site, which was kindly provided by Celia Pavri and James Ridsdill-Smith (CSIRO Entomology).

All plots were sampled at the start of the next season (May 2001), after mites had emerged and were at the adult stage. Nine suction samples were taken randomly per plot. The mites were placed in 70% ethanol, identified and counted under a dissecting microscope.

2.3.2 Field plots (2001-2002)

The experiment was repeated in 2001, except that *P. major* and *P. falcatus* were compared and all treatments were replicated. Six additional plots (1 m²) were constructed in early March, for a total of 30 plots. *Picris echioides* ('bristly ox-

tongue') plants were collected along a roadside near Calder (37° 40' 38 S, 144° 46' 07 E) and transplanted into 14 of the plots, which contained established pasture.

Penthaleus falcatus prefers common weeds (Weeks and Hoffmann 1999), and has been frequently collected from *P. echioides* (Weeks and Hoffmann 1999; Chapter 3). To increase survival of mites placed into the plots, the height of the corflute sheeting was reduced to 55 cm and the vegetation was cut regularly throughout winter.

In June, all plots were sprayed with Imidan® (0.5 ml/L) twice, two weeks apart, to eliminate resident mites. Two weeks after the second spray, 1000 adult *P. major* were placed into each of 14 plots, and 1000 adult *P. falcatus* placed into another 14. Two plots acted as controls with no mites added. *Penthaleus major* were collected from the surrounding pasture paddock from *Poaceae* spp., while *P. falcatus* were collected from *P. echioides* near Calder.

Two plots containing *P. major* and two with *P. falcatus* were sprayed with Imidan® (0.5 ml/L) in mid-July. The remaining plots were sprayed approximately three weeks apart, until the end of the season. Four plots, two with *P. major* and two *P. falcatus*, were not sprayed and acted as controls.

All plots were sampled at the start of the next season (June 2002), after mites had emerged and were at the adult stage. Nine suction samples were taken randomly per plot. The mites were placed in 70% ethanol, identified and counted.

2.3.3 Shade-house experiment

A different approach was also undertaken in 2001 to examine diapause in *P. sp. x* from northwestern Victoria as well as diapause in the other species. At the beginning of the season, six field sites were identified where *Penthaleus* spp. occurred. At two sites, Whittlesea (37° 33' 14 S, 145° 06' 27 E) and Wooroonook (36° 16' 23 S, 143° 14' 39 E), *P. major* was the most abundant species. *Penthaleus falcatus* was prevalent at Calder (37° 40' 38 S, 144° 46' 07 E) and Gooroc (36° 28' 32 S, 143° 12' 05 E), while *P. sp. x* was common at the remaining two sites, Dooboobetic (36° 23' 29 S, 143° 12' 00 E) and Yeungroon West (36° 21' 32 S, 143° 17' 44 E). The major plant types at all sites except Calder and Gooroc were phalaris (*Phalaris* spp.), wild oats (*Avena fatua*) and various thick-bladed grasses. The Calder and Gooroc sites consisted mainly of *P. echioides*.

The six sites were sampled approximately three weeks apart throughout the season. At two sites (Whittlesea and Calder, both near Melbourne), the first samples were taken in early June 2001. Due to the later emergence of mite populations in northwestern Victoria, sampling from the remaining four sites (Wooroonook, Gooroc, Dooboobetic and Yeungroon West) was started a month later (July 2001). A sample constituted an area approximately 35 cm × 45 cm and 10 cm deep that was removed from each site and placed into a clear plastic tub. Each tub was enclosed with a clear plastic lid that had a large gauze window for ventilation. The tubs were brought back to the laboratory and sprayed two times (two weeks apart) with Imidan[®] applied at the rate of 0.5 ml/L. A control tub (not sprayed) was also collected from each site at the beginning of the season and brought back to the laboratory. All tubs were placed in a shade-house in a randomised arrangement and watered throughout the season to mimic field conditions

and to maintain vegetation inside the tubs. At the end of the mite season, watering ceased and tubs were left until the following autumn.

Seeds were collected from each site over the summer months and sown into tubs from the same sites in autumn. Commercially bought seeds, phalaris (*Phalaris aquatica*), rye grass (*Lolium perenne*) and plantain (*Plantago lanceolata*), were also sown into each tub. The tubs were then watered to simulate autumn rains and to germinate seeds. Mites emerged at the beginning of May. Each tub was sampled three times (10 days apart) by suction of the entire tub, using a Ryobi Sweepervac 1100A. Adult mites were removed and placed into vials, with the remaining contents replaced into each tub. Most vials were filled with 70% ethanol to preserve earth mites for identification, while some mites from Wooroonook were stored at -80°C for electrophoresis (see below). Mites were classified into different species at $\times 40$ magnification using dorsal bristle morphology (Weeks *et al.* 1995; Qin and Halliday 1996; Weeks and Hoffmann 1999) and counted.

2.3.4 Electrophoresis and comparisons of clones

To investigate diapause differences among clones of *Penthaleus* spp., samples of mites emerging from all shade-house tubs from Wooroonook were collected and subjected to allozyme electrophoresis. Clones were identified with five enzymes and six polymorphic loci: malate dehydrogenase (MDH-1 and MDH-2); isocitrate dehydrogenase (IDH); glutamate-oxaloacetate transaminase (GOT); phosphoglucose isomerase (PGI); and phosphoglucomutase (PGM). The electrophoresis procedures followed Weeks *et al.* (1995) and Weeks and Hoffmann (1998). The distributions of

clones in the samples were compared using a Monte Carlo contingency test, after rare clones (with fewer than five individuals) were excluded from the analysis.

2.4 RESULTS

2.4.1 Field plots

In the 2000-2001 experiment, a few mites were discovered in one of the two control plots (with no mites added), while no earth mites were collected in either control plot in the 2001-2002 experiment. Therefore, barriers were effective in excluding most mites from the surrounding pasture. It is unlikely that mites actively crossed the barriers, as no mites were collected in any of the samples taken during the respective seasons. The most likely source of contamination was through aerial dispersal of aestivating eggs over the summer period. There was also some cross-contamination of species in a few plots during both years in which the experiment was run. However, numbers were always low and constituted < 15% of the total earth mite population.

2.4.1.1 2000-2001

At the Whittlesea site in 2000, *P. major* produced diapause eggs earlier in the season than *H. destructor* (Figure 2.1), as *P. major* were collected from all treatments including the unsprayed control. This indicates *P. major* started producing diapause eggs in August or earlier. Mite numbers were generally higher in plots sprayed later in the season, suggesting that diapause egg production starts early and extends throughout the season, resulting in a gradual accumulation of diapausing eggs. Nevertheless there was substantial variability in numbers among the plots, which may reflect the effect of suitable vegetation on mite numbers. For instance, the plot sprayed on 14 October mainly contained clover (*Trifolium* spp.) and capeweed (*Arctotheca calendula*), but very few grasses which are the preferred plant hosts of *P. major* (Narayan 1962; Jeppson *et al.* 1975; Weeks and Hoffmann 1999). *Halotydeus destructor* were sampled

in large numbers from all plots sprayed after 13 October, which corroborates the spray date recommended by Timerite[®] (Figure 2.1). This suggests that *H. destructor* delayed the production of most diapause eggs until mid- to late spring, although low numbers were collected from several plots sprayed prior to the recommended Timerite[®] date.

2.4.1.2 2001-2002

In 2002, mite numbers in all plots were substantially lower than their initial density of 1000 per m². This may reflect the dry autumn and winter conditions experienced in 2001 and 2002. In the surrounding paddock, mite populations were also noticeably smaller than in 2001, indicating that conditions were unfavourable. Despite the low numbers (in particular *P. falcatus*), patterns of diapause egg production were apparent (Figure 2.2). Diapause eggs were produced early in *P. major* (before mid-July) and continued throughout the mite season. *Penthaleus falcatus* exhibited a similar pattern, producing diapause eggs from an early stage in the season (start of August), although not as early as *P. major*.

2.4.2 Shade-house experiment

At the Yeungroon West site, *P. sp. x* comprised > 85% of the mite population at the beginning of the 2001 season. However, the species composition at this site changed, with *P. major* and *H. destructor* becoming prevalent. At the remaining five sites (Whittlesea, Calder, Wooroonook, Gooroc, and Dooboobetic), one species predominated throughout the season and constituted > 90% of the mite population.

The numbers of mites emerging in each plot sampled from the six locations in 2001 were plotted against the date of spraying (Figure 2.3). Consistent with the field experiments, the results indicate that unlike *H. destructor*, *P. major* and *P. falcatus* started producing diapause eggs in winter, well before the recommended Timerite[®] date. *Penthaleus* sp. *x* also produced diapause eggs earlier than *H. destructor* and before the Timerite[®] date in spring. *Penthaleus major* was collected in all tubs sampled from Whittlesea and Wooroonook, indicating this species starts producing diapause eggs in late autumn or early winter. At Calder and Gooroc diapause egg production in *P. falcatus* began in mid- to late June, slightly later than *P. major*. *Penthaleus* sp. *x* emerged from Dooboobetic samples sprayed after 2 July, indicating this species started producing diapause eggs slightly later than *P. major* and *P. falcatus*.

Emergence patterns at Yeungroon West confirmed that *P. major* produced diapause eggs in early winter and throughout the season, while *H. destructor* delayed the development of most diapause eggs until spring. As in the 2000-2001 field plot experiment, some *H. destructor* diapause eggs were produced prior to the recommended Timerite[®] date. Some *H. destructor* also emerged in several shade-house tubs from other locations that were sprayed prior to the recommended spring Timerite[®] date (data not presented).

2.4.3 Clonal patterns

Samples of *P. major* emerging from each Wooroonook shade-house tub were collected and the clonal type of each individual mite was identified by electrophoresis. Sixteen *P. major* clones were identified, though half of these were present at a frequency of <

5%. The distribution of the eight common clones (Table 2.1) within the tubs (excluding the control) was compared using a Monte Carlo contingency test. Differences among clones were significant ($\chi^2 = 230.23$; $df = 35$; $P < 0.001$), which suggests that some clones (such as 2 and 7) produce diapause eggs early in the season and others (such as 4) delay the production of diapause eggs.

2.5 DISCUSSION

Results show the three *Penthaleus* spp. differ markedly in their timing of diapause egg production from *H. destructor*. This indicates Timerite[®] will not be effective in the control of blue oat mites. Indeed, Ridsdill-Smith and Pavri (2000) reported an increase in blue oat mites in autumn, following spring spraying in eastern Australia. Due to interspecific competition between *H. destructor* and at least one of the blue oat mite species (Weeks and Hoffmann 2000), suppression of *H. destructor* through carefully timed spring spraying will likely lead to an increase in relative abundance of one or more *Penthaleus* species.

In the 2000-2001 field experiment at Whittlesea, large numbers of *H. destructor* were collected in all plots sprayed after 13 October. Therefore, it appears that *H. destructor* produce the majority of diapause eggs in mid-spring, which is consistent with previous findings (James and O'Malley 1993). Annells and Ridsdill-Smith (1991) suggest that *H. destructor* produce diapause eggs in the third generation, with the first two generations laying non-diapausing eggs. These results suggest that a few diapause eggs are also produced by an earlier generation, as some *H. destructor* were collected from plots sprayed earlier in the season. Other researchers have also recorded the presence of a few *H. destructor* diapause eggs in southern Australia produced prior to spring (Wallace 1970a; James and O'Malley 1993). This strategy may provide insurance against unpredictable conditions. Danks (1994) provides numerous examples of arthropod species demonstrating such intraspecific variability with respect to diapause and other traits.

Selection pressures imposed by spring spraying are likely to be intense in areas where farmers spray at the same time every year. Because of this pressure and the very large population sizes of *H. destructor* (numbers can exceed 60 000 per m² - Ridsdill-Smith and Pavri 1998), it is possible that populations will evolve to alter their diapause strategy, more closely resembling that of the *Penthaleus* species. *Halotydeus destructor* populations have already developed tolerance to chemicals in the field (Hoffmann *et al.* 1997), demonstrating the capacity of this species to adapt to changed circumstances. Other pest species, such as corn rootworms (*Diabrotica* spp.), have circumvented control attempts by evolving different diapause strategies (Krysan *et al.* 1984; Levine *et al.* 2002). Therefore, it would be sensible to rotate Timerite[®] with other control strategies to reduce the likelihood of *H. destructor* evolving an altered life history strategy. Understanding the genetic variability of the diapause response in this species would seem critical to prolonging the effectiveness of Timerite[®].

For the *Penthaleus* spp., the results were generally consistent between the field plots and shade-house experiment and suggest minor differences in the timing of diapause egg production. In *P. major* this started early in the season, and is likely to have occurred in the first generation of mites, as reported by James and O'Malley (1993). Production of diapause eggs in *P. falcatus* also occurs early in the season, although not as early as in *P. major*. Data for *P. sp. x* were limited to the Dooboobetic site, but suggest that *P. sp. x* start producing diapause eggs later than *P. major* and *P. falcatus*, but earlier than *H. destructor*. More sites across several years need to be examined for this species. Moreover, I did not obtain information about *P. sp. x* from northern New

South Wales where there is a disjunct population of this species (Robinson and Hoffmann 2001).

These results show that from the time of emergence in autumn, a fraction of all eggs produced by *P. major* are diapause eggs. Results from the field plot experiments suggest a linear association between the number of diapause eggs produced by *P. major* and treatment dates during the season. This may reflect a fairly constant production of diapause eggs throughout the season. The fact that *P. major* does not deposit large numbers of diapause eggs towards the end of spring, in the same way as *H. destructor*, supports this conjecture. The data for *P. falcatus* also suggest a constant rate of production of diapause eggs, although the trend is less clear. Producing diapause eggs throughout a season will reduce population growth, and may contribute to the relative abundance of *Penthaleus* increasing less dramatically in early spring compared with *H. destructor* (Weeks and Hoffmann 2000). To further examine the diapause egg production of *Penthaleus* using a similar experimental approach, the number of mites within an area should be monitored throughout the season.

Alternatively, the percentage of diapause and non-diapause eggs at any given stage of the season could be investigated by examining eggs of individual mites (eg. James and O'Malley 1993). *Halotydeus destructor* diapause eggs can be distinguished from non-diapause eggs by their size and colour (Ridsdill-Smith 1997), although preliminary attempts to identify *Penthaleus* eggs in this manner have proved difficult (A.R. Weeks, personal communication).

Penthaleus spp. may start to produce diapause eggs earlier in the season than *H. destructor* because they respond to environmental cues differently. Due to its

reliability, photoperiod is the most common seasonal cue used by invertebrates to control diapause (Lees 1955; Tauber *et al.* 1984). Climatic variables (in particular photoperiod) appear to be important regulatory factors of diapause induction in *H. destructor* (Ridsdill-Smith and Annells 1997). Wallace (1970a) suggested the induction of diapause in this species was also influenced by increasing maturity of food plants in spring. Although food quality is the primary factor regulating summer diapause in some phytophagous species (Masaki 1980; Tauber *et al.* 1984), it more often modifies the diapause-inducing effects of another stimulus such as photoperiod or temperature (Tauber *et al.* 1984). The token stimuli used by *Penthaleus* spp. for diapause induction have not been identified, although it is likely that they are different from *H. destructor*. Since *Penthaleus* spp. generally hatch from diapause in autumn earlier than *H. destructor* (James and O'Malley 1993; unpublished data), *Penthaleus* spp. may also respond differently from *H. destructor* to environmental cues that signal the termination of diapause.

Diapause in *Penthaleus* spp. is further complicated by the fact that they are obligate parthenogens, with populations made up of clones that are genetically different. Weeks and Hoffmann (1998) provided evidence of spatial and temporal differences in the clonal distribution of *P. major*. This may reflect selective factors such as host plants, moisture and temperature. Some *Penthaleus* clones also differ in their tolerance levels to pesticides (Umina and Hoffmann 1999). There is some potential for the evolution of different diapause characteristics among clones of *Penthaleus*. Clones of many species, such as *Daphnia pulex* and *Heterocypris incongruens*, have been shown to regulate diapause differently, in some cases differing in the token stimuli used to signal approaching seasonal changes (Larsson 1991; Yampolsky 1992; Rossi *et al.* 1996). My

results indicate that different genotypes emerge when plots are sprayed at different times, suggesting that clones of *P. major* may differ in the timing of diapause egg production. This provides another potential selective factor contributing to the maintenance of clonal diversity within and between populations (Weeks and Hoffmann 1998; Umina and Hoffmann 1999). However, because the clonal composition of mites in each shade-house tub prior to spraying was unknown, differences in clonal composition in the ensuing season could also reflect changes in relative clonal frequencies at the sampling sites. To clarify clonal differences in diapause induction, additional experiments are needed, ideally using pure cultures of each clone.

The differences in timing of diapause egg production are likely to contribute to the distribution and abundance of the earth mite species. Robinson and Hoffmann (2001) recently mapped the distribution of earth mites within southeastern Australia.

Halotydeus destructor is found throughout much of Victoria and southern New South Wales, while *P. sp. x* is restricted to northeastern New South Wales and northwestern Victoria. *Penthaleus major* and *P. falcatus* have a broader distribution than the other species. Early production of diapause eggs may enable *Penthaleus* spp. to inhabit less favourable and unpredictable regions. For instance, *P. major* has been collected from Mt. Buller before the onset of snow (Weeks and Hoffmann 1999). In alpine regions, the period that mites are active is curtailed because of cold temperatures and snow cover. The early production of diapause eggs would be an adaptive strategy ensuring survival into the next season. *Halotydeus destructor* is absent from dry inland regions and occurs sporadically in marginal areas (Wallace and Mahon 1971). In these areas, autumn rains often arrive late and are unpredictable. *Penthaleus* spp. are therefore likely to be at an advantage by producing diapause eggs directly after emergence.

In conclusion, these results show earth mite species differ in their timing of diapause egg production. *Halotydeus destructor* in particular has a very different diapause response from *Penthaleus* species. Timerite[®] is unlikely to be effective against *Penthaleus* species. This highlights the importance of correctly distinguishing blue oat mites from redlegged earth mites prior to implementing control strategies. *Halotydeus destructor* could evolve to change the timing of diapause egg production in response to continued spring spraying, necessitating the need to consider additional control methods. There also appears to be variation in diapause induction among the three *Penthaleus* spp. that could contribute to their relative distributions. Spraying *Penthaleus* within two to three weeks of emergence in autumn may be the most effective means of reducing the impact of these pests in crops and pastures.

2.6 REFERENCES CITED

- Annels A. J. and Ridsdill-Smith T. J. 1991. The effect of moisture on aestivating eggs of *Halotydeus destructor* (Tucker) (Acari: Penthaleidae). *In*: Ridsdill-Smith T.J. (ed.), Proceedings of a National Workshop on Redlegged Earth Mite, Lucerne Flea and Blue Oat Mite. Department of Agriculture, Perth, Australia, pp. 7-9.
- Danks H. V. 1994. Diversity and integration of life-cycle controls in insects. *In*: Danks H.V. (ed.), Insect life-cycle polymorphism: theory, evolution and ecological consequences for seasonality and diapause control. Kluwer Academic Publishers, The Netherlands, pp. 5-40.
- Gillespie D. J. 1991. Identification of resistance to redlegged earth mite *Halotydeus destructor* in pasture legumes. *Plant Protection Quarterly* 6: 170-171.
- Grimm M., Michael P., Hyder M. and Doyle P. 1995. Effects of pasture pest damage and grazing management on efficiency of animal production. *Plant Protection Quarterly* 10: 62-64.
- Halliday R. B. 1991. Taxonomic background of the redlegged earth mite *Halotydeus destructor* (Tucker) (Acarina: Penthaleidae). *Plant Protection Quarterly* 6: 162-165.
- Hoffmann A. A., Porter S. and Kovacs I. 1997. The response of the major crop and pasture pest, the red-legged earth mite (*Halotydeus destructor*) to pesticides: dose-

response curves and evidence for tolerance. *Experimental and Applied Acarology* 21: 151-162.

James D. G. 1991. Environmental factors affecting autumn egg hatch and population development of *Halotydeus destructor* (Tucker) (Acari: Penthaleidae). *In*: Ridsdill-Smith T.J. (ed.), *Proceedings of a National Workshop on Redlegged Earth Mite, Lucerne Flea and Blue Oat Mite*. Department of Agriculture, Perth, Australia, pp. 32-34.

James D. G. 1995. Biological control of earth mites in pasture using endemic natural enemies. *Plant Protection Quarterly* 10: 58-59.

James D. G. and O'Malley K. J. 1993. Phenology of egg production and diapause in *Halotydeus destructor* Tucker and *Penthaleus major* Dugés (Acari: Penthaleidae) in southern New South Wales during 1988/89. *General and Applied Entomology* 24: 33-38.

Jeppson L. R., Keifer H. H. and Baker E. W. 1975. *Mites Injurious to Economic Plants*. University of California Press, Berkeley, America.

Krysan J. L., Jackson J. J. and Lew A. C. 1984. Field termination of egg diapause in *Diabrotica* with new evidence of extended diapause in *D. barberi* (Coleoptera: Chrysomelidae). *Environmental Entomology* 13: 1237-1240.

Lake A. W. H. and Howie J. H. 1995. Selection for redlegged earth mite resistance in

annual *Medicago* species. Plant Protection Quarterly 10: 45-46.

Larsson P. 1991. Intraspecific variability in response to stimuli for male and ephippia formation in *Daphnia pulex*. Hydrobiologia 225: 281-290.

Lees A. D. 1955. The Physiology of Diapause in Arthropods. Cambridge University Press, London, UK.

Levin E., Spencer J. L., Isard S. A., Onstad D. W. and Gray M. E. 2002. Adaptation of the western corn rootworm to crop rotation: evolution of a new strain in response to a management practice. American Entomologist 48: 94-107.

Masaki S. 1980. Summer diapause. Annual Review Entomology 25: 1-25.

Merton E., McDonald G. and Hoffmann A. 1995. Cultural control of redlegged earth mite, blue oat mite and lucerne flea in canola. Plant Protection Quarterly 10: 65-66.

Michael P. 1995. Biological control of redlegged earth mite and lucerne flea by predators *Anystis wallacei* and *Nemologus capillatus*. Plant Protection Quarterly 10: 55-57.

Moritz K. and McDonald G. 1995. Developing redlegged earth mite resistance in canola. In: Potter T.D. (ed.), Proceedings of the Australian Research Assembly on Brassicas. South Australian Research and Development Institute, Adelaide, Australia, pp. 30-35.

Narayan D. S. 1962. Morphological, biological and ecological studies on the winter grain mite, *Penthaleus major* (Dugés), Penthaleidae; Acarina Part 1. Journal of Zoological Society of India 14: 45-63.

Qin T. K. and Halliday R. B. 1996. The Australian species of *Chromotydeus* Berlese and *Penthaleus* Koch, C.L. (Acarina, Penthaleidae). Journal of Natural History 30: 1833-1848.

Ridsdill-Smith T. J. 1997. Biology and control of *Halotydeus destructor* (Tucker) (Acarina: Penthaleidae) – a review. Experimental and Applied Acarology 21: 195-224.

Ridsdill-Smith T. J. and Annells A. J. 1997. Seasonal occurrence and abundance of redlegged earth mite *Halotydeus destructor* (Acari, Penthaleidae) in annual pastures of south western Australia. Bulletin of Entomological Research 87: 413-423.

Ridsdill-Smith T. J. and Pavri C. 1998. Spring spraying for redlegged earth mites. Western Focus (Australian Grain), October-November: 1-4.

Ridsdill-Smith T. J. and Pavri C. 2000. Single spring spray protects pastures. Farming Ahead 103: 60-63.

Ridsdill-Smith T. J., Jiang Y. and Ghisalberti E. L. 1995. A method to test compounds for feeding deterrence towards redlegged earth mites (Acarina: Penthaleidae). Annals Applied Biology 127: 593-600.

Robinson M. R. and Hoffmann A. A. 2001. The pest status and distribution of three cryptic blue oat mite species (*Penthaleus* spp.) and the redlegged earth mite (*Halotydeus destructor*) in southeastern Australia. *Experimental and Applied Acarology* 25: 699-716.

Rossi V., Gandolfi A. and Menozzi P. 1996. Egg diapause and clonal structure in parthenogenetic populations of *Heterocypris incongruens* (Ostracoda). *Hydrobiologia* 320: 45-54.

Tauber M. J., Tauber C. A. and Masaki S. 1984. Adaptations to hazardous seasonal conditions: dormancy, migration, and polyphenism. *In*: Huffaker C.B. and R.L. Rabb (eds.), *Ecological Entomology*. John Wiley and Sons, New York, America, pp. 149-183.

Umina P. A. and Hoffmann A. A. 1999. Tolerance of cryptic species of blue oat mites (*Penthaleus* spp.) and the redlegged earth mite (*Halotydeus destructor*) to pesticides. *Australian Journal of Experimental Agriculture* 39: 621-628.

Wallace M. M. H. 1970a. Diapause in the aestivating egg of *Halotydeus destructor* (Acari: Eupodidae). *Australian Journal of Zoology* 18: 295-313.

Wallace M. M. H. 1970b. The influence of temperature on post-diapause development and survival in the aestivating eggs of *Halotydeus destructor* (Acari: Eupodidae). *Australian Journal of Zoology* 18: 315-329.

Wallace M. M. H. and Mahon J. A. 1971. The distribution of *Halotydeus destructor* and *Penthaleus major* (Acari: Eupodidae) in Australia in relation to climate and land use. Australian Journal of Zoology 19: 65-76.

Weeks A. R. and Hoffmann A. A. 1998. Intense selection of mite clones in a heterogeneous environment. Evolution 52: 1325-1333.

Weeks A. R. and Hoffmann A. A. 1999. The biology of *Penthaleus* species in southeastern Australia. Entomologia Experimentalis et Applicata 92: 179-189.

Weeks A. R. and Hoffmann A. A. 2000. Competitive interactions between two pest species of earth mites, *Halotydeus destructor* and *Penthaleus major* (Acarina: Penthaleidae). Journal of Economic Entomology 93: 1183-1191.

Weeks A. R., Fripp Y. J. and Hoffmann A. A. 1995. Genetic structure of *Halotydeus destructor* and *Penthaleus major* populations in Victoria (Acari: Penthaleidae). Experimental and Applied Acarology 19: 633-646.

Yampolsky L. Y. 1992. Genetic variation in the sexual reproduction rate within a population of cyclic parthenogen, *Daphnia magna*. Evolution 46: 833-873.

2.7 TABLE AND FIGURES

Table 2.1: Number of *Penthaleus major* individuals belonging to common clonal types sampled from Wofoonook shade-house tubs in 2002. Dates indicate when each tub was sprayed with pesticide the previous year (2001).

Clone no.	Control	02-July	03-Aug.	17-Aug.	07-Sept.	26-Sept.	14-Oct.
1	14		21	8	5	4	2
2	15	1	8	6			
3	1			3	5		
4	6				4		2
5	1		2	5	5		4
6	20			6			
7	1	11					
8	9		1		1	8	

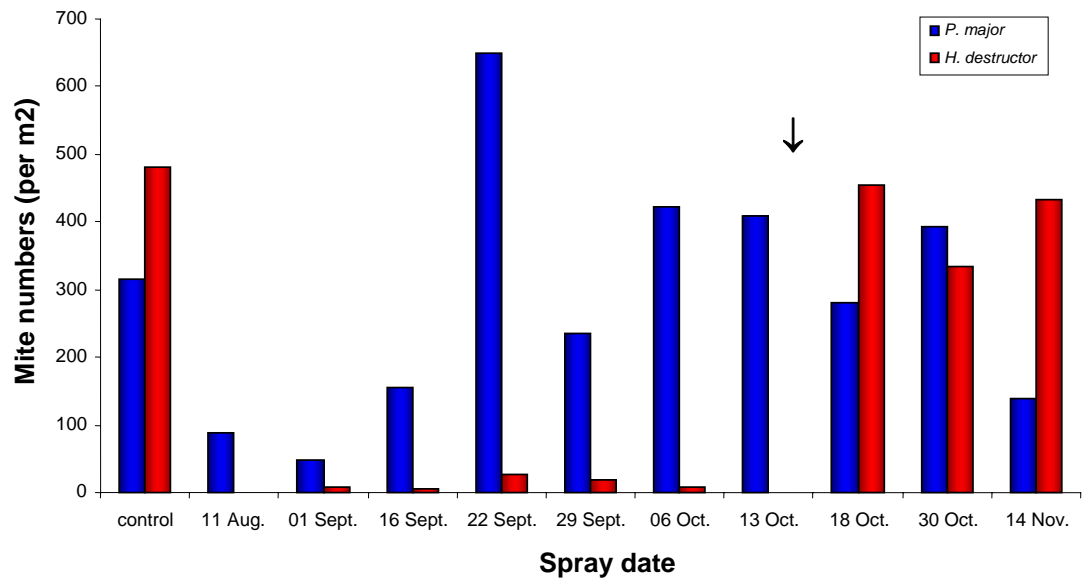


Figure 2.1: Number of *Penthaleus major* and *Halotydeus destructor* adults sampled from Whittlesea plots in 2001. Plots were sprayed at intervals throughout 2000 and then sampled the following autumn (2001). The arrow indicates the optimum Timerite[®] spring spraying date for *Halotydeus destructor* at this location.

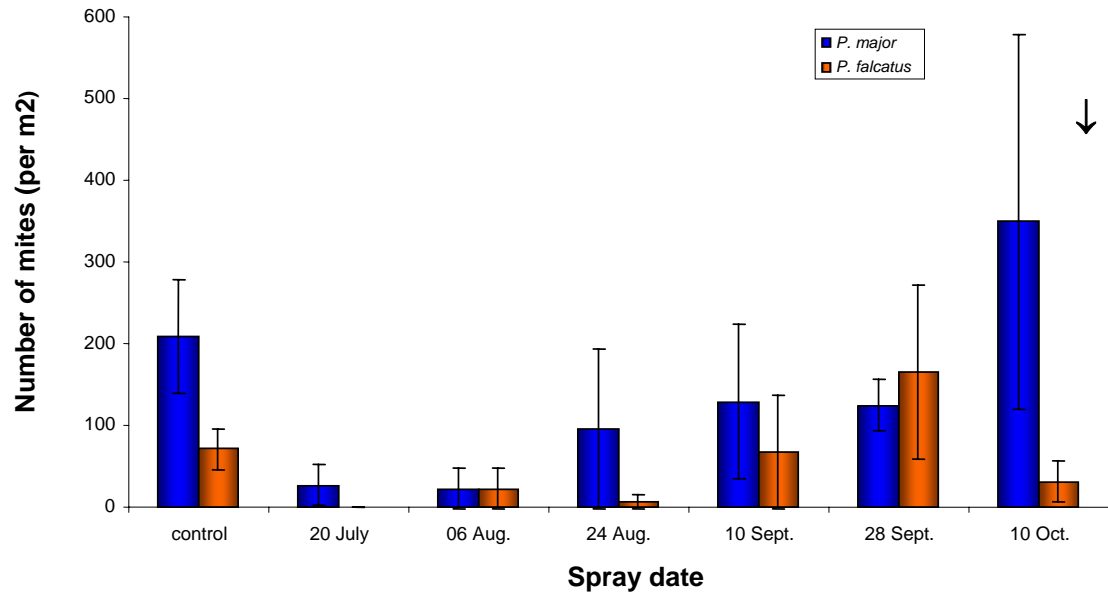
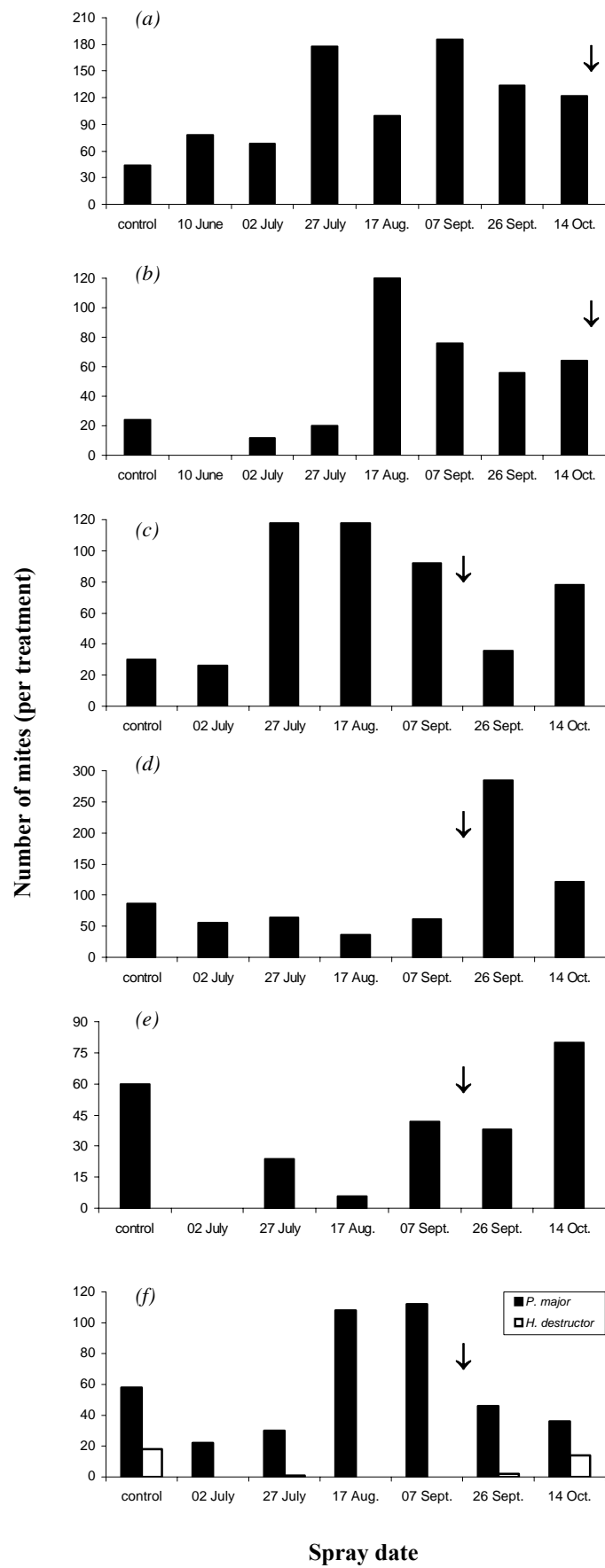


Figure 2.2: Mean number of *Penthaleus major* and *Penthaleus falcatus* adults sampled from Whittlesea plots in 2002. Plots were sprayed at intervals throughout 2001 and sampled the following autumn (2002). Error bars represent one standard deviation. The arrow indicates the optimum Timerite[®] spring spraying date for *Halotydeus destructor* at this location.

Figure 2.3: Number of post-diapause mites collected from shade-house tubs in 2002. Letters represent: (a) *Penthaleus major*, Whittlesea; (b) *Penthaleus falcatus*, Calder; (c) *Penthaleus major*, Wooroonook; (d) *Penthaleus falcatus*, Gooroc; (e) *Penthaleus* species *x*, Dooboobetic; and (f) *Penthaleus major* and *Halotydeus destructor*, Yeungroon West. Tubs were sprayed at intervals throughout 2001 and sampled the following autumn (2002) three times (ten days apart). Arrows indicate the optimum Timerite[®] spring spraying date for *Halotydeus destructor* at each location.



CHAPTER 3.

Plant host associations of *Penthaleus* species and *Halotydeus destructor* and implications for integrated pest management

3.1 ABSTRACT

Integrated pest management programs seek to minimise reliance on pesticides and provide effective long-term control of pests. Cultural control strategies, such as crop rotations, trap and border crops, and weed management, require a thorough understanding of pest host associations. This chapter examines the effects of different plant hosts on the persistence and reproduction of blue oat mites, *Penthaleus* spp., and the redlegged earth mite, *Halotydeus destructor* (Tucker), which are major agricultural pests in southern Australia. Field and shade-house experiments were conducted testing several crop and plant types. All species survived and reproduced from one mite season to the next when confined to pasture. Canola and a common weed, ‘bristly ox-tongue’, were suitable hosts for *H. destructor* and *Penthaleus falcatus* (Qin and Halliday), whereas *Penthaleus* sp. *x* and *Penthaleus major* (Dugés) failed to persist on these plants. A mixture of wheat and oats sustained *P. sp. x* and *H. destructor*, but not *P. falcatus* or *P. major*. Lentils were a poor host plant for all mite species. These findings show that earth mite species differ in their ability to persist on different plant types, highlighting the importance of distinguishing mite species before implementing control strategies. Results are discussed with respect to cultural control options for the management of these winter pests.

3.2 INTRODUCTION

Blue oat mite species, *Penthaleus* spp. (Acari: Penthaleidae), and the redlegged earth mite, *Halotydeus destructor* (Tucker) (Acari: Penthaleidae), are some of the most serious and widespread agricultural pests in Australia. These species attack a variety of pasture plants and virtually all winter crops, feeding mainly on the dorsal surface of cotyledons and leaves (Jeppson *et al.* 1975; Ridsdill-Smith 1997). Mite feeding reduces available feed for livestock and severely damages young seedlings. Most damage is incurred at the establishment phase and this may lead to entire crops needing re-sowing. *Penthaleus* spp. and *H. destructor* (commonly referred to as earth mites) are approximately 1 mm in size when adults, and can reach densities of 60 000 mites per square metre in the field (Ridsdill-Smith and Pavri 1998). They are active between April and October producing two or three generations before entering diapause throughout the summer months. The life cycle is completed with diapause eggs hatching in autumn when conditions are optimal following cool temperatures and rainfall (Wallace 1970; Ridsdill-Smith and Annells 1997).

Until recently *H. destructor* was assumed to be the main agricultural pest earth mite species in southern Australia. As a consequence, most research has tended to focus on *H. destructor*, with little attention directed towards *Penthaleus* species. However, recent surveys have demonstrated that *Penthaleus* spp. are also important pests (Robinson and Hoffmann 2001). Three *Penthaleus* species have been found in southeastern Australia: *Penthaleus major* (Dugés); *Penthaleus falcatus* (Qin and Halliday); and a recently discovered species referred to as *Penthaleus* sp. *x*. These species and *H. destructor* differ markedly. Laboratory tests indicate differences in

pesticide responses, with *P. falcatus* having a much higher tolerance to chemicals registered for earth mite control (Umina and Hoffmann 1999). Mite species differ in their distributions, host plant preferences (Weeks and Hoffmann 1999; Robinson and Hoffmann 2001) and timing of diapause egg production (Chapter 2). The mode of reproduction of earth mites also varies. *Halotydeus destructor* is diploid and reproduces sexually (diplodiploid), while *Penthaleus* spp. reproduce by thelytokous parthenogenesis, with populations made up of distinct clonal types (Annells 1994; Weeks *et al.* 1995; Weeks and Hoffmann 1999).

At present, chemical sprays are the main method of controlling earth mites in Australia. The different pesticide responses between species and the identification of some *H. destructor* populations resistant to a commonly used pesticide (Hoffmann *et al.* 1997; Umina and Hoffmann 1999) indicate the need for alternative control practices. Several approaches such as biological control (James 1995; Michael 1995) and host plant resistance (Gillespie 1991; Moritz and McDonald 1995; Lake and Howie 1995; Ridsdill-Smith *et al.* 1995) have been investigated with limited success. The effects of various cultural control methods have also been explored. McDonald *et al.* (1995) examined the survival and reproductive rate of *H. destructor* on various crop and pasture plants. Canola, lupins, wheat and oats were inferior hosts compared with other plant types, suggesting such crops used in rotations could minimise *H. destructor* infestations. However, Merton *et al.* (1995) showed the use of a wheat border was not effective in preventing movement of mites into a susceptible crop, and that *H. destructor* could persist on both wheat and canola. Inconsistencies between plant host preferences have also been observed for *Penthaleus* species (Robinson and Hoffmann 2001). Potted plant experiments suggest that *P. major* and *P. sp. x* can reproduce on

oats and wheat, while *P. falcatus* does not successfully reproduce on oats, wheat, barley or canola (Robinson and Hoffmann 2001). However, surveys in southeastern Australia show that all species cause feeding damage to these crops in the field. Non-host factors may also influence mite numbers. For instance, broad-leaved weeds and/or other non-vascular flora can provide an alternative food source, particularly for juvenile stages (McDonald *et al.* 1995; Maclellan *et al.* 1998).

In this chapter, the effects of different plant hosts on the persistence and reproduction of *H. destructor* and the three *Penthaleus* spp. are examined. This information is important for developing sustainable management practices that do not rely solely on chemical control, such as weed management and crop rotations. Using both shade-house and field experiments, I considered pasture and crops (canola, wheat and oats) where these mites are pests. I also examined mite performance on lentils, and on the common weed, 'bristly ox-tongue'. Lentils might suppress mite numbers when used in crop rotations, while weeds could act as a refuge for mite populations. Feeding damage to plants was assessed as well as mite numbers. I provide additional survey data on field associations of mites with pasture, weed and crop plants.

3.3 MATERIALS AND METHODS

3.3.1 Shade-house host plant experiment

The persistence and reproduction of earth mite species on several plant types was tested under shade-house conditions. Five treatments were examined: (1) pasture, containing an equal volume of phalaris (*Phalaris aquatica* cv. Holdfast), rye grass (*Lolium perenne* cv. Avalon), plantain (*Plantago lanceolata* cv. Tonic), cocksfoot (*Dactylis glomerata* cv. Porto), white clover (*Trifolium repens* cv. Waverly) and subterranean clover (*Trifolium subterraneum* cv. Gosse) seeds; (2) a mixture containing an equal volume of wheat (*Triticum aestivum*) and oat (*Avena sativa*) seeds; (3) canola (*Brassica napus* cv. Hobson rape); (4) red lentils (*Lens culinaris* cv. Digger); and (5) bristly ox-tongue (*Picris echioides*). This provided a range of agriculturally important plants that are commonly attacked by earth mites in southern Australia (Weeks and Hoffmann 1999; Ridsdill-Smith and Pavri 2000).

In June 2002, seeds were sown into clear plastic tubs (approximately 45 cm long, 35 cm wide and 25 cm deep) using sterilised sandy loam (3:1) soil. The tubs were enclosed with a clear plastic lid that had a large gauze window for ventilation and placed in a shade-house in a randomised block arrangement. Tubs were then watered to germinate seedlings. Due to their longer germination time, pasture and bristly ox-tongue seeds were sown three weeks prior to wheat and oats, canola and red lentils.

In late July 2002, earth mites were collected and brought back to the laboratory where they were sorted and counted. *Halotydeus destructor* and *P. major* were both collected from a pasture paddock near Whittlesea (37° 33' 14 S, 145° 06' 27 E) containing

mostly thick-bladed grasses (*Poaceae* spp.). *Penthaleus* sp. *x* was collected from wild oats (*Avena fatua*) and thick-bladed grasses adjacent to a wheat crop in Dooboobetic (36° 23' 29 S, 143° 12' 00 E). *Penthaleus falcatus* was obtained from bristly ox-tongue, along a roadside near Gooroc (36° 28' 32 S, 143° 12' 05 E). Samples were collected by suction using a STIHL Blowervac BG55 and transported in plastic containers with vegetation and paper towelling to absorb excess moisture.

For each mite species, five plant treatments replicated four times were assigned to the plastic tubs (80 tubs in total). Over a three-day period, 400 adult mites were transferred to each tub and the lids replaced. The tubs were watered regularly throughout the mite season to ensure plant growth, although care was taken not to saturate the tubs.

Overwatering can lead to excess condensation and the build up of fungal growth, which causes mite deaths (Thackray *et al.* 1997). The number of mites in each tub was counted directly three times a week until all mites had disappeared from the tubs.

Feeding damage to plants (which appears as silvery patches on leaves and cotyledons) was rated based on a 0-10 scale, where 0 indicates no visible damage, 5 indicates 50% of the leaves damaged and 10 indicates all plants dead or dying. This method of assessing plant damage has been validated by several authors working on earth mites (Gillespie 1993; Liu and Ridsdill-Smith 2000; Chapman *et al.* 2000). Throughout the experiment, random samples were also taken and mites identified to confirm species composition. In the ox-tongue treatments, feeding by *P. falcatus* resulted in the death of nearly all plants within ten days of release. As a consequence, fresh ox-tongue leaves were added daily to maintain *P. falcatus* populations. Flowering time (number of days from sowing to opening of the first flower) of canola plants in each tub was also recorded. At the end of the mite season (late spring), watering ceased and tubs

were left until the following autumn. Earth mite species survived the dry summer conditions as diapause eggs on the soil surface and/or base of plants.

In April 2003, the same seed types as in the previous year were sown into each tub. The tubs were then watered to simulate autumn rains and germinate seeds. Mites started to emerge at the beginning of May. Each tub was sampled six times (one week apart) by suction of the entire tub, using a Blowervac. Adult mites were removed and placed into vials with 70% ethanol. Mites were classified into species at $\times 40$ magnification using dorsal bristle morphology (Weeks *et al.* 1995; Qin and Halliday 1996; Weeks and Hoffmann 1999), and counted. During the sampling period, four canola tubs became infected with fungal mycelium. Two of these contained *P. major*, one *P. falcatus*, and one contained *P. sp. x*. Because this contamination was detrimental to canola plants and mite numbers, these tubs were excluded from the analyses.

3.3.2 Mite persistence on plants in the field

Between 2001-2002, the persistence of mites on various plant types was examined in a pasture paddock near Yeungroon (36° 21' 32 S, 143° 17' 44 E). In March 2001, 32 plots (1 m by 2 m) were constructed at this site using white corflute sheeting (95 cm high, 0.5 cm thick), following Weeks and Hoffmann (2000). The sheets were placed approximately 15 cm into the ground and fixed to wooden stakes that provided a frame for the sheeting. To prevent mite movement, Tac-Gel[®] (Rentokil, active ingredient polybutene) was applied to the upper edge of the corflute. In May, the soil was cultivated in 24 plots and seeds sown. Eight plots were sown with a mixture of wheat and oats, eight plots with canola and another eight were sown with ox-tongue seeds.

Eight remaining plots contained established pasture, although an equal volume of phalaris, rye grass and subterranean clover seeds were also added. When mites emerged from diapause, the plots were sprayed twice, two weeks apart, with the organophosphate Imidan[®] (Crop Care, active ingredient 150 g/L Phosmet) at a rate of 0.5 ml/L. The plots were then left for an additional two weeks and sampled to ensure all resident earth mites were eliminated before introducing mites (the residual effect of Imidan[®] is approximately one week).

Penthaleus major and *H. destructor* were both collected from thick-bladed grasses along a roadside near Charlton (36° 17' 56 S, 143° 19' 25 E) and Jeffcott North (36° 18' 03 S, 143° 03' 44 E), respectively. *Penthaleus* sp. *x* were collected at Yeungroon East (36° 21' 31 S, 143° 20' 58 E) from a paddock containing vetch and oats, while *P. falcatus* was obtained from bristly ox-tongue, along a roadside near Gooroc (36° 28' 32 S, 143° 12' 05 E). Over a four-day period, mites were sorted, counted and transferred to plots in mid-July. For each mite species, approximately 1500 adults were added separately to each of eight plots. Two plots were set up for each mite species and plant host type.

All plots were sampled for mite abundance in early October, which corresponded with the concluding stages of the active season for each species. Sampling, which involved nine random suctions, each with a diameter of 12 cm and lasting 5-10 seconds, was performed in the morning when mites were most active. Preliminary experiments indicated that after 5 seconds, regardless of vegetation and soil type, > 90% of mites are captured. Mites were placed in 70% ethanol and later sorted into species and counted. Plant damage as a result of mite feeding was also noted.

In May 2002, plots were cultivated and sown with the same seed types as the previous year. Tac-Gel[®] was also reapplied to the upper edges of the barriers where needed. Each plot was sampled at the start of the season (July), after diapause had broken and mites were at the adult stage. Nine samples were taken randomly per plot and mites placed in 70% ethanol for later identification. Plant damage was also recorded. Plots were sampled again at the end of the 2002 season (late September) in the same manner.

During the experiment, grasses and weeds (mainly capeweed, *Arctotheca calendula*), emerged within the cultivated field plots. Although removed by hand every one to two months, these plants could not be eliminated altogether and may have provided mites with alternative hosts. Weed infestations were most prevalent in 2002 but always constituted < 5% of the total vegetation.

3.3.3 Host plant survey

During 2000-2002, field surveys were conducted to investigate the plant hosts of earth mites in southeastern Australia. Over one hundred locations were randomly sampled (one sample per site) by suction using a STIHL Blowervac BG55. Samples were taken from wheat, oats, canola, vetch, peas, and pasture, as well as areas predominantly comprising ox-tongue or capeweed. Adult mites were placed in 70% ethanol and plant material from which they were collected was identified. Species were later identified and counted. All samples were taken from areas where the three *Penthaleus* spp. and *H. destructor* are known to occur (either northwestern Victoria or northern New South Wales - Robinson and Hoffmann 2001). Samples were also taken from a variety of other plants during this period, however in all cases < 20 mites per square metre were

sampled from collections. These numbers were considered below damaging levels, and therefore excluded from the analysis.

3.3.4 Fitness measures

Shade-house experimental data were used to calculate two relative fitness measures for each species on the different plant types. Firstly, regression coefficients (and standard errors) were estimated for mite densities over time in each treatment during the seedling stage (first three weeks after mite application). This provides a measure of the ability of mites to persist after introduction. Because regression coefficients were multiplied by -1 to obtain a positive measure, smaller values indicate a higher fitness. The second fitness measure was the maximum (log) number of adult mites (and standard errors) of the second population peak, which corresponds to the second generation of mites (see Figure 3.1). This provides a measure of the ability of mites to reproduce on each plant type. Plant damage indices were also computed to examine the effects of mite feeding on the different plants. These indices included: (1) the average plant damage score during the seedling stage (first three weeks after mite application); and (2) the average plant damage score for the mite season, calculated until 23 October (this date represents the end of the active season for *Penthaleus* spp. during the experiment).

3.3.5 Data analysis

In all shade-house tubs, mite numbers decreased immediately after introduction. In most cases numbers fell to $< 50\%$ within the first week. Some mites may have died because of stress during handling and transportation or because of the unsuitability of plants as hosts. Consequently, data over the first week were excluded from the

analysis. The numbers of mites in the shade-house experiment were not normally distributed according to Kolmogorov-Smirnov tests. Therefore, mite numbers were log transformed to achieve normality and homogenise variances before analysis of the data set. Linear regression coefficients were estimated for mite numbers during the seedling stage using the statistical program SPSS 11.0. Differences between treatments were tested using Analysis of Variance (ANOVA), and if significant, a Tukey's B test was used for *aposteriori* comparisons. The relative change in population size (Δ_N), which incorporates reproductive output as well as mortality, was estimated for each species on each plant type. This was calculated as the number of mites collected in 2003 (post-diapause) divided by the number placed into each tub in 2002. Multiple regression was used to examine the association between mite numbers and plant damage in each treatment.

For the plant host survey data, contingency tests were used to assess whether plant associations differed between species. Due to small sample sizes for some plant types, the Monte Carlo procedure (SPSS 11.0) was used to determine the significance of the chi-square statistic.

3.4 RESULTS

3.4.1 Shade-house experiment – mite numbers

Differences between species in response to the various plant types were evident (Figure 3.1). All species survived and reproduced on pasture, although *H. destructor* (and to a lesser extent *P. major*) numbers were much greater than the other species. *Halotydeus destructor* and *P. sp. x* survived and bred successfully on wheat/oats, while *P. major* and *P. falcatus* numbers were variable and low. In general, red lentils were a poor host. Despite some mites of each species persisting and reproducing on lentils during 2002, survivorship of all species, especially *P. major* and *P. falcatus*, was low. *Penthaleus falcatus* and *H. destructor* were the only species to survive and successfully reproduce on canola and ox-tongue. It should be noted that as mites were not reared through a generation on a common host before the experiment, prior experience (or exposure) in the field may have confounded the results.

Regression coefficients were estimated based on mite densities in each treatment during the seedling stage (Figure 3.2), and used as fitness measures. There was a significant interaction between mite species and treatment ($F_{12,79} = 23.959$; $P < 0.001$), indicating that differences in the performance of species was dependent on plant type. On pasture, coefficients varied significantly between species ($F_{3,15} = 4.066$; $P = 0.033$), with post-hoc tests suggesting a faster decline in *P. major* numbers compared with *H. destructor*. There was no significant difference between mite species on wheat/oats ($F_{3,15} = 1.122$; $P = 0.379$). Coefficients differed significantly between species on canola ($F_{3,15} = 169.738$; $P < 0.001$), with *H. destructor* and *P. falcatus* showing the slowest rate of decline. On lentils there were significant differences among species

($F_{3,15} = 14.445$; $P < 0.001$), with *P. major* numbers decreasing faster than those of the other species. Regression coefficients differed significantly between species on ox-tongue ($F_{3,15} = 72.777$; $P < 0.001$). *Penthaleus falcatus* numbers decreased at a slower rate compared with *H. destructor* and *P. sp. x*, which were slower again compared with *P. major*.

The maximum number of mites in the second generation (log transformed) was estimated for all treatments (Figure 3.3). There was a significant interaction between mite species and treatment ($F_{12,79} = 40.724$; $P < 0.001$), indicating that species differences were dependent on plant type. There were significant differences among species on pasture ($F_{3,15} = 18.644$; $P < 0.001$) and wheat/oats ($F_{3,15} = 17.316$; $P < 0.001$). In both treatments, post-hoc tests indicated a higher second-generation peak in *H. destructor* than other species. On lentils, there were significant differences between species ($F_{3,15} = 38.841$; $P < 0.001$), with *P. major* having a lower second-generation peak compared with *P. falcatus* and *P. sp. x*, which were lower still compared with *H. destructor*. There were significant differences between species on canola ($F_{3,15} = 865.025$; $P < 0.001$) and ox-tongue ($F_{3,15} = 637.793$; $P < 0.001$), with *P. falcatus* and *H. destructor* having higher second-generation peaks compared with *P. major* and *P. sp. x*.

The relatively low soil moisture and surface humidity (necessary to avoid fungal growth) inside the tubs probably contributed to the low mite returns in 2003 (eg. Gaull and Ridsdill-Smith 1996; Thackray *et al.* 1997). Nevertheless, there were differences in mite numbers and Δ_N values between treatments (Table 3.1). *Halotydeus destructor*

reproduced on all plant types, although numbers recovered from lentils were very low. *Penthaleus falcatus* survived and reproduced on pasture, canola and ox-tongue, but not on wheat/oats or red lentils. *Penthaleus major* successfully reproduced on pasture and the wheat/oat mixture, while *P. sp. x* reproduced on pasture, wheat/oats and red lentils.

3.4.2 Shade-house experiment - plant damage

Mite feeding damage, seen as silvery to grey patches on plant foliage, was recorded throughout the 2002 season (Figure 3.4). In pasture, plant damage was found for all mite species, most notably during the seedling stage. For *H. destructor*, high damage scores were recorded later in the year than for all *Penthaleus* species. This is most likely due to the extended active period of *H. destructor* in the pasture tubs (see Figure 3.1). I also observed species differences in the plant types attacked within pasture tubs. *Penthaleus major* avoided the clovers, primarily damaging rye and phalaris. *Penthaleus falcatus* and *P. sp. x* mainly attacked plantain and, to a lesser extent, phalaris and rye grass, while *H. destructor* caused most damage to plantain and both clover varieties.

In the wheat/oats treatments, all mite species caused damage during the seedling stage, with *H. destructor* and *P. sp. x* also causing damage later in the season. Feeding by *H. destructor* and *P. falcatus* resulted in significant damage to canola, while *P. sp. x* and *P. major* (in particular) caused little damage. Generally, lentils had minimal damage as a result of mite feeding. In the ox-tongue treatments, feeding by *P. falcatus* caused the majority of plants to die and as a consequence, fresh ox-tongue leaves were added to maintain mite populations. This likely affected the number of mites surviving inside the tubs in 2002, as well as the number of diapause eggs produced and, hence, the

number of mites that emerged in 2003. *Halotydeus destructor* also caused feeding damage to ox-tongue, while *P. sp. x* and *P. major* caused little damage.

Damage indices were used to examine the impact of mite feeding (Figure 3.5). On pasture, there were no significant differences in plant damage between species during the seedling stage ($F_{3,15} = 1.102$; $P = 0.386$), however, there were differences on wheat/oats ($F_{3,15} = 7.570$; $P = 0.004$), lentils ($F_{3,15} = 15.578$; $P < 0.001$), canola ($F_{3,15} = 58.157$; $P < 0.001$), and ox-tongue ($F_{3,15} = 504.596$; $P < 0.001$). The average damage scores over a season (until 23 October) differed significantly among species on all plant types: pasture ($F_{3,15} = 29.474$; $P < 0.001$); wheat/oats ($F_{3,15} = 141.398$; $P < 0.001$); lentils ($F_{3,15} = 53.339$; $P < 0.001$); canola ($F_{3,15} = 225.344$; $P < 0.001$); and ox-tongue ($F_{3,15} = 777.544$; $P < 0.001$). Post-hoc tests indicate that *H. destructor* caused significantly more feeding damage than other species on both pasture and wheat/oats over the mite season. On lentils, most plant damage during the seedling stage and over the season was due to *P. sp. x*. *Halotydeus destructor* and *P. falcatus* caused the greatest seedling damage and average season damage to canola plants, while *P. falcatus* caused significantly more damage to ox-tongue than other species both during the seedling stage and over the mite season.

For each species, the relationship between mite numbers and plant damage during the season was examined. In all cases, plant damage increased as mite numbers increased, although there was a time delay between mite densities and damage. As an example, the data for *P. falcatus* on each plant type are plotted in Figure 3.6. When the size of *P. falcatus* populations declined and then increased again with the next generation, plant damage decreased and then increased with a lag effect. The association between mite

numbers and damage was examined directly using regression analysis. There was a significant association for each species on all plant types, except in two cases where species failed to persist for longer than two weeks (data not presented). As an example, for *P. falcatus*, the regression of mite numbers onto plant damage was significant in pasture ($r^2 = 0.187$; $P = 0.031$), wheat/oats ($r^2 = 0.281$; $P = 0.003$), canola ($r^2 = 0.612$; $P < 0.001$), lentils ($r^2 = 0.473$; $P < 0.001$), and ox-tongue ($r^2 = 0.804$; $P < 0.001$). Therefore, plant damage during the experiment was most likely a direct reflection of mite numbers feeding on the plants. To examine the effect of feeding damage on plant development, the number of days from sowing to flowering of canola plants was examined. Flowering time was delayed as a result of mite feeding in *P. falcatus* treatments (average = 76.25 days) compared with both *P. major* (65.5 days) and *P. sp. x* (66.75 days) treatments ($F_{3,15} = 6.575$; $P = 0.007$).

3.4.3 Field plots

For each species, average mite numbers fluctuated during the field experiment (Figure 3.7). In most treatments, numbers decreased during the 2001 season from their initial starting numbers. This probably reflected the dry autumn and winter conditions experienced at Yeungroon in 2001. In the surrounding paddocks, mite populations were lower than in previous years, suggesting that conditions were unfavourable for the mites. Winter rainfall in 2002 was also well below average and likely to have reduced mite numbers.

The results were generally consistent between the field plots and the shade-house experiment. In pasture, all four species survived and reproduced, with mite densities highest in *H. destructor* and *P. major* treatments. In the canola and ox-tongue

treatments, numbers of *P. falcatus* and *H. destructor* were significantly greater than *P. major* and *P. sp. x* at each sampling date. The small number of *P. major* and *P. sp. x* surviving inside field plots was most likely due to the presence of unwanted grasses and weeds. This is supported by the fact that feeding damage (recorded throughout the experiment) to ox-tongue and canola plants in both *P. major* and *P. sp. x* treatments was negligible (data not presented). In the wheat/oat field plots, all four species persisted throughout the experiment. This contradicts the shade-house data, which indicated that wheat/oats could not support the development of *P. major* and *P. falcatus*. Annual grasses and weeds may have facilitated the survival of these species in the field.

3.4.4 Host plant survey

The survey indicates that all earth mite species are found on crops and pastures, while *P. falcatus* and *H. destructor* are the only species to commonly feed on weeds (Table 3.2). There were significant differences in the plant hosts among species ($\chi^2 = 84.076$; $df = 21$; $P < 0.001$). *Halotydeus destructor* attacked a wide variety of plant types but was most commonly found in pasture and wheat. *Penthaleus major* was commonly found attacking pasture and oats, while *P. sp. x* was mainly associated with wheat and oats. The most common species feeding on canola was *P. falcatus*, which was also the most abundant species on bristly ox-tongue.

3.5 DISCUSSION

Plant host availability is an important biotic factor influencing earth mite abundance in southern Australia (McDonald *et al.* 1995), and is likely to be more important than other biotic factors such as pathogenic fungi and predators, at least under current agricultural management practices (Ridsdill-Smith and Annells 1997). In the current experiments, each mite species responded differently when confined to various plant treatments. This reinforces the view that mite species differ in their biology and that control strategies need to be developed which consider each species separately.

All species survived on pasture and caused feeding damage, particularly *H. destructor*. Of the *Penthaleus* spp., *P. major* was the most successful, while *P. sp. x* numbers were quite low. This is consistent with findings from the field host survey and plant outbreak data conducted by Robinson and Hoffmann (2001) in southeastern Australia. Differences were also observed in the types of legumes and grasses attacked by mites within pasture treatments. *Penthaleus* spp. preferred grasses and rarely attacked clovers, while *H. destructor* mainly fed on clovers and plantain. Therefore, a healthy pasture paddock containing a large proportion of clover may be relatively more susceptible to *H. destructor*. This has implications for control in northern New South Wales where *H. destructor* is completely absent (Robinson and Hoffmann 2001), as pastures with a high proportion of clover may be relatively more resistant to earth mite attack.

Canola was a suitable host for both *P. falcatus* and *H. destructor*, but not for *P. major* or *P. sp. x*. In the shade-house experiment, canola was particularly vulnerable to *P.*

falcatus attack, and feeding damage suffered by this species resulted in delayed flowering time. These findings are supported by field survey data, where the majority of associations on canola were attributed to *P. falcatus* and then *H. destructor*. *Penthaleus major* and *P. sp. x* are occasionally found attacking canola in the field (Robinson and Hoffmann 2001; this chapter). However, these species cannot successfully breed on canola and it has been suggested that, in such instances, mites move in from adjacent paddocks harbouring large populations (Merton *et al.* 1995; Weeks *et al.* 2000). Therefore, canola may be an ideal crop for use in rotation programs when either *P. major* and *P. sp. x* are prevalent. Ox-tongue sustained both *P. falcatus* and *H. destructor* populations, but not *P. major* or *P. sp. x*. This is consistent with previous findings that *H. destructor* and *P. falcatus* are commonly found on, and can survive on broad-leaved weeds such as ‘cats ear’ (*Hypochoeris* spp.), capeweed and ox-tongue (Swan 1934; Gaull and Ridsdill-Smith 1996; Weeks and Hoffmann 1999). This supports conjecture that broad-leaved weeds can provide alternative plant hosts and favourable habitats enabling survival of mites within non-host crops. Weed management inside crops is therefore likely to be important, particularly when *P. falcatus* and/or *H. destructor* are the prevalent mite species.

In laboratory studies, McDonald *et al.* (1995) found that *H. destructor* was unable to survive and reproduce in small pots of either wheat or oats. In contrast, Robinson and Hoffmann (2001) showed that *H. destructor*, *P. major* and *P. sp. x* could persist on these two crops, although mite numbers were variable. My shade-house results suggest that a mix of wheat and oats is a suitable host for *H. destructor* and *P. sp. x*, although the current data for *P. falcatus* and *P. major* are inconclusive. *Penthaleus falcatus* survived during the 2002 season, however, the reproductive output measured in 2003

was negligible. Wheat and oats may therefore not sustain *P. falcatus* populations in the long term. *Penthaleus major* numbers and feeding damage scores were relatively low during 2002, however a number of mites emerged from diapause in 2003. This was surprising and may be due to the early production of diapause eggs in this species (Chapter 2). Results from the field experiment indicate that all species can persist on wheat/oats. While this supports the shade-house data for *H. destructor* and *P. sp. x*, data for *P. major* and *P. falcatus* are inconsistent. This could be explained by grasses and weeds dispersed within the field plots enabling mite survival (Ridsdill-Smith and Pavri 2000). Further studies are required on the host suitability of wheat and oats, although control of weeds and annual grasses in these crops seems important, particularly in situations when *P. falcatus* is abundant.

The host capabilities of red lentils were only tested in the shade-house. Lentils were a poor host for mites, corroborating previous findings (McDonald *et al.* 1996). In particular, *P. major* and *P. falcatus* populations were very low and caused minimal feeding damage. Relatively few *H. destructor* emerged from diapause in 2003, despite this species persisting in 2002. Perhaps adult mites did not obtain sufficient nutrients to produce viable diapause eggs in spring, or the larvae that emerged from diapause failed to develop into adults because they were unable to penetrate the outer plant cuticle (MacLennan *et al.* 1998). *Penthaleus sp. x* caused feeding damage to lentil plants and bred successfully on this host, albeit in low numbers. In a field survey conducted in southeastern Australia < 2% of reported mite outbreaks were on lentils (Robinson and Hoffmann 2001). Therefore, lentils may be a suitable rotation crop in many instances, although further field-based studies are needed to determine the potential benefits of such an approach when *P. sp. x* is problematic.

Apparent contradictions in the survival of mites on ‘non-host’ plants (McDonald *et al.* 1995; Robinson and Hoffmann 2001; this chapter) may in part be due to microflora on the soil surface. Maclennan *et al.* (1998) showed that the presence of microflora on the soil surface permits *H. destructor* to survive, develop and reproduce on an otherwise ‘unsuitable’ host. They suggested the major effect of microflora is that it increases survivorship during the early development stages, providing both food and a favourable microclimate for the larvae, and a microhabitat for adults. Although the impact of microflora was not examined here, free-living filamentous green algae and mosses were identified within most shade-house tubs. This biota may have played an important role in the survivorship of juvenile mites. Microflora could also explain why some lentil varieties are relatively poor hosts for mites. The tall growth characteristics of lentil plants allow air movement through the canopy, leaving the surrounding soil surface exposed. Consequently, the relative humidity and microflora within lentils is reduced compared with pastures and some crops. It is also possible that lentils are a poor host because they are resistant to mite attack. Various lectins, including lentil lectins, can kill or retard the growth of some plant-eating insects (Czapla and Lang 1990; Eisemann *et al.* 1994).

In summary, these results show that earth mite species differ in their ability to persist and reproduce on different plant types. This information could be used to develop cultural strategies that would reduce the need for chemical sprays and form part of a more sustainable and environmentally acceptable means of control. When *P. major* is an important pest, canola and lentils are potentially very useful rotation crops. Pastures containing predominantly thick-bladed grasses should also be carefully monitored and rotated with other crops when practical. In situations where *P. falcatus* and/or *H.*

destructor are the most abundant mite species, farmers should consider rotating crops with lentils and remain diligent about weed management. It is recommended that pastures with high clover content be avoided in regions where *H. destructor* occurs at high densities. Crop rotations that involve canola are likely to be the most effective means of reducing the impact of *P. sp. x*. Such recommendations may be an attractive option for minimising earth mite damage within Australian agricultural ecosystems, as they are relatively straightforward, economically desirable, environmentally acceptable, and perhaps most importantly, willingly adopted by farmers (Dent 2000). There are several other crops not examined in this study that could be useful in rotation programs. For instance, chickpea and some lupin varieties are poor hosts of *H. destructor* (McDonald *et al.* 1996; Thackray *et al.* 1997; Liu and Ridsdill-Smith 2000). Lupins may prove especially valuable in rotations with pastures because of their association with the herbicide simazin, which allows efficient control of annual grass weeds (Dowling and Ridings 1993). Additionally, cultivars of the same plant may differ in their suitability as hosts and in their susceptibility to earth mite attack (eg. Liu and Ridsdill-Smith 2000).

3.6 REFERENCES CITED

- Annells A. J. 1994. The reproductive biology and mating behaviour of redlegged earth mite: an overview. *In*: McDonald G. and A.A. Hoffmann (eds.), Proceedings of the Second National Workshop on Redlegged Earth Mite, Lucerne Flea and Blue Oat Mite. Department of Agriculture, Rutherglen, Australia, pp. 29-31.
- Chapman R., Ridsdill-Smith T. J. and Turner N. C. 2000. Water stress and redlegged earth mites affect the early growth of seedlings in a subterranean clover/capeweed pasture community. *Australian Journal of Agricultural Research* 51: 361-370.
- Dent D. 2000. Insect pest management, 2nd edition. CABI Publishing, Cambridge, UK.
- Czapla T. H. and Lang B. A. 1990. Effect of plant lectins on the larval development of European Corn Borer (Lepidoptera: Pyralidae) and Southern Corn Rootworm (Coleoptera: Chrysomelidae). *Journal of Economic Entomology* 83: 2480-2485.
- Dowling P. M. and Ridings H. I. 1993. Crop rotations and annual grass control. *In*: Delfosse E. (ed.), Pests of pastures: weed, invertebrate and disease pests of Australian sheep pastures. CSIRO, Melbourne, Australia, pp. 193-196.
- Eisemann C. H., Donaldson R. A., Pearson R. D., Cadogan L. C., Vuocolo T. and Tellam R. L. 1994. Larvicidal activity of lectins on *Lucilia cuprina*: mechanism of action. *Entomologia Experimentalis et Applicata* 72: 1-10.

Gaull K. R. and Ridsdill-Smith T. J. 1996. The foraging behaviour of redlegged earth mite, *Halotydeus destructor* (Acarina: Pentheleidae), in an annual subterranean clover pasture. Bulletin of Entomological Research 86: 247-252.

Gillespie D. J. 1991. Identification of resistance to redlegged earth mite *Halotydeus destructor* in pasture legumes. Plant Protection Quarterly 6: 170-171.

Gillespie D. J. 1993. Redlegged earth mite (*Halotydeus destructor*) resistance in annual pasture legumes. In: Delfosse E. (ed.), Pests of pastures: weed, invertebrate and disease pests of Australian sheep pastures. CSIRO, Melbourne, Australia, pp. 211-213.

Hoffmann A. A., Porter S. and Kovacs I. 1997. The response of the major crop and pasture pest, the red-legged earth mite (*Halotydeus destructor*) to pesticides: dose-response curves and evidence for tolerance. Experimental and Applied Acarology 21: 151-162.

James D. G. 1995. Biological control of earth mites in pasture using endemic natural enemies. Plant Protection Quarterly 10: 58-59.

Jeppson L. R., Keifer H. H. and Baker E. W. 1975. Mites Injurious to Economic Plants. University of California Press, Berkeley, America.

Lake A. W. H. and Howie J. H. 1995. Selection for redlegged earth mite resistance in annual *Medicago* species. Plant Protection Quarterly 10: 45-46.

Liu A. and Ridsdill-Smith T. J. 2000. Feeding by redlegged earth mite (*Halotydeus destructor*) on seedlings influences subsequent plant performance of different pulse crops. Australian Journal of Experimental Agriculture 40: 715-723.

MacIennan K. E., McDonald G. and Ward S. A. 1998. Soil microflora as hosts of redlegged earth mite (*Halotydeus destructor*). Entomologia Experimentalis et Applicata 86: 319-323.

McDonald G., Moritz K., Merton E. and Hoffmann A. A. 1995. The biology and behaviour of redlegged earth mite and blue oat mite on crop plants. Plant Protection Quarterly 10: 52-55.

McDonald G., Ballinger D. and Hoffmann A. 1996. Integrated pest management of earth mites in canola. Western Focus (Australian Grain), February-March: 6-8.

Merton E., McDonald G. and Hoffmann A. 1995. Cultural control of redlegged earth mite, blue oat mite and lucerne flea in canola. Plant Protection Quarterly 10: 65-66.

Michael P. 1995. Biological control of redlegged earth mite and lucerne flea by predators *Anystis wallacei* and *Nemologus capillatus*. Plant Protection Quarterly 10: 55-57.

Moritz K. and McDonald G. 1995. Developing redlegged earth mite resistance in canola. In: Potter T.D. (ed.), Proceedings of the Australian Research Assembly on

Brassicas. South Australian Research and Development Institute, Adelaide, Australia, pp. 30-35.

Qin T. K. and Halliday R. B. 1996. The Australian species of *Chromotydeus* Berlese and *Penthaleus* Koch, C.L. (Acarina, Penthaleidae). Journal of Natural History 30: 1833-1848.

Ridsdill-Smith T. J. 1997. Biology and control of *Halotydeus destructor* (Tucker) (Acarina: Penthaleidae) – a review. Experimental and Applied Acarology 21: 195-224.

Ridsdill-Smith T. J. and Annells A. J. 1997. Seasonal occurrence and abundance of redlegged earth mite *Halotydeus destructor* (Acari, Penthaleidae) in annual pastures of southwestern Australia. Bulletin of Entomological Research 87: 413-423.

Ridsdill-Smith T. J. and Pavri C. 1998. Spring spraying for redlegged earth mites. Western Focus (Australian Grain), October-November: 1-4.

Ridsdill-Smith T. J. and Pavri C. C. 2000. Feeding life style of redlegged earth mite, *Halotydeus destructor* (Acari: Penthaleidae), in pastures and the role of broad-leafed weeds. Experimental and Applied Acarology 24: 397-414.

Ridsdill-Smith T. J., Jiang Y. and Ghisalberti E. L. 1995. A method to test compounds for feeding deterrence towards redlegged earth mites (Acarina: Penthaleidae). Annals of Applied Biology 127: 593-600.

Robinson M. R. and Hoffmann A. A. 2001. The pest status and distribution of three cryptic blue oat mite species (*Penthaleus* spp.) and the redlegged earth mite (*Halotydeus destructor*) in southeastern Australia. *Experimental and Applied Acarology* 25: 699-716.

Swan D. C. 1934. The red-legged earth mite *Halotydeus destructor* (Tucker) in South Australia: with remarks upon *Penthaleus major* (Dugés). *Journal of Agriculture South Australia* 38: 353-367.

Thackray D. J., Ridsdill-Smith T. J. and Gillespie D. J. 1997. Susceptibility of grain legume species to redlegged earth mite (*Halotydeus destructor*) damage at the seedling stage. *Plant Protection Quarterly* 12(3): 141-144.

Umina P. A. and Hoffmann A. A. 1999. Tolerance of cryptic species of blue oat mites (*Penthaleus* spp.) and the redlegged earth mite (*Halotydeus destructor*) to pesticides. *Australian Journal of Experimental Agriculture* 39: 621-628.

Wallace M. M. H. 1970. The influence of temperature on post-diapause development and survival in the aestivating eggs of *Halotydeus destructor* (Acari: Eupodidae). *Australian Journal of Zoology* 18: 315-329.

Weeks A. R. and Hoffmann A. A. 1999. The biology of *Penthaleus* species in southeastern Australia. *Entomologia Experimentalis et Applicata* 92: 179-189.

Weeks A. R. and Hoffmann A. A. 2000. Competitive interactions between two pest species of earth mites, *Halotydeus destructor* and *Penthaleus major* (Acarina: Penthaleidae). Journal of Economic Entomology 93: 1183-1191.

Weeks A. R., Fripp Y. J. and Hoffmann A. A. 1995. Genetic structure of *Halotydeus destructor* and *Penthaleus major* populations in Victoria (Acari: Penthaleidae). Experimental and Applied Acarology 19: 633-646.

Weeks A. R., Turelli M. and Hoffmann A. A. 2000. Dispersal patterns of pest earth mites (Acari: Penthaleidae) in pastures and crops. Journal of Economic Entomology 93: 1415-1423.

3.7 TABLES AND FIGURES

Table 3.1: Average change in population size (expressed as a % of input) (\pm SE) of each earth mite species on different plant types. Standard errors are based on 4 replicates (unless indicated by and asterisk - * = 3 replicates and ** = 2 replicates).

Plant type	Relative population change (%) \pm SE			
	<i>P. major</i>	<i>P. falcatus</i>	<i>P. sp. x</i>	<i>H. destructor</i>
Pasture	51.5 \pm 26.6	41.2 \pm 8.1	14.0 \pm 3.8	211.9 \pm 31.0
Wheat/oats	52.5 \pm 21.6	0.5 \pm 0.3	18.9 \pm 3.0	64.8 \pm 12.5
Canola	0**	174.3 \pm 23.7*	0*	109.3 \pm 1.7
Lentils	6.6 \pm 1.7	1.0 \pm 0.4	27.2 \pm 6.6	2.9 \pm 0.8
Ox-tongue	0.3 \pm 0.2	159.0 \pm 11.0	0	70.4 \pm 23.4

Table 3.2: Number of field host associations on pasture, weed and crop plants recorded for each mite species in surveys conducted between 2000-2002 in southeastern Australia.

Species [†]	Oats	Wheat	Canola	Field peas	Vetch	Pasture	Ox- tongue	Capeweed	Total
<i>H. destructor</i>	3	6	3	1	1	14	3	3	34
<i>P. major</i>	8	2	1			24			35
<i>P. falcatus</i>		1	6			2	7		16
<i>P. sp. x</i>	4	8			2	3			17
Total	15	17	10	1	3	43	10	3	102

[†] = predominant earth mite species in each sample (comprising > 80% of the mite population).

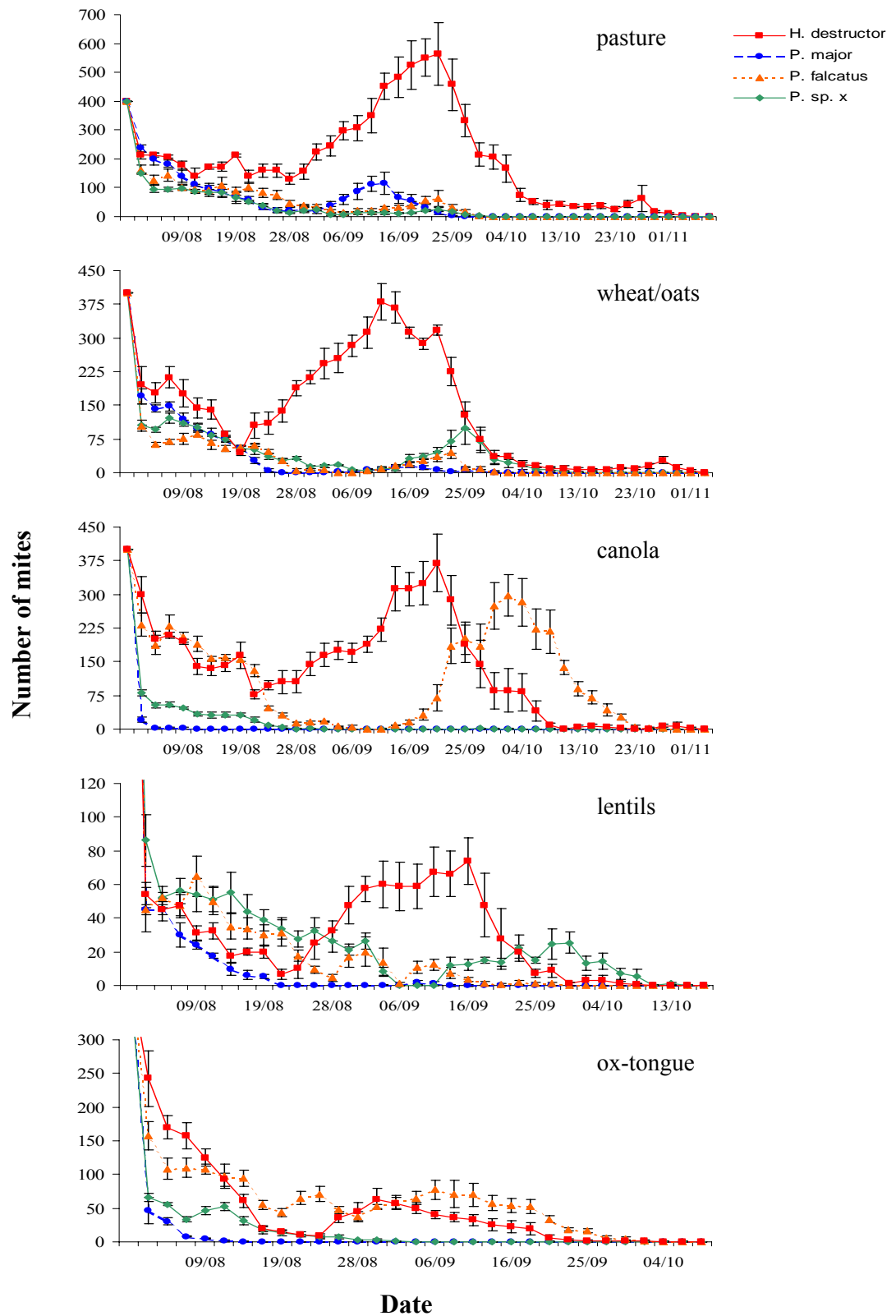


Figure 3.1: Average numbers (\pm SE) of each mite species when confined to different plant treatments. Note the graphs have different scales.

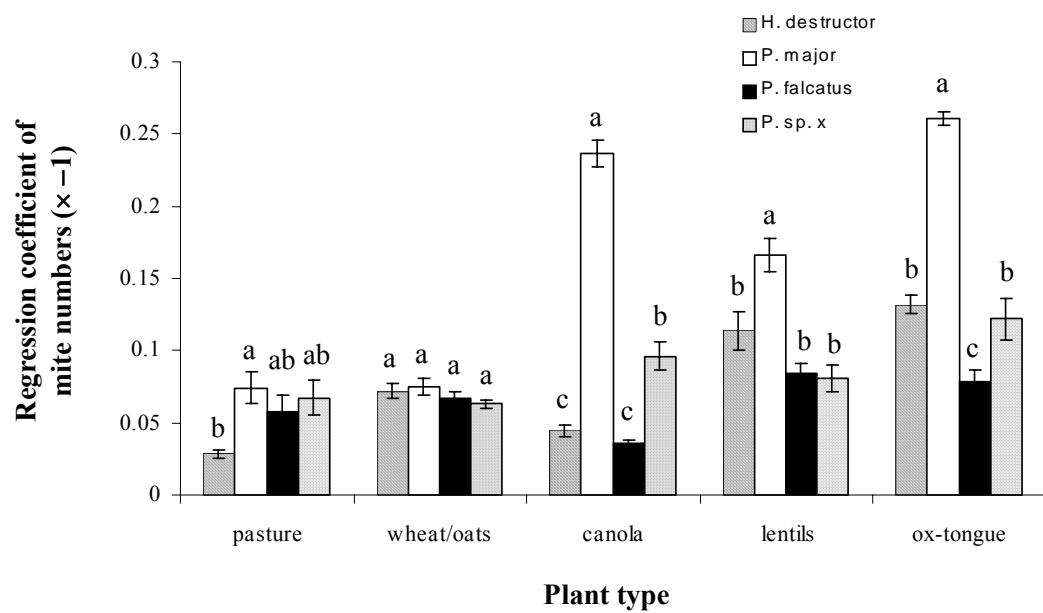


Figure 3.2: Regression coefficients (\pm SE) of average mite numbers during the seedling stage (first 3 weeks after mite application) for each species on various plant types. Regression coefficients are multiplied by -1 to obtain a positive fitness measure. Bars with the same letter are not significantly different according to a Tukey B post-hoc test.

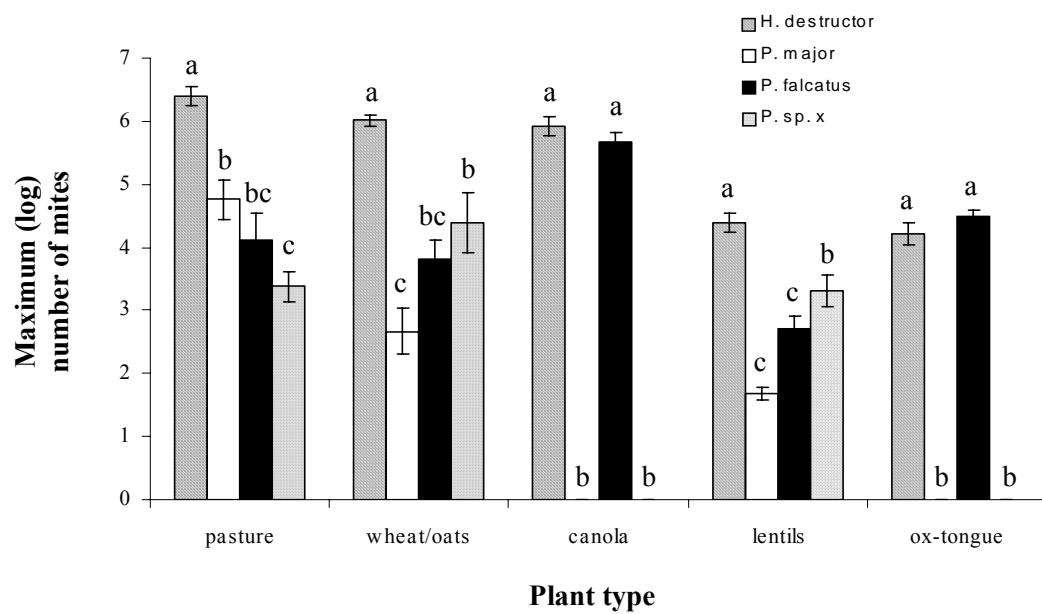


Figure 3.3: Average maximum number (log transformed) (\pm SE) of second-generation mites on various plant types. Bars with the same letter are not significantly different according to a Tukey B post-hoc test.

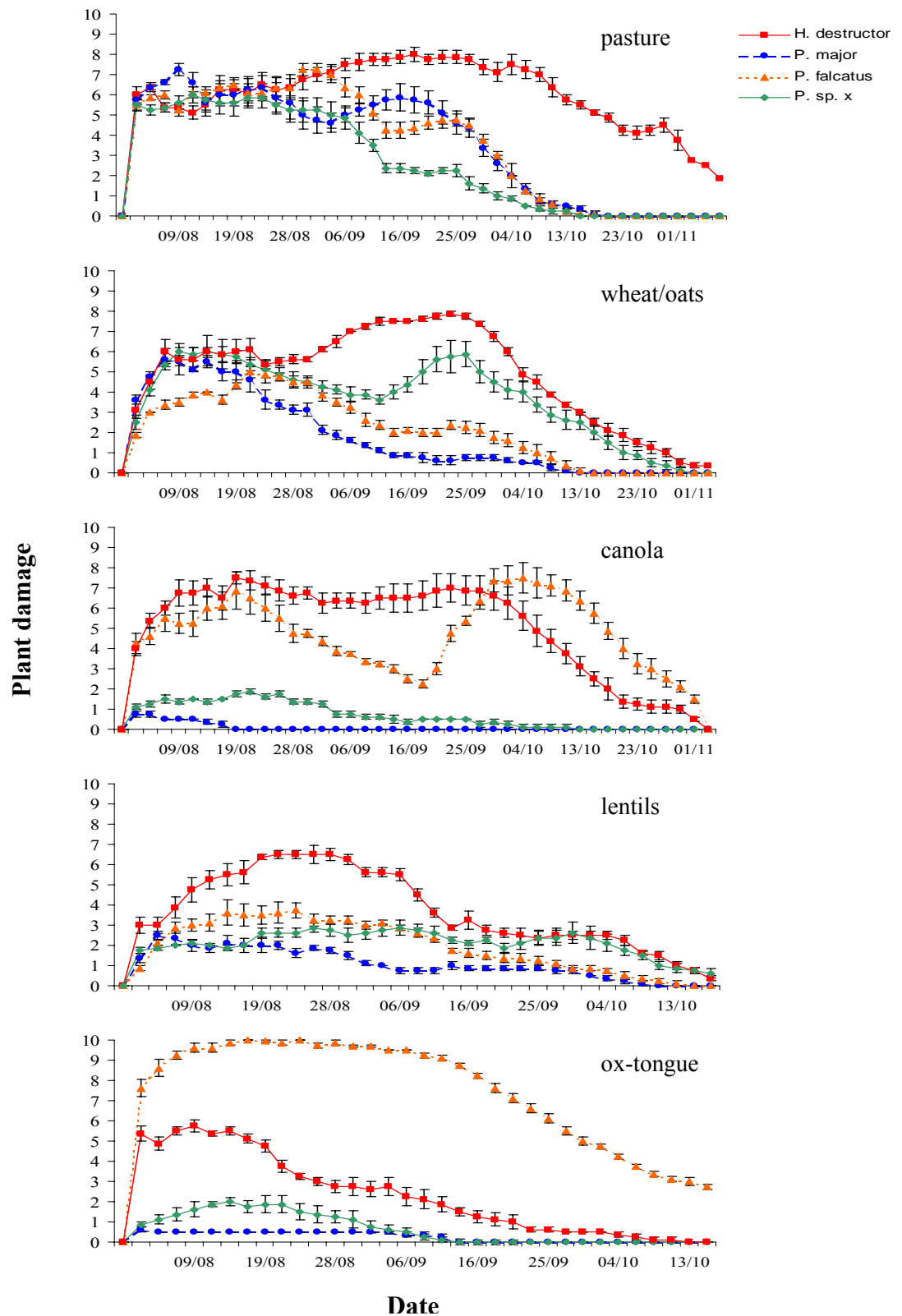


Figure 3.4: Average plant damage (\pm SE) to the different plant treatments after feeding by each mite species. Note the graphs have different scales.

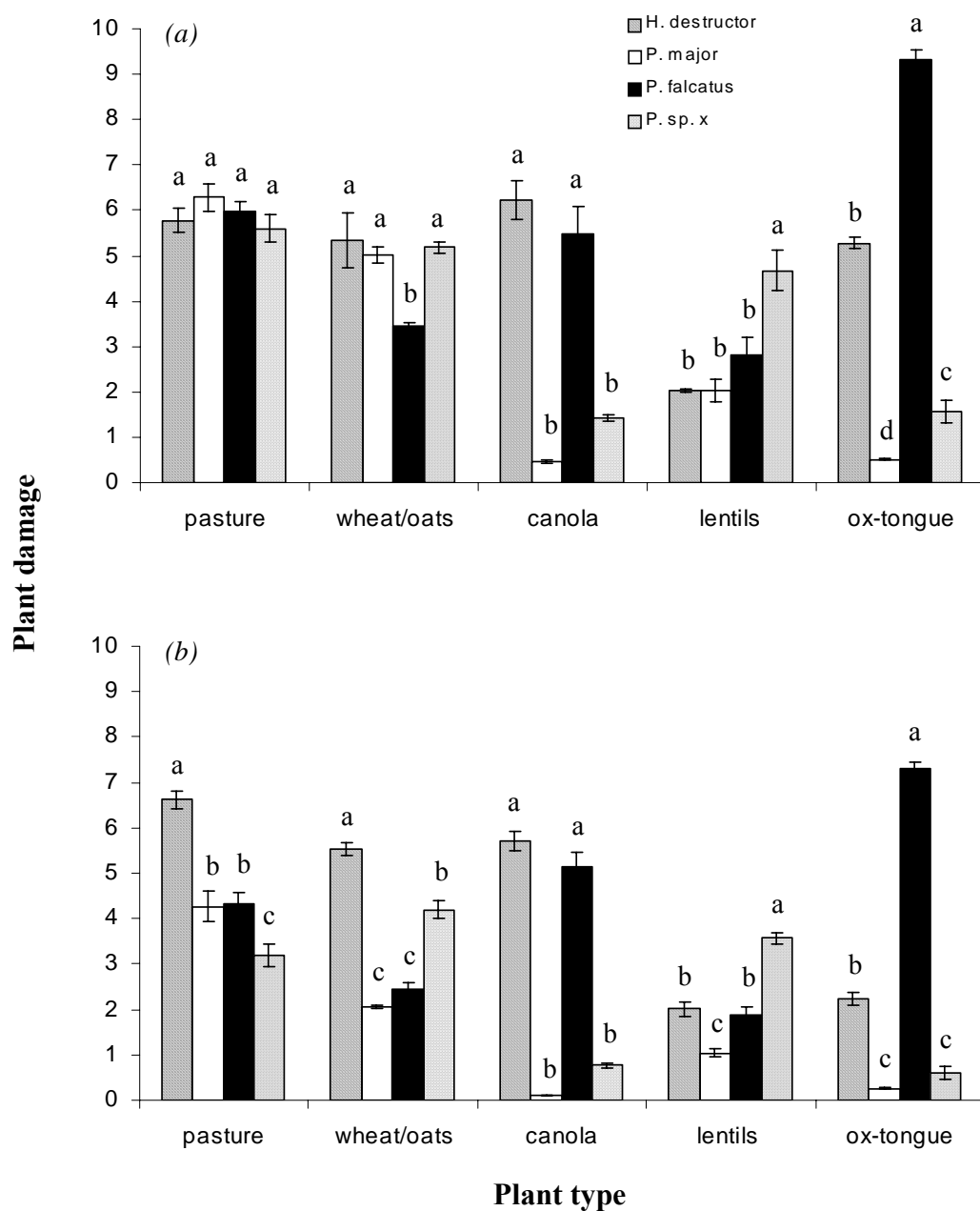


Figure 3.5: Average plant damage (\pm SE) caused by mite feeding during (a) the seedling stage (first 3 weeks after mite application) and (b) the active period for *Penthaleus* spp. during the experiment (until 23 October). Bars with the same letter are not significantly different according to a Tukey B post-hoc test.

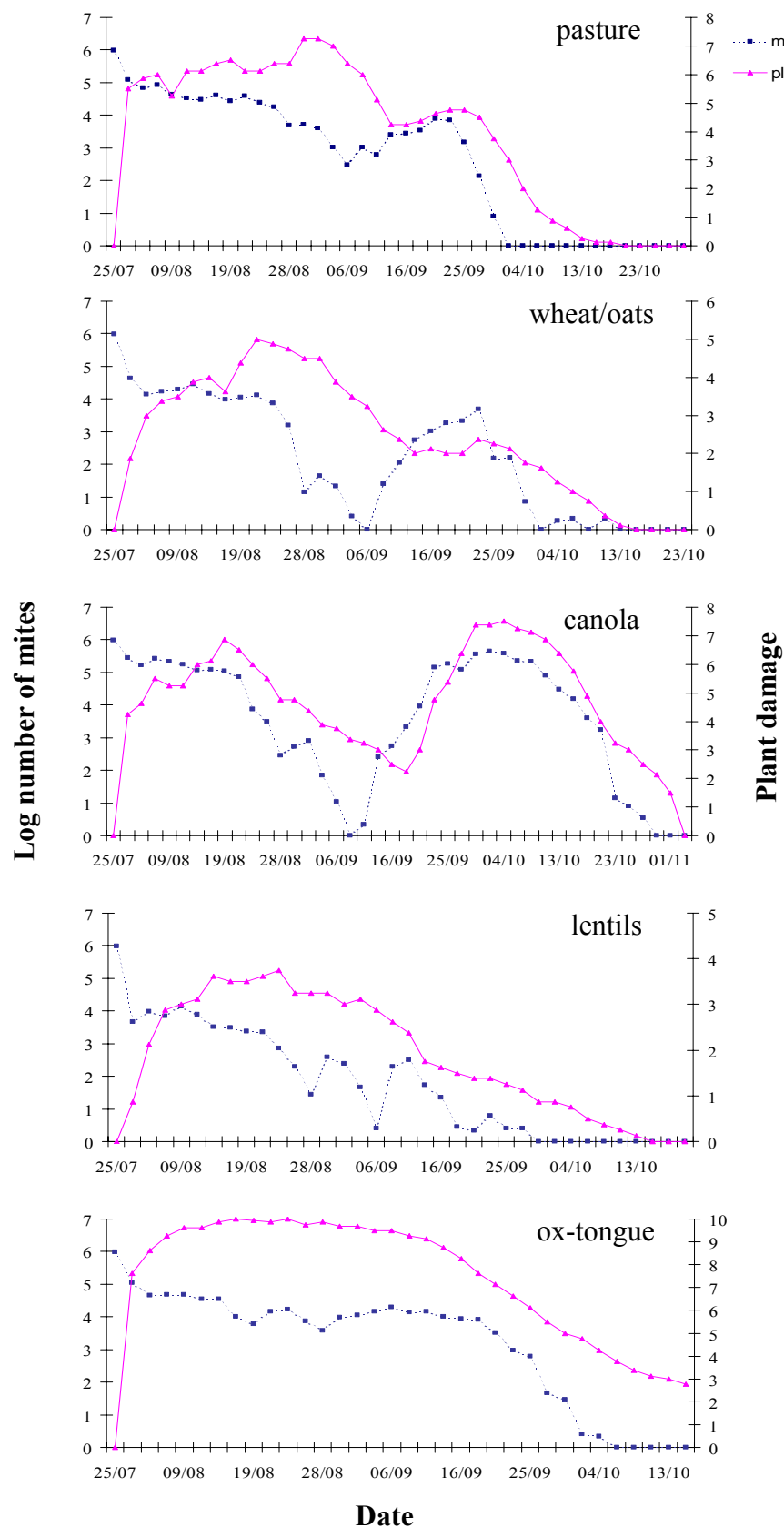


Figure 3.6: Relationship between average numbers of *P. falcatus* (log transformed) and plant damage during the 2002 season. Note the graphs have different scales.

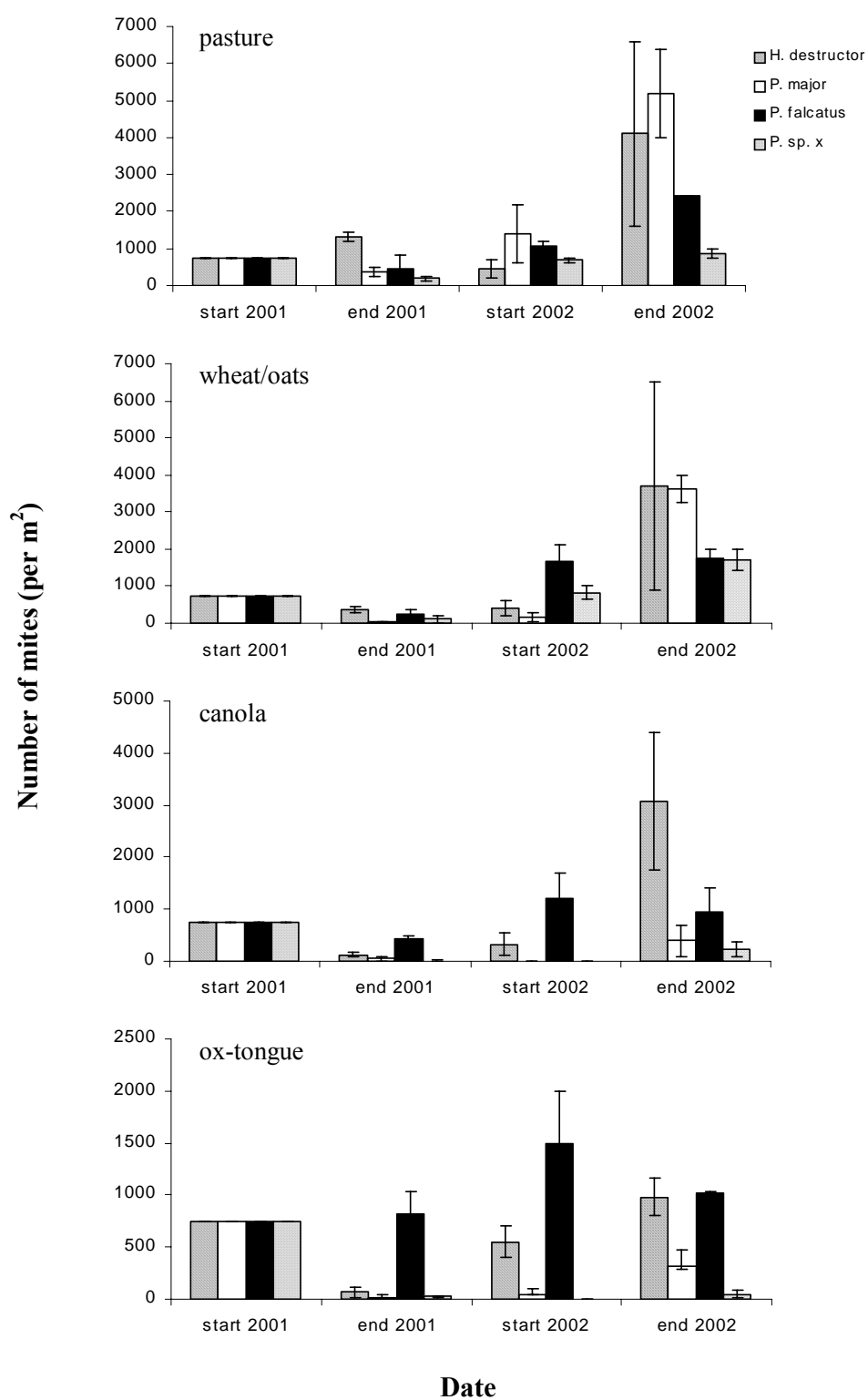


Figure 3.7: Average number of mites per square metre (\pm SE) in Yeungroon field plots at release and three sampling dates.

CHAPTER 4.

Competitive interactions among four pest species of earth mites

4.1 ABSTRACT

Earth mites are major winter pests of a variety of crops and pastures in southern Australia. Competition between four earth mite species was investigated using field and shade-house experiments. The influence of different host plant species on the frequency and intensity of competitive interactions were also examined. This information is important, as control attempts that eradicate one species of mite could be directly followed by an increase in abundance of another earth mite species. There were strong effects of intraspecific competition on the reproductive rate of species, while interspecific interactions between *Halotydeus destructor* (Tucker) and *Penthaleus* spp. and between the three *Penthaleus* species were also detected. Competitive abilities were altered on the different plant types. On pasture, the competitive advantage swayed between *Penthaleus major* (Dugés), *H. destructor*, and *Penthaleus falcatus* (Qin and Halliday). *Penthaleus* sp. *x* was the strongest competitor in a mixture of wheat and oats, while on canola and bristly ox-tongue, *P. falcatus* and *H. destructor* were superior competitors. These results suggest that competition is a strong force influencing the abundance of earth mites in the field and that host plant factors are important in shaping the type of interactions. This highlights the importance of identifying mite species when considering control options and suggests that effective control recommendations need to be developed for each individual species.

4.2 INTRODUCTION

The influence of competition in determining community structure has caused considerable debate among ecologists for several decades (Hariston *et al.* 1960; Connell 1983; Underwood 1986; Ferrenberg and Denno 2003). In part this is due to poorly designed experiments, where competition is described but results are confounded by external factors such as weather, predation and parasitism (Connell 1983; Underwood 1986). Recent reviews and field experiments, however, have demonstrated that competition is important in the population dynamics and performance of various animal and plant communities. In particular, clear evidence of interspecific competition now exists among many phytophagous insects and herbivorous species (see Denno *et al.* 1995).

In this chapter, I examine the competitive interactions of earth mite species, which are important plant-sucking pests in many parts of the world. Within Australia, there are four earth mite species of agricultural importance: *Halotydeus destructor* (Tucker); *Penthaleus major* (Dugés); *Penthaleus falcatus* (Qin and Halliday); and a recently discovered species referred to as *Penthaleus* sp. *x*. Earth mites are approximately 1 mm in size when adults, and in the field have been recorded at densities of up to 60 000 mites per square metre (Ridsdill-Smith and Pavri 1998). They are active between April and October, producing two or three generations before entering diapause throughout the summer months. Diapause eggs hatch in autumn when conditions are optimal following cool temperatures and rainfall (Wallace 1970; Ridsdill-Smith and Annells 1997). *Halotydeus destructor* is highly polyphagous, feeding on most agriculturally important pasture and crop plants, as well as vegetables and other market garden

produce (Annells and Ridsdill-Smith 1994). The three *Penthaleus* species are also polyphagous, although they tend to be associated with particular plant hosts (Weeks and Hoffmann 1999). *Penthaleus major* primarily feeds on pasture, wheat and oats. *Penthaleus falcatus* prefers pasture, canola and broad-leaved weeds, while *P. sp. x* prefers wheat, oats and barley (Robinson and Hoffmann 2001; Chapter 3).

Earth mite species are presently treated identically in terms of control, even though the species differ markedly in their biology. *Halotydeus destructor* reproduces sexually (diplo-diploid), while *Penthaleus* spp. reproduce via obligate parthenogenesis and consist of genetically distinct clones (Annells 1994; Weeks *et al.* 1995; Weeks and Hoffmann 1999). Species also differ in their distribution, response to pesticides and timing of diapause egg production (Weeks and Hoffmann 1999; Umina and Hoffmann 1999; Chapter 2). Therefore, control strategies based on one species may not be adequate for the other earth mite species. Using field pasture plots, Weeks and Hoffmann (2000) demonstrated competition between the two earth mite species, *P. major* and *H. destructor*. This suggests that suppression or eradication of one species of earth mite may not lead to a reduction in pest pressure because another species could increase in abundance.

In this study, I extend the findings of Weeks and Hoffmann (2000) to include the four earth mite species. Competitive interactions between species were investigated under field and shade-house conditions using four different plant types: pasture; canola; bristly ox-tongue; and a mixture of wheat and oats. This provided a range of agriculturally important plants that are also commonly attacked by earth mites in southern Australia (Weeks and Hoffmann 1999; Ridsdill-Smith and Pavri 2000).

Examining competition on different plant types is important because plants vary in their suitability as hosts and mite species differ in their preferences for plants in the field (Weeks and Hoffmann 1999; Robinson and Hoffmann 2001; Chapter 3).

Understanding the likely competitive interactions under different conditions is, therefore, imperative for the development of successful pest management programs.

4.3 MATERIALS AND METHODS

4.3.1 Field competition experiment

At the start of 2001, a field experiment was set up in a pasture paddock near Yeungroon (36° 21' 32 S, 143° 17' 44 E). This location was chosen because it lies within the distribution of *H. destructor* and the three *Penthaleus* species. Forty-eight plots (1 m by 2 m) were constructed at this site using white corflute sheeting (95 cm high, 0.5 cm thick), following Weeks and Hoffmann (2000). Briefly, the sheets were placed in the ground approximately 15 cm and fixed to wooden stakes that provided a frame for the sheeting. To prevent mite movement, Tac-Gel[®] (Rentokil, active ingredient polybutene) was applied to the upper edge of the corflute. Field plots are ideal because they permit experimental manipulation of mite density and composition under natural abiotic conditions (Weeks and Hoffmann 2000).

In May, the soil was cultivated in 36 plots and seeds sown. Twelve plots were sown with a mixture of wheat (*Triticum aestivum*) and oats (*Avena sativa*), 12 plots with canola (*Brassica napus*), and another 12 were sown with seeds of a common weed, bristly ox-tongue (*Picris echioides*). Twelve remaining plots contained established pasture, although a few phalaris (*Phalaris aquatica*), rye grass (*Lolium perenne*) and subterranean clover (*Trifolium subterraneum*) seeds were also added. When mites emerged from diapause, the plots were sprayed twice, two weeks apart, with the organophosphate Imidan[®] (Crop Care, active ingredient 150 g/L Phosmet) at a rate of 0.5 ml/L, to eliminate all resident mites. The plots were then left for an additional two weeks before introducing mites (the residual effect of Imidan[®] is approximately one week).

In mid-July, adult mites were introduced to the plots. For each plant type, five different treatments were randomly assigned to the plots: (1) 1500 *H. destructor* (750 mites per square metre); (2) 1500 *P. major*; (3) 1500 *P. falcatus*; (4) 1500 *P. sp. x*; and (5) 1500 *H. destructor* + 1500 *P. major* + 1500 *P. falcatus* + 1500 *P. sp. x* (3000 mites per square metre). Treatments 1-4 were replicated twice and Treatment 5 was replicated four times within each plant type. This simple design has been used successfully by many authors and allows the assessment of interspecific competition without confounding effects (see Underwood 1986). *Penthaleus major* and *H. destructor* were both collected from thick-bladed grasses (*Poaceae* spp.) along a roadside near Charlton (36° 17' 56 S, 143° 19' 25 E) and Jeffcott North (36° 18' 03 S, 143° 03' 44 E), respectively. *Penthaleus sp. x* were collected at Yeungroon East (36° 21' 31 S, 143° 20' 58 E) from a paddock containing vetch (*Vicia sativa*) and oats, while *P. falcatus* was obtained from bristly ox-tongue, along a roadside near Gooroc (36° 28' 32 S, 143° 12' 05 E). Samples were collected by suction using a STIHL Blowervac BG55 and transported in plastic containers with vegetation and paper towelling to absorb excess moisture. Mites were sorted, counted and transferred to the plots over a four-day period.

All plots were sampled for mite abundance towards the end of the season (early October). Sampling involved nine random suctions, each with a diameter of 12 cm and lasting 5-10 seconds. Mites were placed in 70% ethanol and later classified into species at ×40 magnification using dorsal bristle morphology (Weeks *et al.* 1995; Qin and Halliday 1996; Weeks and Hoffmann 1999) and counted.

In May 2002, plots were sown with the same seed types as the previous year. Tac-Gel[®] was also reapplied to the upper edges of the barriers where needed. Each plot was sampled at the start of the season (early July), after diapause had broken in each species and mites were at the adult stage. Nine samples were randomly taken per plot and mites placed in 70% ethanol for later identification. At the start of 2002, there was some cross-contamination of mite species in plots where only one species had been introduced. This was probably caused by the release of low numbers of the incorrect species although aerial dispersal of diapause eggs over the summer period cannot be discounted (Chapter 2). As a consequence, a range of densities of each species existed over the plots at the start of the 2002 season. All plots were therefore treated as independent data points. Each plot was sampled again at the end of the 2002 season (late September) in the same manner described above.

In May 2003, the plots were again sown with appropriate seeds. Each plot was sampled in late June, after most mites had emerged and were at the adult stage. Nine samples were taken randomly per plot and mites placed in 70% ethanol. Species were later identified and counted.

4.3.2 Shade-house experiment

Competition between earth mites was further investigated under shade-house conditions. Again, four plant types were examined: (1) pasture, containing an equal quantity of phalaris, rye grass, plantain (*Plantago lanceolata*), cocksfoot (*Dactylis glomerata*), white clover (*Trifolium repens*) and subterranean clover seeds; (2) a mixture of wheat and oats; (3) canola; and (4) bristly ox-tongue. In June 2002, seeds were sown into clear plastic tubs (45 cm long, 35 cm wide and 25 cm deep) using

sterilised sandy loam (3:1) soil. The tubs were covered with a clear plastic lid that had a gauze window for ventilation, and placed in a shade-house in a randomised block arrangement. Tubs were then watered to germinate seedlings. Due to their longer germination time, pasture and bristly ox-tongue seeds were sown three weeks prior to wheat/oats and canola.

In late July 2002, earth mites from the field were collected and brought back to the laboratory where they were sorted, counted and released into the tubs. *Halotydeus destructor* and *P. major* were both collected from a pasture paddock near Whittlesea (37° 33' 14 S, 145° 06' 27 E) containing mostly thick-bladed grasses. *Penthaleus* sp. *x* was collected from wild oats (*Avena* spp.) and thick-bladed grasses adjacent to a wheat crop in Dooboobetic (36° 23' 29 S, 143° 12' 00 E). *Penthaleus falcatus* was obtained from bristly ox-tongue, along a roadside near Gooroc (36° 28' 32 S, 143° 12' 05 E).

For each plant type, five different treatments replicated four times were assigned to the plastic tubs (80 tubs in total). The treatments were as follows: (1) 400 *H. destructor* (2500 mites per square metre); (2) 400 *P. major*; (3) 400 *P. falcatus*; (4) 400 *P. sp. x*; and (5) 400 *H. destructor* + 400 *P. major* + 400 *P. falcatus* + 400 *P. sp. x* (10 000 mites per square metre). Over a three-day period, adult mites were transferred to each tub and the lids replaced to prevent mite movement. The tubs were watered regularly throughout the mite season to ensure plant growth, although care was taken not to saturate the tubs. Over-watering can lead to condensation and fungal growth, which causes mite mortality (Thackray *et al.* 1997).

In April 2003, the same seed types as in the previous year were sown into each tub. The tubs were then watered to simulate autumn rains and germinate seeds. Mites started to emerge at the beginning of May. Each tub was sampled six times (one week apart) by suction, using a Blowervac. Adult mites were removed, placed into vials with 70% ethanol, and later sorted to species and counted. During the sampling period, four canola tubs became infected with fungal mycelium. Two of these contained *P. major*, one *P. falcatus* and one contained *P. sp. x*. Because this contamination was detrimental to canola plants and mite numbers, these tubs were excluded from the analyses.

4.3.3 Data analysis

To achieve normality, mite numbers were log transformed (\log_{n+1}) prior to analysis of the data set. For the field competition data, samples were converted to numbers of mites per square metre. During 2001, the reproductive output, R_O (Stearns 1992), was calculated for each species on each plant type as the number of mites collected at the end of the season divided by the number placed into each tub at the start of the season. R_O was also calculated for the second season (number of adult mites at the end of 2002 divided by the number at the beginning of 2002) and through diapause (number at the start of 2003 divided by the number at the end of 2002). Before running Analysis of Variance (ANOVA), R_O values were tested for normality and equality of variances through the Kolmogorov-Smirnov and Scheffe-Box tests (Sokal and Rohlf 1995).

Interspecific competition was determined in the first season using the mean R_O values for the pure and mixed treatments. For each species ANOVAs were undertaken, unless the data were not normally distributed after transformation. In such cases, non-parametric analyses (Kruskal-Wallis tests) were used to compare groups. At the start of

2002, a range of densities of mite species existed across the field plots. Each plot was treated as an independent data point and used in multiple regression, allowing both intra- and interspecific competitive interactions during the 2002 season to be examined. For each species on the different plant types, R_O values were regressed against the number of mites of all four species at the start of 2002. Regression coefficients describe the impact of numbers of each species on R_O taking into account the effects of the other species. Multiple regression was also used to analyse data through diapause (2002-2003), with R_O values regressed against the number of adult mites at the end of the 2002 season. Zero values, which represent plots where a species was completely absent, were excluded from the analyses. The Dunn-Sidak method (Sokal and Rohlf 1995) was utilized to adjust significance levels for multiple comparisons ($k = 4$), while t-tests were undertaken to determine if the slope of regression coefficients (b) were significantly different from -1 . Under contest competition, b does not differ from a slope of -1 , whereas under scramble competition b should differ from -1 (Varley *et al.* 1975).

For the shade-house experiment, R_O was calculated for each species on each plant type as the number of mites collected at the beginning of 2003 divided by the number placed into each tub at the start of 2002. To determine if interspecific competition occurred in the shade-house tubs, the mean R_O values for the pure and mixed treatments were compared using two-way ANOVAs. All analyses were conducted with the statistical program SPSS 11.0.

4.4 RESULTS

4.4.1 Field competition experiment

Competition was evident on all plant types during the 2001 season (Figure 4.1). For *H. destructor*, the mean R_O in pure plots was significantly higher than in mixed plots ($F_{1,16} = 22.996$; $P < 0.001$), indicating other mite species had a negative impact on the reproductive output of *H. destructor*. There was no significant effect of plant type on R_O during 2001 ($F_{3,16} = 1.803$; $P = 0.187$), and no significant interaction between plant type and pure versus mixed plots ($F_{3,16} = 0.239$; $P = 0.868$). This suggests that the fitness and competitive ability of *H. destructor* was the same on the four plant types. For *P. falcatus*, there was no significant difference in the mean R_O between pure and mixed plots ($F_{1,16} = 2.377$; $P = 0.143$), among plant types ($F_{3,16} = 2.645$; $P = 0.085$) or interaction between these factors ($F_{3,16} = 0.938$; $P = 0.445$). Therefore, the reproductive output of *P. falcatus* was not affected by the presence of other species or influenced by plant type during the 2001 season.

For *P. major*, R_O values in wheat/oats and canola treatments were not normally distributed after log transformation, so one-way ANOVAs were only performed on pasture and ox-tongue data. There were no significant differences detected among pure and mixed plots for either pasture ($F_{1,4} = 3.936$; $P = 0.118$) or ox-tongue ($F_{1,4} = 1.748$; $P = 0.257$) treatments. Kruskal-Wallis tests indicated R_O values of *P. major* in pure plots were significantly higher than in mixed plots for both wheat/oats ($\chi^2 = 4.800$; df = 1; $P = 0.028$) and canola ($\chi^2 = 4.800$; df = 1; $P = 0.028$). Therefore, the reproductive output of *P. major* was negatively affected by other species on both canola and

wheat/oats, but not on pasture or ox-tongue. As for *P. major*, one-way ANOVAs were only performed for *P. sp. x* in pasture and wheat/oats treatments. Interspecific competition was demonstrated in pasture treatments ($F_{1,4} = 9.461$; $P < 0.05$), but not in wheat/oats ($F_{1,4} = 0.008$; $P = 0.935$). On the other plant types there was a significant difference between pure and mixed plots containing ox-tongue ($\chi^2 = 4.800$; $df = 1$; $P = 0.028$), but not canola ($\chi^2 = 2.000$; $df = 1$; $P = 0.157$). The reproductive output of *P. sp. x* was, therefore, reduced by competition with other mite species in ox-tongue and pasture. However, it should be noted that mite numbers on canola were variable and low.

Multiple regression was used to examine the frequency and intensity of competitive interactions over the 2002 season and diapause period. On pasture, there was a significant negative relationship between R_O of *H. destructor* and the number of *H. destructor* at the beginning of 2002 (Table 4.1). Therefore, plots with higher densities of *H. destructor* had a lower reproductive output over the season, indicating intraspecific competition or ‘density-dependence’. For *H. destructor*, the regression coefficient was significantly different from -1 ($t = 22.324$; $df = 10$; $P < 0.001$), suggesting scramble competition. Because the slope was significantly less than -1 , undercompensation is suggested (Begon *et al.* 1996). Density-dependence negatively affected the reproductive output of *P. major* during the 2002 season. For *P. major* intraspecific competition may have involved undercompensation because b was significantly less than -1 ($t = 12.310$; $df = 10$; $P < 0.001$). In the generation following diapause, density-dependent effects were again found for *H. destructor* and *P. major*. The slope of the regression lines was significantly less than -1 in *H. destructor* ($t =$

28.437; $df = 10$; $P < 0.001$) and *P. major* ($t = 33.023$; $df = 10$; $P < 0.001$), suggesting undercompensation.

There was evidence of competitive interactions within and between mite species in the wheat/oats treatments during the 2002 season (Table 4.2). Intraspecific competition was suggested for both *H. destructor* and *P. major*, as a significant negative relationship between R_O and density for each species was demonstrated. The slope of b was significantly less than -1 for *H. destructor* ($t = 19.965$; $df = 10$; $P < 0.001$) and *P. major* ($t = 6.579$; $df = 9$; $P < 0.001$), suggesting undercompensation. Regression coefficients for R_O of *P. major* regressed against the number of *P. falcatus* revealed a significant negative slope, with R_O decreasing as *P. falcatus* numbers at the start of 2002 increased. This suggests interspecific competition, with *P. falcatus* limiting the reproductive output of *P. major*.

Multiple regression analysis of data from canola treatments indicated intraspecific competition in *P. falcatus* during the 2002 season (Table 4. 3). Undercompensation was the likely density-dependent effect as the slope of b was significantly less than -1 ($t = 31.943$; $df = 10$; $P < 0.001$). There was some suggestion of other competitive interactions, however, these cases failed to remain significant after correction for multiple comparisons. For example, there appeared to be a positive association between the reproductive output of *H. destructor* and the number of *P. sp. x* at the preceding sampling date, which could indicate negative or ‘inverse’ competition between mite species (Begon *et al.* 1996). Because only four field plots contained *P. sp. x* at the end of 2002, regression analysis on this species over the diapause period was not undertaken.

Competition within and between species was also evident in ox-tongue plots (Table 4.4). The coefficient for R_O of *P. falcatus* in 2002 regressed against the number of *P. falcatus* was significant, indicating intraspecific competition. Intraspecific competition was also demonstrated for this species during the diapause period; the slope of b was significantly less than -1 during 2002 ($t = 32.652$; $df = 10$; $P < 0.001$) and through diapause ($t = 24.717$; $df = 10$; $P < 0.001$), suggesting undercompensation. There was a significant negative relationship when R_O of *P. major* during 2002 was regressed against the number of *P. falcatus* at the start of 2002. Therefore, the reproductive output of *P. major* during the season was limited by *P. falcatus*. Again, there was a suggestion of other interactions although these failed to remain significant after the Dunn-Sidak correction for multiple comparisons.

4.4.2 Shade-house experiment

The results were generally consistent with those of the field plots. Mean R_O values of each species for pure and mixed shade-house tubs suggest competitive interactions (Figure 4.2). For *H. destructor* the mean R_O in pure plots was significantly higher than mixed plots ($F_{1, 23} = 33.915$; $P < 0.001$), indicating the reproductive output of *H. destructor* was reduced by other species. There was also an effect of plant type ($F_{3, 23} = 4.262$; $P = 0.016$), with post-hoc tests indicating a lower R_O in ox-tongue treatments than on pasture and canola. No significant interaction between factors was evident ($F_{3, 23} = 1.583$; $P = 0.221$). For *P. major* there was a significant difference between plant types ($F_{3, 21} = 69.439$; $P < 0.001$), R_O being highest on pasture and wheat/oats, while R_O in pure plots was higher than in mixed plots ($F_{1, 21} = 5.475$; $P = 0.029$), indicating interspecific competition. There was no interaction between these factors ($F_{3, 21} =$

2.334; $P = 0.103$). For *P. falcatus*, the overall R_O differed significantly between plant types ($F_{3, 22} = 82.825$; $P < 0.001$), but there was no difference between pure and mixed plots ($F_{1, 22} = 0.613$; $P = 0.442$), and no interaction between factors ($F_{3, 22} = 2.049$; $P = 0.136$). Therefore, the reproductive output of *P. falcatus* was not influenced by other species.

The shade-house experiment indicated that *P. sp. x* was unable to breed successfully on canola or ox-tongue as no mites of this species were recovered from any pure or mixed tubs in 2003. For this reason, no analyses were undertaken. The overall R_O for *P. sp. x* on pasture and wheat/oats was not significantly different ($F_{1, 12} = 0.532$; $P = 0.480$), while there was no evidence of significant differentiation between the mean R_O in pure and mixed plots ($F_{1, 12} = 4.663$; $P = 0.052$). Furthermore, there was no interaction among plant type and R_O in pure and mixed plots ($F_{1, 12} = 0.713$; $P = 0.415$), suggesting that the reproductive output of *P. sp. x* on pasture and wheat/oats was not limited by interspecific competition.

4.5 DISCUSSION

The present experiments indicate differences in the competitive abilities of earth mite species. The intensity, frequency, and type of competition varied with plant type and from year-to-year, providing strong evidence that competitive interactions influence the population dynamics of earth mites. These results support and extend those of Weeks and Hoffmann (2000) who found competitive interactions between *H. destructor* and *P. major* on pasture in southeastern Australia. Competitive interactions appear to influence the dynamics of all earth mite species in this region.

The reproductive rate of *H. destructor* was limited by interspecific competition on all plant types. The effects of density-dependence, which were only determined at Yeungroon in the second season and through diapause, indicated that intraspecific competition also affected the reproductive output of *H. destructor*. There are two extreme forms of intraspecific competition, which can be assessed by determining if regression coefficients differ from a slope of -1 (Nicholson 1954; Varley *et al.* 1975). Firstly, in scramble competition, all individuals are similarly affected. The resources are shared equally among the competitors, and when density exceeds a critical value all individuals die because there are not enough resources to complete development. Conversely, in contest competition some individuals take an adequate and equal share of resources, while others fail to gain the required amount and die. The relationship between density and reproductive rate of *H. destructor* was characteristic of undercompensation (scramble competition). A reduction in individual output occurred, but this did not counter the increasing density and therefore the total output increased (Begon *et al.* 1996).

On each plant type, the effects of competitive interactions involving *H. destructor* were variable. For example, on wheat/oats at Yeungroon, the reproductive output was reduced by interspecific competition during the 2001 season. However, *H. destructor* was not affected by other species during 2002. The competitive ability of *H. destructor* (and other species) is also likely to vary with different environmental conditions, and competition is likely to be affected by non-host plant factors. There is ample evidence of competitive interactions changing with environmental factors in other species. For instance, in bruchid beetles, *Callosobruchus chinensis* excludes *Callosobruchus maculatus* at 30°C, but a 2°C increase completely reverses the outcome (Fujii 1967). Temporal variability for interspecific competition due to changes in environmental conditions has also been found in other organisms (Schoener 1983).

For *P. major*, the reproductive output on pasture was not affected by interspecific interactions, although there was evidence of intraspecific competition in the Yeungroon field plots during 2002. The relationship between R_0 and numbers of *P. major* was characteristic of undercompensation. Density-dependent effects were also found for *P. major* in the generation following diapause. Although competition will not occur when eggs are in the diapause stage, it may occur among juvenile mites, which tend to feed on soil microflora rather than plants (MacLennan *et al.* 1998). On wheat/oats, the reproductive output of *P. major* was limited by other species in the field, although there was no clear evidence of this in the shade-house experiment. On both canola and bristly ox-tongue treatments, *P. major* was a poor competitor and in some instances was completely excluded from plots. Therefore, *P. major* appears to have a competitive advantage on pasture, but not on ox-tongue or canola. This is

consistent with the fact that *P. major* is commonly present in mite outbreaks on pasture and is occasionally found attacking cereal crops (Robinson and Hoffmann 2001; Chapter 3).

Penthaleus falcatus was a strong competitor in many treatments, particularly on canola and bristly ox-tongue. This has important implications for control not only in canola, but also in crops and pastures that contain a high proportion of weeds or in crops that border weedy roadsides. On pasture, there was no evidence that *P. falcatus* was adversely affected by interspecific competition, except perhaps in the first season at Yeungroon. The reproductive output was lower in the mixed plots than pure plots. Although this difference was not significant, variability was high. This variability may reflect the patchy presence of weeds within some pasture plots, which can enable survival of mites when other resources are limiting (Ridsdill-Smith and Pavri 2000). The lack of interspecific interactions involving *P. falcatus* on pasture indicates that competition with other species may be avoided. *Penthaleus falcatus* may feed from different grass types (Chapter 3) or parts of the plant not utilized by other species. It has been suggested that *P. falcatus* has some degree of tolerance to plant defences (Umina and Hoffmann 1999), allowing it to feed from material with relatively high levels of toxins. However, strong interspecific competition could still exist from the sharing of plant nutrients. Even foliar-feeding and root-feeding insects can compete intensively (Moran and Whitham 1990).

Penthaleus sp. *x* performed poorly on pure treatments of canola and bristly ox-tongue, and was a poor competitor on pasture. Therefore, outbreaks of this species on canola crops are unlikely. Results from the shade-house experiment indicate that *P. sp. x*

cannot successfully survive on canola or ox-tongue, although a low number persisted inside some field plots with these plant types. These inconsistencies could be explained by grasses and weeds dispersed within the field plots enabling mite survival (Ridsdill-Smith and Pavri 2000). The reproductive output of *P. sp. x* on wheat/oats was not affected by interspecific competition, suggesting *P. sp. x* was a relatively strong competitor. These findings, and the fact that *P. sp. x* attacks relatively few plant types (Robinson and Hoffmann 2001), indicate a specialist species. *Penthaleus sp. x* also emerges from diapause later than other species (unpublished data), limiting its ability to build up to damaging levels during winter. These factors may contribute to the restricted distribution and relatively low abundance of *P. sp. x*, which is only found in northeastern New South Wales and northwestern Victoria (Robinson and Hoffmann 2001). Outside this range, the differences in phenology may give other species a decisive advantage in competition. In his work on blowflies, Hanski (1976) found an earlier seasonal start gave *Lucilia illustris* an advantage and most probably led to the eventual exclusion of *Lucilia silvarum*.

In a review of interspecific interactions in phytophagous insects, Denno *et al.* (1995) found that interspecific competition was more likely between species that were closely related. On the basis of phylogenetic similarity, *Penthaleus* spp. would be expected to compete more intensively with one another than with *H. destructor*. Weeks and Hoffmann (2000) postulated that *Penthaleus* spp. might be less likely to compete with one another than with *H. destructor* because of differences in host plant preference. Interspecific competition in the past may have led to divergence in resource utilisation among the *Penthaleus* species. However, recent findings suggest some overlap in plant hosts (Robinson and Hoffmann 2001; Chapter 3). In the current experiments,

interspecific interactions were found between *H. destructor* and a single *Penthaleus* species, and among the *Penthaleus* species. This indicates *Penthaleus* spp. do compete with one another for food resources and this probably occurs on a variety of plant types. Therefore, competition appears to be an important component structuring the population dynamics of the *Penthaleus* complex.

There is a cornucopia of explanations why competitive interactions among earth mites vary temporally and on different plant types. Genetic and environmental variability in plant resistance can mediate the intensity and frequency of competitive interactions (Denno *et al.* 1995). For example, the survival of budworms, *Choristoneura fumiferana*, was negatively affected by aphids, *Mindarus abietinus*, but only on trees susceptible to aphid attack (Mattson *et al.* 1989). Plant phenological events can also mediate interactions between phytophagous insects, while changes in plant nutrition or allelochemistry can intensify interspecific competition (Denno *et al.* 1995). In earth mites, juveniles feed on microflora, avoiding competition with adults feeding on plant material. There are also differences in the feeding behaviour of earth mite species (Narayan 1962; Ridsdill-Smith 1997). Therefore, different development rates, generation times, and behavioural characteristics between species could act to limit the amount of interspecific competition.

In conclusion, these results show competition exists within and between earth mite species and corroborate the view that intraspecific effects are often stronger than interspecific effects for sap-feeding organisms (Denno *et al.* 1995). Because predators and pathogenic fungi have minimal impact on mite numbers, unless artificially increased to high numbers (Michael *et al.* 1991; Michael 1995; Ridsdill-Smith and

Annelis 1997), it appears competitive interactions can impact on the population dynamics of mite species. Competitive pressures could influence the relative distribution, abundance, plant preference, and even diapause response of earth mites. Host plant factors influence competition, while environmental heterogeneity probably plays a major role in the coexistence of competing mite species. In pasture, the competitive advantage is likely to alter between *P. major*, *H. destructor*, and in some instances, *P. falcatus*. *Pentthaleus* sp. *x* may be the strongest competitor in wheat and oats, while in canola and bristly ox-tongue, *P. falcatus* and *H. destructor* are likely to dominate. This information is important when devising long-term pest management programs for resident mite populations, and also highlights the importance of devising control strategies that are specific to species complexes and different cropping situations.

4.6 REFERENCES CITED

Annells A. J. 1994. The reproductive biology and mating behaviour of redlegged earth mite: an overview. *In*: McDonald G. and A.A. Hoffmann (eds.), Proceedings of the Second National Workshop on Redlegged Earth Mite, Lucerne Flea and Blue Oat Mite. Department of Agriculture, Rutherglen, Australia, pp. 29-31.

Annells A. J. and Ridsdill-Smith T. J. 1994. Host plant species and carbohydrate supplements affecting rate of multiplication of redlegged earth mite. *Experimental and Applied Acarology* 18: 521-530.

Begon M., Mortimer M. and Thompson D. J. 1996. Population ecology: a unified study of animals and plants. Blackwell Science, London, UK.

Connell J. H. 1983. On the prevalence and relative importance of interspecific competition: evidence from field experiments. *American Naturalist* 122: 661-696.

Denno R. F., McClure M. S. and Ott J. R. 1995. Interspecific interactions in phytophagous insects: competition re-examined and resurrected. *Annual Review of Entomology* 40: 297-331.

Ferrenberg S. M. and Denno R. F. 2003. Competition as a factor underlying the abundance of an uncommon phytophagous insect, the salt-marsh planthopper *Delphacodes penedecta*. *Ecological Entomology* 28: 58-66.

Fujii K. 1967. Studies on the interspecific competition between the azuki bean weevil, *Callosobruchus chinensis*, and the southern cowpea weevil, *C. maculatus*. II Competition under different environmental conditions. Researches on Population Ecology 9: 192-200.

Hanski I. 1976. Breeding experiments with carrion flies (Diptera) in natural conditions. Annales Entomologici Fennici 43: 113-121.

Hariston N. G., Smith F. E. and Slobodkin L. B. 1960. Community structure, population control, and competition. American Naturalist 44: 421-425.

MacIennan K. E., McDonald G. and Ward S. A. 1998. Soil microflora as hosts of redlegged earth mite (*Halotydeus destructor*). Entomologia Experimentalis et Applicata 86: 319-323.

Mattson W. J., Haack R. A., Lawrence R. K. and Herms D. A. 1989. Do balsam aphids (Homoptera: Aphididae) lower tree susceptibility to spruce budworm? Canadian Entomologist 121: 93-103.

Michael P. 1995. Biological control of redlegged earth mite and lucerne flea by predators *Anystis wallacei* and *Nemologus capillatus*. Plant Protection Quarterly. 10: 55-57.

Michael P. J., Dutch M. E. and Pekin C. J. 1991. A review of the predators of redlegged earth mite, blue oat mite and lucerne flea. In: Ridsdill-Smith T.J. (ed.),

Proceedings of a National Workshop on Redlegged Earth Mite, Lucerne Flea and Blue Oat Mite. Department of Agriculture, South Perth, Australia, pp. 115-120.

Moran N. A. and Whitham T. G. 1990. Interspecific competition between root-feeding and leaf-galling aphids mediated by host-plant resistance. *Ecology* 71: 1050-1058.

Narayan D. S. 1962. Morphological, biological and ecological studies on the winter grain mite, *Penthaleus major* (Dugés), Penthaleidae; Acarina Part 1. *Journal of Zoological Society of India* 14: 45-63.

Nicholson A. J. 1954. An outline of the dynamics of animal populations. *Australian Journal of Zoology* 2: 9-65.

Qin T. K. and Halliday R. B. 1996. The Australian species of *Chromotydeus* Berlese and *Penthaleus* Koch, C.L. (Acarina, Penthaleidae). *Journal of Natural History* 30: 1833-1848.

Ridsdill-Smith T. J. 1997. Biology and control of *Halotydeus destructor* (Tucker) (Acarina: Penthaleidae) – a review. *Experimental and Applied Acarology* 21: 195-224.

Ridsdill-Smith T. J. and Annells A. J. 1997. Seasonal occurrence and abundance of redlegged earth mite *Halotydeus destructor* (Acari, Penthaleidae) in annual pastures of southwestern Australia. *Bulletin of Entomological Research* 87: 413-423.

Ridsdill-Smith T. J. and Pavri C. 1998. Spring spraying for redlegged earth mites.

Western Focus (Australian Grain), October-November: 1-4.

Ridsdill-Smith T. J. and Pavri C. C. 2000. Feeding life style of redlegged earth mite, *Halotydeus destructor* (Acari: Pentheleidae), in pastures and the role of broad-leafed weeds. Experimental and Applied Acarology 24: 397-414.

Robinson M. R. and Hoffmann A. A. 2001. The pest status and distribution of three cryptic blue oat mite species (*Penthaleus* spp.) and the redlegged earth mite (*Halotydeus destructor*) in southeastern Australia. Experimental and Applied Acarology 25: 699-716.

Schoener T. W. 1983. Field experiments on interspecific competition. American Naturalist 122: 240-285.

Sokal R. R. and Rohlf F. J. 1995. Biometry: The principles and practice of statistics in biological research, 3rd ed. W. H. Freeman, New York, America.

Stearns S. C. 1992. The evolution of life histories. Oxford University Press, New York, America.

Thackray D. J., Ridsdill-Smith T. J. and Gillespie D. J. 1997. Susceptibility of grain legume species to redlegged earth mite (*Halotydeus destructor*) damage at the seedling stage. Plant Protection Quarterly 12(3): 141-144.

- Umina P. A. and Hoffmann A. A. 1999. Tolerance of cryptic species of blue oat mites (*Penthaleus* spp.) and the redlegged earth mite (*Halotydeus destructor*) to pesticides. Australian Journal of Experimental Agriculture 39: 621-628.
- Underwood T. 1986. The analysis of competition by field experiments. *In*: Kikkawa J. and D.J. Anderson (eds.), Community ecology: pattern and process. Blackwell Scientific, Oxford, UK, pp. 29-31.
- Varley G. C., Gradwell G. R. and Hassell M. P. 1975. Insect population ecology: an analytical approach. Blackwell Scientific, Oxford, UK.
- Wallace M. M. H. 1970. Diapause in the aestivating egg of *Halotydeus destructor* (Acari: Eupodidae). Australian Journal of Zoology 18: 295-313.
- Weeks A. R. and Hoffmann A. A. 1999. The biology of *Penthaleus* species in southeastern Australia. Entomologia Experimentalis et Applicata 92: 179-189.
- Weeks A. R. and Hoffmann A. A. 2000. Competitive interactions between two pest species of earth mites, *Halotydeus destructor* and *Penthaleus major* (Acarina: Penthaleidae). Journal of Economic Entomology 93: 1183-1191.
- Weeks A. R., Fripp Y. J. and Hoffmann A. A. 1995. Genetic structure of *Halotydeus destructor* and *Penthaleus major* populations in Victoria (Acari: Penthaleidae). Experimental and Applied Acarology 19: 633-646.

4.7 TABLES AND FIGURES

Table 4.1: Coefficients ($b \pm \text{SE}$) ($\times 10^2$) from multiple regressions testing if the number of adult mites of each species influenced reproductive output (R_O) in pasture field plots. (a) R_O during the 2002 season regressed against the number of adult mites at the start of 2002; (b) R_O through diapause (2002-2003) regressed against the number of adult mites before diapause (end 2002).

Species (R_O)	Species (regressed against)			
	<i>H. destructor</i>	<i>P. major</i>	<i>P. falcatus</i>	<i>P. sp. x</i>
^a Start 2002 - end 2002				
<i>H. destructor</i>	$-13.83 \pm 3.86^{**\dagger}$	-1.21 ± 1.95	-0.11 ± 3.19	2.45 ± 1.04
<i>P. major</i>	25.04 ± 11.62	$-27.74 \pm 5.87^{**\dagger}$	-22.56 ± 9.60	-5.91 ± 3.14
<i>P. falcatus</i>	23.51 ± 15.47	-10.32 ± 7.82	23.24 ± 12.79	4.25 ± 4.18
<i>P. sp. x</i>	$-109.57 \pm 38.99^*$	41.85 ± 17.48	15.21 ± 29.93	$-65.62 \pm 20.98^*$
^b End 2002 - start 2003				
<i>H. destructor</i>	$-12.13 \pm 3.09^{**\dagger}$	0.37 ± 1.35	0.23 ± 0.79	0.73 ± 0.76
<i>P. major</i>	1.25 ± 5.95	$-14.14 \pm 2.60^{***\dagger}$	-0.47 ± 1.53	1.13 ± 1.45
<i>P. falcatus</i>	65.61 ± 30.19	0.38 ± 14.09	10.61 ± 19.12	4.39 ± 7.13
<i>P. sp. x</i>	0.86 ± 42.76	-15.21 ± 23.45	17.44 ± 15.69	43.72 ± 55.45

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

[†], remained significant after correction for multiple comparisons.

Table 4.2: Coefficients ($b \pm SE$) ($\times 10^2$) from multiple regressions testing if the number of adult mites of each species influenced reproductive output (R_o) in wheat/oats field plots. (a) R_o during the 2002 season regressed against the number of adult mites at the start of 2002; (b) R_o through diapause (2002-2003) regressed against the number of adult mites before diapause (end 2002).

Species (R_o)	Species (regressed against)			
	<i>H. destructor</i>	<i>P. major</i>	<i>P. falcatus</i>	<i>P. sp. x</i>
^a Start 2002 - end 2002				
<i>H. destructor</i>	$-15.55 \pm 4.23^{**\dagger}$	-0.70 ± 2.63	-3.64 ± 6.17	5.71 ± 6.71
<i>P. major</i>	$-26.54 \pm 7.78^{*\dagger}$	$-42.63 \pm 8.72^{**\dagger}$	$-34.99 \pm 9.18^{**\dagger}$	$38.73 \pm 11.72^*$
<i>P. falcatus</i>	43.32 ± 27.18	17.63 ± 16.53	43.71 ± 39.12	-59.68 ± 46.81
<i>P. sp. x</i>	5.99 ± 21.76	-3.75 ± 13.24	-38.59 ± 31.33	27.12 ± 37.49
^b End 2002 - start 2003				
<i>H. destructor</i>	21.33 ± 9.00	5.35 ± 11.06	3.32 ± 4.38	$-7.93 \pm 3.32^*$
<i>P. major</i>	-20.04 ± 12.81	-19.11 ± 15.75	-1.85 ± 6.23	-1.37 ± 4.74
<i>P. falcatus</i>	51.30 ± 34.35	59.62 ± 45.58	17.74 ± 21.79	-22.25 ± 15.37
<i>P. sp. x</i>	26.64 ± 17.64	-13.01 ± 26.49	0.12 ± 7.97	-5.36 ± 12.51

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

[†], remained significant after correction for multiple comparisons.

Table 4.3: Coefficients ($b \pm SE$) ($\times 10^2$) from multiple regressions testing if the number of adult mites of each species influenced reproductive output (R_O) in canola field plots. (a) R_O during the 2002 season regressed against the number of adult mites at the start of 2002; (b) R_O through diapause (2002-2003) regressed against the number of adult mites before diapause (end 2002).

Species (R_O)	Species (regressed against)			
	<i>H. destructor</i>	<i>P. major</i>	<i>P. falcatus</i>	<i>P. sp. x</i>
^a Start 2002 - end 2002				
<i>H. destructor</i>	$-14.57 \pm 6.04^*$	0.79 ± 1.49	-0.02 ± 2.99	-2.07 ± 1.94
<i>P. major</i>	-10.25 ± 16.06	-19.32 ± 16.17	$-38.79 \pm 12.13^*$	11.29 ± 5.86
<i>P. falcatus</i>	$-16.09 \pm 5.67^*$	-1.02 ± 1.40	$-10.24 \pm 2.81^{**\dagger}$	-1.97 ± 1.82
<i>P. sp. x</i>	-20.32 ± 50.24	3.78 ± 17.79	-44.45 ± 29.77	40.41 ± 56.24
^b End 2002 - start 2003				
<i>H. destructor</i>	1.91 ± 6.40	-0.85 ± 1.88	9.87 ± 4.45	$5.79 \pm 2.07^*$
<i>P. major</i>	-52.66 ± 55.89	54.79 ± 41.15	-2.01 ± 39.27	-6.89 ± 11.82
<i>P. falcatus</i>	24.73 ± 18.48	10.13 ± 6.32	$-41.88 \pm 14.86^*$	-8.84 ± 6.28

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

[†], remained significant after correction for multiple comparisons.

Table 4.4: Coefficients ($b \pm SE$) ($\times 10^2$) from multiple regressions testing if the number of adult mites of each species influenced reproductive output (R_o) in bristly ox-tongue field plots. (a) R_o during the 2002 season regressed against the number of adult mites at the start of 2002; (b) R_o through diapause (2002-2003) regressed against the number of adult mites before diapause (end 2002).

Species (R_o)	Species (regressed against)			
	<i>H. destructor</i>	<i>P. major</i>	<i>P. falcatus</i>	<i>P. sp. x</i>
^a Start 2002 - end 2002				
<i>H. destructor</i>	$-9.50 \pm 3.24^*$	-0.16 ± 1.48	$-5.98 \pm 2.19^*$	2.11 ± 1.43
<i>P. major</i>	-15.82 ± 10.51	$44.64 \pm 13.77^*$	$-38.01 \pm 7.97^{**\dagger}$	9.14 ± 4.77
<i>P. falcatus</i>	3.05 ± 3.90	0.452 ± 1.78	$-13.8 \pm 2.64^{***\dagger}$	-0.39 ± 1.72
<i>P. sp. x</i>	-10.49 ± 15.72	-4.49 ± 13.52	-27.14 ± 19.65	45.60 ± 28.59
^b End 2002 - start 2003				
<i>H. destructor</i>	-18.03 ± 11.15	4.61 ± 3.31	-26.94 ± 17.72	-4.19 ± 4.00
<i>P. major</i>	3.72 ± 29.26	-27.93 ± 15.52	-24.90 ± 35.01	-0.72 ± 8.10
<i>P. falcatus</i>	-3.58 ± 2.11	$1.68 \pm 0.63^*$	$-16.95 \pm 3.36^{**\dagger}$	0.81 ± 0.76
<i>P. sp. x</i>	-26.61 ± 68.63	23.06 ± 44.94	-7.29 ± 105.21	-149.77 ± 264.19

*, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

[†], remained significant after correction for multiple comparisons.

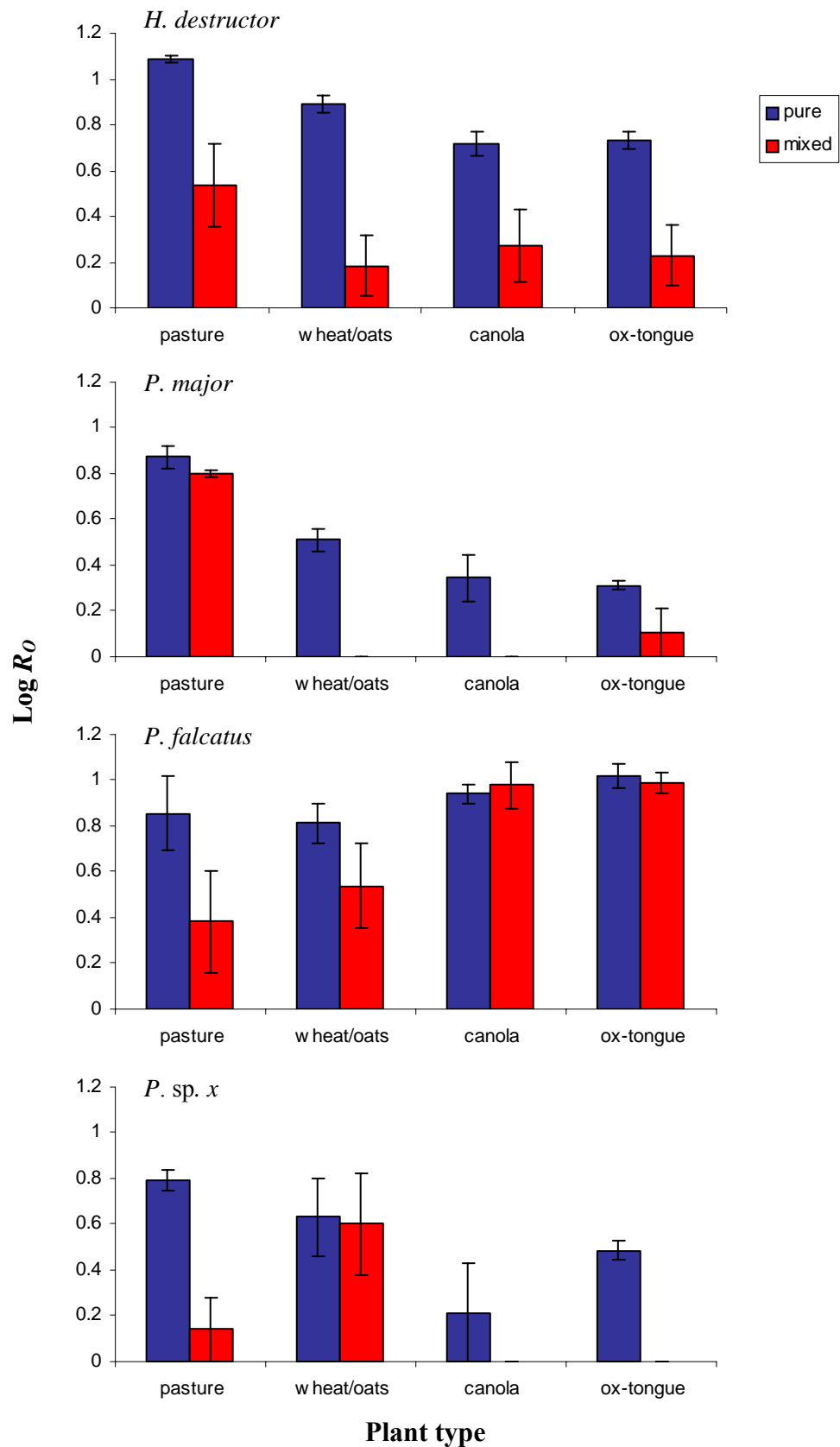


Figure 4.1: Average reproductive output (R_0) (\pm SE) of *H. destructor*, *P. major*, *P. falcatus* and *P. sp. x* in pure and mixed plots of pasture, wheat/oats, canola and bristly ox-tongue at Yeungroon during the 2001 season.

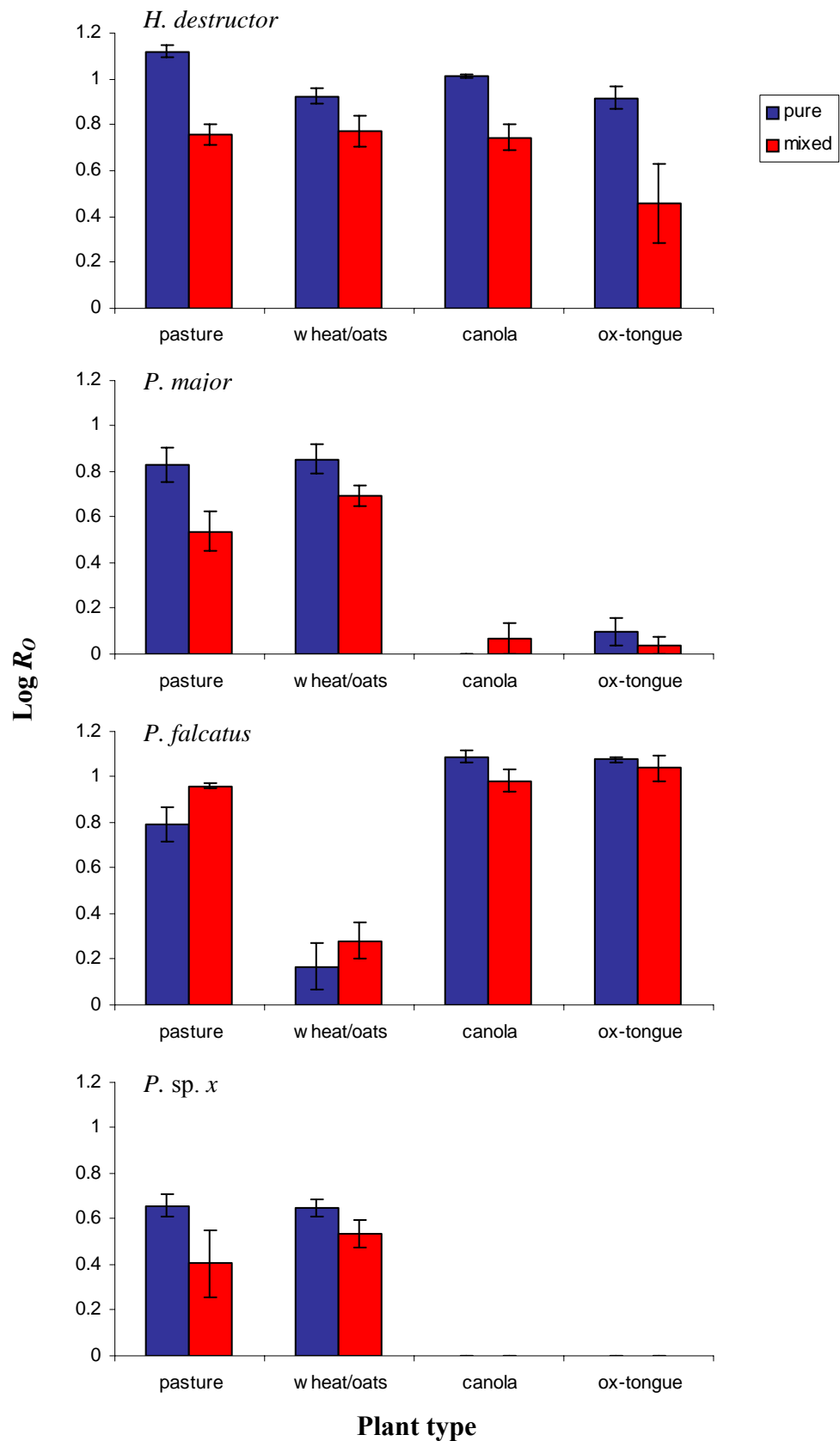


Figure 4.2: Average reproductive output (R_0) (\pm SE) of *H. destructor*, *P. major*, *P. falcatus* and *P. sp. x* in pure and mixed shade-house tubs of pasture, wheat/oats, canola and bristly ox-tongue from the start of 2002 to the start of the 2003 season.

CHAPTER 5.

**Biochemical and molecular analysis of earth mite protein extracts
with the view of developing a field-based diagnostic kit**

List of abbreviations

Bis-acrylamide	N, N'-methylenebisacrylamide
BLAST	basic local alignment search tool
BSA	bovine serum albumin
CNBr	cyanogen bromide
DTT	dithiothreitol
EDTA	ethylenediamine tetra-acetic acid
ELISA	enzyme-linked immunosorbent assay
HAT	hypoxanthine, aminopterin, thymidine
HIV	human immunodeficiency virus
HPLC	high-performance liquid chromatography
IEF	isoelectric focusing
IgG	immunoglobulin G
IPG	immobilised pH gradient
kDa	kilo Dalton
LDS	lithium dodecyl sulfate
M	moles per litre
mM	millimoles per litre
MOPS	3-(N-morpholino) propane sulfonic acid
MW	molecular weight
NCBI	National Centre for Biotechnology Information
OD	optical density
OPD	<i>o</i> -phenylenediamine
PAGE	polyacrylamide gel electrophoresis

PAS	periodic acid/Schiff's
PBS	phosphate buffered saline
pI	isoelectric point
PVDF	polyvinylidene difluoride
RP-HPLC	reverse phase-high performance liquid chromatography
RT	room temperature
SDS	sodium dodecyl sulphate
Tris	Tris (hydroxymethyl) aminomethane
v/v	volume per volume
Vhrs	voltage hours
w/v	weight per volume

5.1 ABSTRACT

Earth mite species in Australia consist of the redlegged earth mite, *Halotydeus destructor* (Tucker) and the blue oat mite complex, which includes *Penthaleus major* (Dugés), *Penthaleus falcatus* (Qin and Halliday), and an undescribed species referred to as *Penthaleus* species *x*. Recent studies show earth mite species differ markedly in their biology and confusion between species could be responsible for ineffective control in crops and pastures. This highlights the importance of identifying the mite species before implementing management strategies. In this study, protein extracts of the three *Penthaleus* spp. were examined with the aim of identifying species-specific proteins suitable for use in developing a diagnostic kit. I revealed important information about the complex nature of the *Penthaleus* group, the similarity between the three species, as well as identified methods for isolating and characterising species-specific proteins. Antibodies specific to one species were isolated and several candidate polypeptide bands identified. Preliminary experiments were also performed analysing protein extracts of *H. destructor*. Directions that this project may take in the future are discussed.

5.2 INTRODUCTION

Blue oat mites, *Penthaleus* spp., and the redlegged earth mite, *Halotydeus destructor* (Tucker) (Acari: Penthaleidae), are serious pasture and crop pests throughout southern agricultural areas of Australia (Jeppson *et al.* 1975; Ridsdill-Smith 1997). They are active during the winter months where they go through two or three generations, reaching densities of up to 60 000 mites per square metre (Ridsdill-Smith and Pavri 1998). Chemical control has been, and continues to be, the only effective management option available for the control of these earth mites (Ridsdill-Smith 1997). All earth mite species are presently treated identically in terms of control even though the species differ markedly in their biology. *Halotydeus destructor* is diploid and reproduces sexually (diplodiploid) (Annells 1994; Weeks *et al.* 1995). It is extremely polyphagous, attacking most agricultural crops within southern Australia (Ridsdill-Smith 1997), and has four active stages between egg and adulthood. In contrast, *Penthaleus* spp. reproduce by thelytokous parthenogenesis, with populations made of several distinct clonal types (Weeks *et al.* 1995). *Penthaleus* spp. are known to attack mainly small grains and grasses (Narayan 1962). They go through three life stages before becoming adults, with a generation time thought to be longer than *H. destructor* (Narayan 1962; Jeppson *et al.* 1975).

Management of earth mites has been further complicated by the recent discovery of three cryptic species of *Penthaleus*, whereas prior research had assumed a single species. *Penthaleus major* (Dugés) and *Penthaleus falcatus* (Qin and Halliday) were previously identified by Qin and Halliday (1996) and Weeks *et al.* (1995), with *Penthaleus* species *x* only recently discovered and not yet formally named (Weeks and

Hoffmann 1999). The three species of *Penthaleus* are vastly different in their distribution, timing of diapause and host plant preferences (Weeks and Hoffmann 1999; Chapters 2 & 3). The species also differ in their tolerance levels to a variety of commonly used pesticides (Umina and Hoffmann 1999; Robinson and Hoffmann 2001). Furthermore, recent findings indicate that suppression or eradication of one species of earth mite may result in another species increasing in relative abundance (Weeks and Hoffmann 2000; Chapter 4). This is because mite species compete with each other and the removal of one species could eliminate competitive pressures, allowing the other species to thrive. Therefore, pest management strategies that consider each species separately need to be developed for effective control of earth mites.

Penthaleus spp. and *H. destructor* can be distinguished in the field quite easily. Although both have eight red legs and are about the size of a pinhead, *Penthaleus* spp. have a dorsal anus, which appears as a red mark on their backs. They also have a blue-black coloured body. *Halotydeus destructor* has a ventrally positioned anus and, hence, lack this mark. They also have a completely black coloured body. Despite these morphological differences, the similarity in life cycle and the fact they occur sympatrically within Australia, has led to the frequent misidentification of blue oat mites, which are often assumed to be redlegged earth mites (see Umina *et al.* 2001).

Moreover, distinguishing the three *Penthaleus* spp. is difficult. The species can only be identified via allozyme electrophoresis or by morphological differences in the length and number of dorsal bristles (Weeks *et al.* 1995; Weeks and Hoffmann 1999).

Separation of *Penthaleus* spp. on the basis of bristle patterns requires considerable skill

and experience, while electrophoretic techniques depend on access to equipment not widely available. Both techniques also require mites to be transported to appropriately equipped laboratories. Therefore, the current methods are not suitable for routine use as management tools. For successful control, a simple, quick, and low cost method for distinguishing the different earth mite species is needed.

Numerous field-based kits have been developed worldwide with varied diagnostic applications (Stephens 1993; Rose *et al.* 1995; Chambers *et al.* 1999; Trowell *et al.* 2000). One such example is a rapid method for detecting storage mites in cereals and their derived products, currently being funded by the HGCA (Home-Grown Cereals Authority, England). This method utilises a monoclonal antibody for the detection of mites by immunoassay (Chambers *et al.* 1999). Immunoassays are a sensitive method based on the detection of specific antigens by antibodies. They are reliable, rapid and do not require expert knowledge and are routinely used in clinical diagnosis (Avrameas *et al.* 1992). Specific assays for other agricultural species using immunochemical methods such as radioimmunoassay (RIA) and enzyme-linked immunosorbent assay (ELISA) have also proven successful in field diagnostics (Luczynska *et al.* 1989; Stephens 1993; Trowell *et al.* 2000).

The aim of this study was to work towards the development of a rapid and simple field test that could be used by agronomists, farmers and others to determine the mite species composition on individual properties. This would allow appropriate control strategies to be devised, as well as reduce the ineffectual application of pesticides. I primarily focused on methods employing antibodies with three main objectives: (1) to examine the protein components of mite extracts with the aim of identifying proteins

that are species-specific; (2) to sequence and characterise unique proteins; and (3) to make antibodies to those proteins found to be species-specific.

5.3 MATERIALS AND METHODS

5.3.1 Preparation of crude mite protein extract for SDS-PAGE

Mites were collected and placed at -80°C until use. Sample preparation was optimised using different solubilisation techniques and detergent concentrations. Adult mites were ground up in milliQ water using a spatula. SDS sample buffer (4% [w/v] SDS, 10% [v/v] β -mercaptoethanol, 20% [v/v] glycerol, 125 mM Tris-glycine, pH 6.8) was added to the mite extract, which was sonicated for 1 min on ice using a Rapidis 600 sonifier (Ultrasonics Ltd) at power setting 3. Samples were then centrifuged at 100 000g for 30 min at 4°C to separate soluble and insoluble material. The supernatant was removed and boiled for 5 min prior to SDS-PAGE.

Water-soluble extracts of mites were also prepared and separated by SDS-PAGE.

Adult mites were ground up in milliQ water and sonicated for 1 min on ice. Insoluble material was removed by centrifugation at 100 000g for 30 min at 4°C. SDS sample buffer was added to the supernatant, which was then boiled for 5 min. The samples were centrifuged at 20 000g for 10 min and the supernatants subjected to SDS-PAGE.

5.3.2 Quantification of total mite protein

Protein quantities were determined against known concentrations of Bovine Serum Albumin (BSA) using the BCA Protein Assay Kit (Pierce), following the manufacturers instructions. The Microwell Plate Protocol with an incubation period at 37°C for 30 min was employed, with absorbance measured at 562 nm.

5.3.3 One-dimensional SDS-PAGE

The separation of proteins was achieved by one-dimensional sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), originally described by Laemmli (1970). Separating gels were either 7.5% or 10% (v/v) acrylamide in 0.375 M Tris, 0.1% (w/v) SDS, pH 8.8. Stacking gels were 5% (v/v) acrylamide in 0.125 M Tris, 0.1% (w/v) SDS, pH 6.8. The molecular weight standards used were either the low silver stain SDS mixture (Sigma) or SeeBlue[®] Plus2 prestained molecular mass markers (Invitrogen Co.).

Proteins were visualised by staining with either Coomassie Brilliant Blue or Silver. Gels stained with Coomassie Brilliant Blue R-250 (0.2% [w/v] in 40% [v/v] ethanol, 10% [v/v] acetic acid) were incubated for at least 2 h before destaining in 40% (v/v) ethanol, 10% (v/v) acetic acid. Silver staining was performed to visualise low abundance proteins. Following SDS-PAGE, gels were soaked in fixative solution (40% [v/v] ethanol, 10% [v/v] acetic acid) for 90 min and then washed in 20% (v/v) ethanol for 10 min. Gels were then washed with milliQ water for 10 min, sensitised in fresh 0.8 mM Na₂S₂O₃ for 1 min and washed 3 times in milliQ water for 20 s. Gels were stained in silver solution (750 µl/L formaldehyde, 11.8 mM AgNO₃) for 20 min, followed by 2 washes in milliQ water. Developing solution (290 mM Na₂CO₃, 0.03 mM Na₂S₂O₃, 500 µl/L formaldehyde) was added until bands appeared. Gels were then rinsed in milliQ water and placed in fixative solution, which stops development.

5.3.4 Invitrogen gradient gels

NuPAGE[®] Bis-Tris pre-cast gels (4-12% polyacrylamide, Invitrogen Co.) were used to

provide a separation and resolution range otherwise unattainable with uniform concentration gels. Gradient gels are also more suitable for resolving glycosylated proteins. Samples were prepared and run at 200 V for 50 min according to the manufacturers instructions. NuPAGE[®] MOPS SDS Running Buffer, Antioxidant and LDS Sample Buffer were used. Gels were either stained with Coomassie Blue, following normal staining procedures, or transferred to polyvinylidene difluoride (PVDF) (Millipore) membranes. Electroblothing of proteins was achieved using NuPAGE[®] Transfer Buffer and Antioxidant.

5.3.5 Electroblothing

Electrophoretic transfer of proteins from acrylamide gels to PVDF was performed using a Bio-Rad Trans-Blot[®] Semi-Dry apparatus. Following SDS-PAGE, gels were equilibrated in transfer buffer (25 mM Tris, 192 mM glycine, 20% [v/v] methanol) for 30 min. PVDF membranes were immersed in 100% (v/v) methanol for 1 min, followed by equilibration in transfer buffer for 5 min. Gel sandwiches were then constructed ensuring no air bubbles were trapped between the gel and membrane. The sandwich was placed on the platinum anode plate with blotting paper (also soaked in transfer buffer) on either side. After the cathode assembly plate was secured, the transfer was run at 15 V for approximately 45 min.

5.3.6 Two-dimensional (2-D) electrophoresis

5.3.6.1 FIRST DIMENSION

13 cm Immobiline DryStrip gels (Amersham Pharmacia) with a pH range of 3-10 were used in the first dimension with the IPGphor Isoelectric Focusing System (Amersham

Pharmacia). Mites were ground up in 50 µl milliQ water and added to 200 µl rehydration solution (8 M urea, 0.5% [v/v] IPG buffer (pH 3-10), 4% [w/v] CHAPS, 100 mM DTT, 10% [v/v] triton). Particulate material which blocks gel pores was removed by centrifugation at 100 000g for 30 min at 4°C. The supernatant was aspirated onto 13 cm strip holders and the Immobiline DryStrips were carefully positioned on top of the gel. After addition of 500 µl of IPG Cover Fluid, the strips were rehydrated for 12 h at 20°C, prior to focusing at 50 µA per strip. Focusing was performed at 500 V for 1 h, 1000 V for 1 h and then 8000 V for 10 h. The total voltage hours of runs varied between 54 000 Vhrs – 68 000 Vhrs. After initial 2D results, a pH range of 6-11 was substituted to facilitate separation of alkaline proteins.

5.3.6.2 SECOND DIMENSION

After IEF, IPG strips were saturated with an equilibration solution (6 M urea, 30% [v/v] glycerol, 10% [w/v] SDS, 50 mM Tris-HCl, pH 8.8). Firstly, 100 mg DTT was added to 10 mL solution for 15 min to preserve the fully reduced state of denatured proteins. 900 mg iodoacetamide in 10 mL equilibration solution was introduced in a second equilibration step for 15 min to remove excess DTT (responsible for point streaking and other artefacts in silver-stained patterns). The PROTEAN II xi cell apparatus (Bio-Rad) was used to separate polypeptides in the second dimension. The equilibrated IPG strips were placed on 10% (v/v) acrylamide gels and sealed with 0.5% (w/v) agarose in 100 mL running buffer (25 mM Tris, 192 mM glycine, 0.1% [w/v] SDS). Low silver stain SDS molecular mass markers were loaded in the appropriate wells and the gels were run at a constant current of 40 mA per gel. All 2-D gels were stained with silver.

5.3.7 N-terminal amino acid sequencing

N-terminal sequence analysis of proteins was performed in an attempt to identify a number of mite proteins. Following electrophoresis, electroblotting onto PVDF was performed. The transferred proteins were briefly stained with Coomassie Blue (1-2 min) and destained in 50% (v/v) methanol. The membrane was washed in several changes of milliQ water and air-dried. Bands of interest were excised, subjected to Edman degradation and sequenced, as described by Hewick *et al.* (1981). Edman degradation is a method of determining the amino acid sequence of peptides by sequentially cleaving the N-terminus and releasing the amino acid residues for identification by HPLC. Samples were loaded directly onto polybrene filters in the sequencing cartridge, and phenylthiohydantion derivatives were detected by high-pressure liquid chromatography using an automated gas-phase sequencer (Applied Biosystems Inc.). Rosemary Condron of La Trobe University, Department of Biochemistry, performed the sequencing.

5.3.8 Internal amino acid sequencing

5.3.8.1 DIGESTION OF PROTEINS WITH TRYPSIN

(i) Gel reduction and alkylation prior to digestion

Reduction and alkylation of gel pieces was performed to convert cysteine residues to more stable derivatives in order to prevent the random formation of disulfide bridges. Following SDS-PAGE, proteins were stained for 40 min in Coomassie Blue. Gels were then destained in several changes of 20% (v/v) ethanol, 7.5% (v/v) acetic acid, until protein bands became visible. Protein bands of interest were excised, cut into 1 mm (approximately) pieces and washed 3 times in 100 µl of 50% (v/v) acetonitrile for 20

min. The gel pieces were dehydrated in a SpeedVac for 15 min and incubated in 150 µl of DTT solution (10 mg/ml in 100 mM NH_4HCO_3) at 56°C. After 50 min the solution was removed and the gel pieces washed with 50 µl milliQ water. The gel pieces were then incubated in 300 µl iodoacetamide solution (50 mg/ml in 100 mM NH_4HCO_3) in the dark, at RT for 50 min. Gel pieces were washed for 10 min in 100 µl 100 mM NH_4HCO_3 and then 100 µl 100% (v/v) acetonitrile for 10 min. The two washing steps were repeated, before the gel pieces were semi-dried in a SpeedVac for 50-60 min.

(ii) 'In-gel' tryptic digestion and peptide recovery

Following reduction and alkylation of the gel pieces, trypsin solution (20 µg/ml in 100 mM NH_4HCO_3) was added until the gel pieces were fully hydrated. Digestion was carried out at 30°C overnight. The resulting peptides were recovered by two extractions of 20 min each, with 100 µl 60% (v/v) acetonitrile, 2% (v/v) formic acid. Extracts were combined and concentrated to approximately 20 µl in a SpeedVac. The peptides were then separated by reverse-phase HPLC.

5.3.8.2 CYANOGEN BROMIDE CLEAVAGE

Cleavage with cyanogen bromide (CNBr) was performed on proteins within the gel matrix. Following SDS-PAGE, gels were stained with Coomassie Blue for 20 min and then destained in 50% (v/v) methanol. Polypeptides of interest were excised and subject to 'in-gel' cleavage, according to Córdoba *et al.* (1997). Briefly, the bands were washed twice with 50% (v/v) acetonitrile for 15 min and then dried under vacuum in a SpeedVac. The gel pieces were rehydrated with 250 µl CNBr solution (16 µg/µl in 70% [v/v] formic acid) and kept in the dark for 48 h. The liquid was

evaporated in a SpeedVac and the pieces washed twice in 150 µl milliQ water, also evaporated each time. 50 µl SDS sample buffer was added to the gel pieces and boiled for 5 min. The cleaved peptides were separated by 10% SDS-PAGE and stained with silver.

5.3.9 Reverse-phase HPLC peptide purification

In situ tryptic digests were separated by reverse phase-high performance liquid chromatography using a Hewlett Packard HP 1090, series II HPLC. Peptides eluted from the polyacrylamide matrices were injected into a C8 (250 x 1 mm) microbore column. The column was eluted with a linear gradient of 70% (v/v) acetonitrile, 0.1% (v/v) trifluoroacetic acid at a flow rate of 40 µl/min. Fractions were collected manually in a minimum volume. Large peaks were then sequenced by Edman degradation.

5.3.10 Production of polyclonal antibodies against mite extracts

Due to the complex protein mixture extracted from the *Penthaeus* spp., water fractionation was undertaken to simplify mite extracts prior to antibody production. Ideally, the antibodies in a diagnostic kit should be produced against an abundant but species-specific water-soluble protein. Targeting of water-soluble proteins would keep the kit simple and cost to a minimum, as the need for a detergent-solubilisation step, followed by a step to remove the detergent/s, would be eliminated. Therefore, antisera against only the water-soluble extracts of all three *Penthaeus* spp. were generated. Firstly, 100 mites (approximately 2000 µg total protein) were crushed in milliQ water. Samples were then centrifuged at 100 000g for 30 min at 4°C. The supernatant was removed and dialyzed overnight against phosphate-buffered saline (PBS; 150 mM

NaCl, 15 mM Na₂HPO₄, 5 mM NaH₂PO₄, pH 7.4). This suspension (typically around 1 mL) was emulsified in an equal volume of Freund's complete adjuvant (Gibco) and injected into rabbits subcutaneously. A total of three inoculations were given for each rabbit (approximately 5 weeks apart). The amount of protein injected into the rabbits varied from 1000-2000 µg. Boost inoculations used Freund's incomplete adjuvant (Gibco).

5.3.11 Serum preparation

Blood was collected into polypropylene tubes 7-10 days after the final injection. The blood was left at RT for 1-2 h to allow clotting. The clot was detached from the surfaces of the tube with a pasture pipette and then stored at 4°C overnight to allow the clot to contract. The following day, the blood was centrifuged at 3000 g for 10 min and the serum was carefully removed and stored in 500 µl aliquots at -80°C until needed.

5.3.12 Enzyme-linked immunosorbent assay to measure antibody titre

Maxisorb microtitre plates (Nunc) were coated overnight with *P. major* water-soluble protein solution diluted to 10 µg/ml in PBS (100 µl per well). The plates were then washed twice with PBS and blocked in 250 µl 10% (w/v) skim milk powder (blotto) in PBS/0.05% Tween-20 (PBS-T) for 2 h. Empty wells were also blocked and used in parallel with protein-coated wells, in order to control for any non-specific binding to the plastic or blocking agent. Anti-*P. major* serum was diluted in PBS-T to 1/100, 1/500, 1/1000, 1/5000, 1/10 000, 1/20 000 and 1/50 000. Dilutions of the control serum (from pre-bleed) were also prepared in PBS-T. Having been washed twice with PBS, the wells were then coated with dilutions of the serum (100 µl/well), added in

duplicate. The wells were incubated for 1 h at RT, with gentle shaking. Unbound serum was washed with PBS-T. Wells were then incubated with 100 µl of horseradish peroxidase-conjugated anti-rabbit IgG (Sigma) at a dilution of 1/1000 (in PBS-T). After 1 h at RT with shaking, the wells were washed extensively with six changes of PBS-T. The assays were developed with 100 µl enzyme-substrate solution (0.5 mg/ml *o*-phenylenediamine [OPD] dihydrochloride [Sigma] in 0.05 M phosphate citrate buffer, pH 5, 0.04% [v/v] H₂O₂) per well. The reaction was stopped with 50 µl 1 M HCl and the absorbance measured at 490 nm. Negative control wells without serum were also included to control for any non-specific binding of the anti-rabbit conjugate. The enzyme-linked immunosorbent assay (ELISA) method is illustrated in Figure 5.1.

ELISAs were also performed for anti-*P. falcatus* and anti-*P. sp. x* sera. Absorbance results were plotted against concentration of test antibody.

5.3.13 Characterisation of antiserum by immunoblotting

The specificity of antibodies made to each mite species was determined by immunoblot analysis. Water-soluble extracts were prepared as described earlier, and separated by 4-12% SDS-PAGE (NuPAGE[®]). Proteins were transferred to PVDF and incubated overnight in 10% (w/v) blotto/PBS-T, to allow the blocking of non-specific binding sites. The blot was washed three times in PBS-T for 15 min with gentle shaking. The membrane was probed with anti-*P. major* serum (1/2000 dilution in blocking solution) for 1 h with shaking. Unbound antibodies were washed away with four washes in PBS-T for 15 min. The membrane was then incubated for 1 h in horseradish peroxidase-conjugated anti-rabbit IgG, diluted 1/1000 in 10% (w/v)

blotto/PBS-T. After three washes with PBS-T for 15 min, ECL reagent (SuperSignal Chemiluminescent Substrate, Pierce) was added. Finally, the membrane was exposed to Hyperfilm[®] (Amersham Life Sciences) under darkness and developed photographically. Routinely, two different exposure times were carried out: 30 seconds and 2 min, depending on the reactivity of the assayed sera. The immunoblot protocol is illustrated in Figure 5.2.

Immunoblots were also repeated using anti-*P. falcatus* (1/2000 dilution) and anti-*P. sp. x* (1/2000 dilution) sera.

5.3.14 Affinity purification of antibodies

Anti-*P. falcatus* antibodies that appeared species-specific following immunoblot analysis, were affinity purified to test their specificity. Water-soluble proteins of *P. falcatus* were subjected to 4-12% SDS-PAGE (NuPAGE) and transferred to PVDF. SeeBlue[®] Pre-Stained Standards were used to identify the region of interest, which was excised and cut into small pieces. The membrane was then blocked with 10% (w/v) blotto/PBS-T overnight at 4°C. Following two 15-min washes with PBS-T, the membrane was incubated in 1.5 mL pure *P. falcatus* antiserum for 1 h at RT. The membrane was then washed three times in PBS-T (15 min each) to remove any unbound antibodies. 150 µl 0.1 M glycine, 0.1 M NaCl, pH 2.6 was added to the membrane pieces to elute bound antibodies. After 15 min, the solution was removed and the process repeated. The two eluants were pooled and neutralised with 0.5 M Tris, pH 10. The antibody specificity was tested by immunoblot analysis (1/1000 dilution). A schematic diagram of the affinity purification procedure is given in Figure 5.3.

5.3.15 Inhibition ELISAs

Before inhibition ELISAs could be conducted, assay conditions were optimised for each mite species. Antisera dilutions were determined by the initial titration ELISAs (5.3.12). Varying concentrations of protein solutions (0.63 µg/ml – 40 µg/ml) were then bound to microtitre plates. Following blocking of unbound sites, polyclonal antiserum (1/1000) was allowed to bind, which was then detected using a secondary antibody and OPD substrate. Absorbance was measured at 490 nm. From the resulting titration curves, an optimum protein coating concentration of 10 µg/ml was estimated for each species.

Inhibition ELISAs were performed by the simultaneous addition of antiserum and protein. Firstly, 100 µl of *P. major* water-soluble extract diluted in PBS to a concentration of 10 µg/ml was added to a 96-well microtitre plate and allowed to bind overnight at 4°C with gentle agitation. The wells were washed twice with PBS and blocked in 250 µl 10% (w/v) blotto/PBS for 2 h. 50 µl of varying concentrations (40 µg/ml to 0.3 µg/ml in PBS) of *P. major* protein was added to 32 wells. 50 µl of the same dilutions of *P. falcatus* protein were added to another 32 wells and 50 µl of *P. sp. x* protein added to the remaining 32 wells. 50 µl of anti-*P. major* serum or pre-immune serum (diluted to 1/500 in PBS) was then immediately added to all the wells and left for 1 h at RT. The wells were then washed four times with PBS-T and incubated with 100 µl of horseradish peroxidase-conjugated anti-rabbit IgG at a dilution of 1/1000 (in PBS-T). After 1 h at RT with shaking, the wells were washed extensively with six changes of PBS-T. The assays were developed with 100 µl of OPD substrate per well. After approximately 3 min the reaction was stopped with 50 µl 1M HCl and the

absorbance measured at 490 nm. Figure 5.4 depicts the inhibition ELISA protocol used.

Inhibition assays were also conducted for anti-*P. falcatus* and anti-*P. sp. x* sera, using protein concentrations ranging from 80 µg/ml – 0.63 µg/ml.

5.3.16 Dialysis of protein samples

Protein solutions were dialyzed in order to remove unwanted low molecular weight solutes and simultaneously introduce a new buffer solution into the sample. Firstly, a strip of dialysis tubing (MW cut-off 12 000, Sigma) was pretreated by boiling in 10 mM NaHCO₃, 2 mM EDTA for 30 min. The tube was then rinsed thoroughly in several changes of milliQ water and clamped tightly at one end. The protein solution was added to the dialysis tube, which was then clamped at the other end and immersed in 2 L buffer (either milliQ water, PBS, or 0.1 M sodium phosphate buffer, pH 7.0). Dialysis was carried out at 4°C for at least 24 h.

5.3.17 Coupling of protein to Amino-Link® gel

100 *P. major* were crushed in 900 µl milliQ water, sonicated and centrifuged at 100 000g for 30 min. The supernatant was then dialyzed overnight at 4°C against 0.1 M sodium phosphate buffer, pH 7.0. A protein column was made by coupling the *P. major* extract to Amino-Link® Coupling Gel (Pierce), following the manufacturers instructions. Briefly, 1 mL protein extract and 1 mL Amino-Link® Gel were added to a disposable column, along with 50 µl 1M NaCNBH₃. The solution was slowly mixed on a spinning wheel for 2 h, removed and then left at RT for an additional 4 h. After

two brief washes with 1M Tris-HCl, pH 7.4, the remaining unbound sites were blocked for 30 min with 1 mL 1M Tris-HCl, pH 7.4 and 50 µl 1M NaCNBH₃. After draining the solution, the gel was washed once with 10 mL 1M NaCl and once with 10 mL 0.05% (w/v) sodium azide. The coupled *P. major* protein was then stored as a 50% slurry in 0.05% (w/v) sodium azide at 4°C.

5.3.18 Immuno-depletion of antiserum using coupled protein

Anti-*P. falcatus* serum was immuno-depleted against *P. major* antigen immobilised to Amino-Link[®] Gel. Firstly, the column containing the covalently attached antigen was drained and washed for 5 min with PBS. This solution was drained and replaced with 15 µl of pure anti-*P. falcatus* serum and 985 µl PBS. After gentle mixing on a spinning wheel for 1 h at RT, the antibody solution was drained into an Eppendorf Tube and placed on ice. The column was then washed in PBS and incubated with a 'stripping' solution (0.1 M glycine, 0.1 M NaCl, pH 2.6) for 15 min to elute bound antibodies. The eluant was collected and neutralised with 0.5 M Tris, pH 10. The column was then washed three times with PBS and the entire procedure was repeated using the antibodies collected from the first immuno-depletion step. This enhanced the probability of removing all antibodies recognising *P. major* antigens. After the final elution process, the eluates were pooled, neutralised and stored in 50% (v/v) glycerol at -20°C.

The specificity of immuno-depleted *P. falcatus* antibodies was tested by ELISA and immunoblot analysis. The bound antibody fraction was also characterised by immunoblot analysis.

5.3.19 Production of monoclonal antibodies

Female BALB/c mice were immunised with 200-300 µg of water-soluble *P. major* extract that was dialyzed against PBS. For the first injections, samples were emulsified 1:1 in Freund's complete adjuvant and administered intraperitoneally. Subsequent boost injections were given with Freund's incomplete adjuvant 38 and 67 days after the primary injection. Mice to be used for hybridoma production were also immunised with protein solution in PBS (without adjuvant) 4-5 days before they were killed. Blood for testing was collected by eyebleeding and then prepared as described for rabbit serum preparation.

The spleen of the mouse with the highest titre was removed and used for hybridoma production, which was kindly performed by Joan Hoogenraad (La Trobe University, Department of Biochemistry). Spleen plasma cells were fused to Ag8 myeloma cells using polyethylene glycol as described by Galfre *et al.* (1977). Hybridomas were selected on HAT medium and screened for secretion of antibody specificity to *P. major* by ELISA. However, no clones were isolated which secreted antibodies that bound specifically to mite extracts.

Another attempt to produce monoclonal antibodies was performed. The spleen of a second mouse was used in a second fusion, which successfully yielded hybridomas. Following selection on HAT medium, hybridomas were again screened by ELISA. However, similar to the first attempt, no clones demonstrated a good antibody response. Since the antibodies sought were amongst a complex mixture of polypeptides, screening by immunoblot analysis was also performed. Groups of ten

supernatants were pooled together and screened against *P. major* protein separated by SDS-PAGE. No positive clones were detected in any of the pooled mixtures and so the production of monoclonal antibodies was discontinued.

5.3.20 Gel filtration (size exclusion) chromatography

Water-soluble *P. major* proteins that had been dialyzed overnight in PBS were separated by size exclusion chromatography. Approximately 200 µg of protein extract was loaded onto a Superose 12 column (MW range 1–300 kDa, Amersham Pharmacia) that was equilibrated in PBS. The *P. major* sample and Bio-Rad's molecular weight standards were run in the abovementioned buffer at a flow rate of 0.4 mL/min.

Molecular weight standards were Thyroglobulin (670 kDa), IgG (158 kDa), Ovalbumin (44 kDa), Myoglobin (17 kDa) and Vitamin B-12 (1.35 kDa). 2-min fractions (approximately 800 µl) were collected into Eppendorf tubes and stored at –80°C. A 75 µl sample was later removed from each Eppendorf tube and concentrated by SpeedVac. After addition of SDS sample buffer, all samples were subjected to SDS-PAGE (10% [v/v] acrylamide) and transferred to PVDF. Immunoblot analysis using anti-*P. major* serum was then performed to characterise each fraction for purity.

5.3.21 Detection of protein glycosylation using periodic acid/Schiff's procedure

Following SDS-PAGE, gels were fixed in several changes of 40% (v/v) ethanol, 7% (v/v) acetic acid for 30 min each and then left in fixative solution overnight. Proteins were then oxidised by immersing gels in 1% (v/v) periodic acid, 3% (v/v) acetic acid for 1 h. Traces of periodic acid were removed after six washes (10 min each) in milliQ water. Gels were stained with Schiff's Fuchsin-sulfite reagent (Sigma) in the dark for 1

h. Gels were then washed in several changes of 0.58% (v/v) potassium metabisulfite, 3% (v/v) acetic acid until bands appeared.

As a control, proteins of erythrocyte membrane ‘ghosts’, depleted of haemoglobin by hypotonic washing similar to that of Dodge *et al.* (1963), were also separated by SDS-PAGE and stained using periodic acid/Schiff’s procedure.

5.4 RESULTS

Penthaleus major is the most abundant and widespread blue oat mite species, commonly found feeding on thick-bladed grasses and cereal crops. Therefore, the collection of large quantities of these mites necessary for this research is relatively straightforward. However, it is far more difficult to collect large numbers of both *P. falcatus* and *P. sp. x*, due to their rare plant hosts and limited distributions (Robinson and Hoffmann 2001; Chapters 1 & 3). For this reason, *P. major* was generally used in initial experimental techniques.

5.4.1 Characterisation of mite proteins

After optimising the preparation of samples for electrophoresis, adult and juvenile mite extracts were separated by SDS-PAGE (10% [v/v] acrylamide) and silver stained (Figure 5.5). A large number of polypeptide bands were seen with a range of apparent molecular weights in both samples. It was evident that the maturity of individual mites significantly altered the protein profile, with a number of additional bands appearing in adult extracts. Therefore, all further experiments used adult mites only. Due to the large number of proteins visualised using SDS-PAGE, sub-fractions of the mite samples were taken. Water-soluble proteins were targeted because antibodies recognising water-soluble proteins would be advantageous for use in a field diagnostic. Water-soluble and water-insoluble/detergent-soluble extracts of each *Penthaleus* spp. were separated by SDS-PAGE (10% [v/v] acrylamide) and stained with Coomassie Blue (Figure 5.6). As expected, the profiles for the water-soluble and water-insoluble extracts were clearly different. However, when comparing extracts of the three mite species, the protein profiles had a number of bands in common. Some bands that were

present in both *P. major* and *P. sp. x* water-soluble extracts were not apparent in *P. falcatus*. Two such bands had approximate molecular weights of 75 kDa and 115 kDa, respectively (Figure 5.6). The complexity of the protein profiles of detergent-soluble and water-soluble extracts meant further separation and/or fractionation of the mite samples was needed.

5.4.2 Two-dimensional electrophoresis

To further characterise the complexity of the mite protein complement, 2-D electrophoresis was employed. Using the BCA Protein Assay Kit, it was estimated that each adult mite contains a total of approximately 20 µg of protein. It is recommended that around 100-200 µg of protein per 13 cm well is loaded in the first dimension of 2-D electrophoresis. Therefore, ten mites were generally used per gel.

The rehydration solution used to solubilise proteins and limit aggregation during isoelectric focusing was derived from a stock solution used in the laboratory (see 5.3.6.1). However, the initial 2-D results were fairly poor with relatively few distinct spots and vertical streaking on the gels. For comparison, a *Plasmodium falciparum* (strain HB3) extract, that was kindly provided by Kaylene Raynes (La Trobe University, Department of Biochemistry), was prepared and run simultaneously with a water-soluble extract of *P. major* (Figure 5.7). In this case, multiple individual spots were resolved. Thus, the poor resolution of the proteins visualised in the mite sample appears to result from a problem in solubilisation and/or focusing of the mite proteins, rather than a more general problem with the experimental procedure. The *P. major* 2-D pattern revealed that the majority of proteins were grouped towards the cathode, which

indicates that they were strongly positively charged. A major drawback of 2-D electrophoresis is that strongly basic proteins are not well resolved due to the limited buffering capabilities currently available for separation in the first dimension (Gorg 1999). Therefore, it is not surprising that it was difficult to obtain a high-resolution map of *Penthaleus* extracts. The 2-D pattern of *P. major* water-soluble extract also indicated a high degree of homogeneity of the mite proteins with respect to their physiochemical properties. The trains of spots differing in pI and MW could be proteins that have the same amino acid sequence but different glycosylation profiles (see Packer and Keatinge 2001).

Some 2-D protein analysis has been performed on other acarids (Osakabe and Sakagami 1993; Osakabe *et al.* 1993) and in these instances the conditions for rehydration of the protein samples differed from those used in the initial experiments. The types and concentrations of detergents used to solubilise mite extracts were therefore altered accordingly. Additionally, β -mercaptoethanol was added to the rehydration solution and the urea concentration was increased to 9.5 M. However, the resolution of the gels did not dramatically improve (data not presented).

5.4.3 N-terminal sequence analysis

Given that the 2-D electrophoresis approach was not successful, one-dimensional SDS-PAGE was pursued. An attempt was made to identify some of the bands that appeared to be species-specific by subjecting the proteins to Edman degradation and N-terminal sequencing. Firstly, mite extracts were separated by SDS-PAGE (7.5% acrylamide), electrophoretically transferred to PVDF and stained with Coomassie Blue. Some

polypeptide bands were identified which migrated with different apparent molecular weights in the three species examined (Figure 5.8). These bands may represent proteins that are unique to the different species. For *P. major*, *P. falcatus* and *P. sp. x*, the excised protein bands had approximate molecular weights of 66 kDa, 77 kDa and 64 kDa, respectively. N-terminal sequence data for each of these protein bands was obtained, following Edman degradation (Table 5.1).

The sequence data generated were compared with protein databases using the 'BLAST' homology search program (<http://www.ncbi.nlm.nih.gov/BLAST/>), maintained by NCBI (National Center for Biotechnology Information, America). The BLAST algorithm compares protein or DNA queries with data in publicly available protein and DNA databases (Altschul *et al.* 1997). No significant sequence similarities were found for any of the mite sequences when using the standard protein-protein BLAST (blastp) search. An alternative to the standard blastp search is the search for short, nearly exact protein sequences, which has parameters set automatically to optimise for searching with short sequences. The sequences were searched again using this algorithm. While homologies were identified for each sequence (Table 5.1), the E values assigned in the BLAST search were very high. For example, an E value in the range of 10^{-5} to 10^{-8} would normally be expected for an exact match of a 14 amino acid peptide. These statistical scores make real matches easier to distinguish from random background hits. These results indicated that further sequence data were needed to identify sequences with significant similarity.

5.4.4 Internal amino acid sequencing

Due to the limited sequence data obtained from N-terminal sequencing and the lack of closely related species in the public databases, no significant homology to known proteins or positive identification could be made. Therefore, an attempt was made to obtain internal sequence information that would help identify some of the different mite proteins. Amino acid sequence data from two separate sites within the polypeptides would also be useful for the design of oligonucleotides if attempts were made to clone the genes encoding these proteins.

5.4.4.1 TRYPTIC DIGESTION OF PROTEINS

Internal sequence information can be obtained by subjecting proteins to proteolytic digestion followed by N-terminal sequencing of polypeptide fragments. An abundant *P. major* protein (approximately 66 kDa), which appeared unique, was enzymatically digested using trypsin. Firstly, *P. major* extracts were applied to four wells of an SDS-PA gel, subjected to electrophoresis and stained with Coomassie Blue. Appropriate bands, containing an estimated 2 µg total protein, were excised, crushed and mixed with trypsin to initiate digestion *in situ*. The enzymatic digests were extracted from the acrylamide and loaded onto a RP-HPLC microbore column. The separated peptide eluants, corresponding to chromatographic peaks 1-9 (Figure 5.9), were then collected and subjected to Edman degradation. Meaningful sequence information was obtained for only two of the eluted peptide fragments corresponding to peaks 5 and 8 (Table 5.2). Sequence information from these eluant fractions was limited due to the small protein amounts collected, with only ten amino acid residues sequenced in both samples. These sequences were used to search the database at NCBI employing the

standard protein BLAST (blastp) algorithm and the search for short, nearly exact protein sequences. Neither sequence was found to have significant similarity to any known proteins using the blastp search, while only weak homologies were identified when using the search for short, nearly exact protein sequences (Table 5.2). For the sample corresponding to peak 8, the first amino acid was not unambiguously identified, with a tentative assignment of valine, alanine or serine. All three possibilities were examined when comparing sequence data to known proteins, with no clear identification able to be made.

The internal amino acid sequences were obtained from the same *P. major* polypeptide as the N-terminal sequence data generated. All three sequences could therefore be used in a combined search of public databases, which was performed using the FASTS program (<http://fasta.bioch.virginia.edu/fasta/>). FASTS compares unordered sets of short peptide fragments against protein databases. Attempts to identify library sequences using this program failed to identify any polypeptides that contained a combination of two (or all three) of the peptide sequences with any significance.

The small quantities of protein retrieved from the trypsin digestion protocols were responsible for the low number of peptides from which sequence data was obtained. This technical problem could be solved using a more sensitive gas phase sequencer or starting with a larger amount of sample to compensate for the high protein losses. However, it is clear that a major problem in terms of identifying proteins by sequence comparison may be the lack of gene sequence data for related species in the public databases.

5.4.4.2 CYANOGEN BROMIDE CLEAVAGE OF PROTEINS

Chemical cleavage of polypeptides generally results in fewer fragments than enzymatic digestion. This simplifies the separation process, often resulting in lower protein losses (Allen 1989). For example, cyanogen bromide specifically cleaves at the C-terminus of methionine residues, which occur at low frequencies in proteins. Thus, on average, fewer fragments are generated and these fragments are relatively large in size. The feasibility of this was examined by performing 'in gel' cleavage using cyanogen bromide (16 µg/µl in 70% formic acid) on the 66 kDa *P. major* polypeptide. Bands containing approximately 2 µg protein were excised and subjected to CNBr treatment over a 48 h period. Relevant controls included proteins incubated without CNBr in the presence or absence of the formic acid solvent. The resulting fragments were separated by SDS-PAGE (10% [v/v] acrylamide) and silver-stained (Figure 5.10). For the control samples, there was some non-specific breakdown of protein when incubated in formic acid alone. Although when the solvent was replaced with milliQ water there was no evidence of this. Cleavage fragments of approximately 32 kDa, 17 kDa and 14 kDa were produced. Smaller fragments may have also been produced. However, peptides less than 10 kDa may not have been visualised, as they tend to migrate with the dye band under the conditions used here for electrophoresis. Although CNBr cleavage was successful, the overall levels of protein available for analysis were very low, with peptide bands only visible after staining with silver. To obtain useful sequence data from samples transferred to PVDF, a strongly stained Coomassie Blue band is usually required. Thus, a greater amount of starting protein would be needed when sequencing fragments of candidate proteins in the future.

5.4.5 Analysis of polyclonal antibodies

Because of difficulties identifying mite polypeptides, I examined an alternative approach to obtaining reagents that could specifically recognise different mite proteins. Water-soluble protein extracts of the three *Penthaleus* spp. were injected into rabbits to generate polyclonal antibodies. Each rabbit was injected three times (roughly 5 weeks apart) with approximately 5-6 mg total protein. The antibody mixtures generated were then characterised by enzyme-linked immunosorbent assays (ELISA) and immunoblot analysis.

ELISAs were conducted to analyse the specificity and to determine the titres of polyclonal antibodies produced against the different mite species. The titre gives a measure of the relative antibody affinity, by testing the capacity of serial dilutions of the antiserum to bind to proteins immobilised on the walls of microtitre plates. In these cases, water-soluble extracts of the three mite species used to immunise rabbits were coated onto microtitre wells. Absorbance results were plotted against antibody dilution (Figure 5.11). Data points are the average of three replicates and standard error bars are indicated. The titration curves showed that maximum signal with the anti-*P. major*, anti-*P. falcatus* and anti-*P. sp. x* sera was retained up to a dilution of 1/1000. This suggests that the rabbits generated a reasonably strong immune response to the mite proteins and revealed a suitable dilution range for immunoblot analysis. Pre-immune sera from the rabbits gave very low signals at all dilutions tested in the ELISA, indicating that the signal obtained was due to antibodies generated in response to the mite proteins.

Immunoblot analysis of antibodies was carried out against water-soluble extracts of the three *Penthaleus* spp. (Figure 5.12), with the aim of identifying one or more species-specific proteins. Antibodies in the sera raised against the three species recognised proteins in each of the mite extracts. Indeed the immunoblot analysis showed similar banding patterns across the three species, suggesting the dominant populations of antibodies in the sera were recognising proteins that are present in all three species. There was also a relatively large amount of smearing and fuzzy bands visualised for all three antisera, which was consistently observed on replicate immunoblots. The polydisperse banding and high cross reactivity between species suggests that some proteins were glycosylated and the antibodies were recognising carbohydrate epitopes.

Immunoblot analysis using anti-*P. major* serum showed two major immunoreactive bands in all three species (Figure 5.12a). A number of bands were polydisperse, with none appearing specific to *P. major*. The band of approximately 52 kDa appears to be immunodominant and migrates as a tight protein band. However, this reactivity was common to all species and, therefore, not a candidate protein for a species-specific diagnostic test. Analysis of the anti-*P. falcatus* serum (Figure 5.12b) also showed some similarities in the banding pattern between all species, although two broad bands that are dominant in the *P. falcatus* sample were identified. These bands had approximate molecular weights of 40 kDa and 50 kDa. Although these bands appeared to be very immunogenic, both bands migrated as broad smeared regions of reactivity. Therefore, it could not be determined if the bands were representative of one or more polypeptides recognised by *P. falcatus* antibodies. Close similarities in the banding patterns for the three species were also seen following immunoblot analysis with anti-*P. sp. x* serum (Figure 5.12c).

In all three immunoblots, the banding pattern for the *P. major* and *P. sp. x* extracts were quite similar, while the banding pattern for *P. falcatus* was somewhat different. Moreover, the banding patterns using *P. major* and *P. sp. x* antibodies were more similar to each other than for antibodies raised against *P. falcatus*. This suggests a closer relationship in the dominant antigens and higher amount of homogeneity between *P. major* and *P. sp. x*.

5.4.6 Purification of antibodies immobilised on PVDF

Immunoblot analysis revealed two bands of approximately 35 kDa and 50 kDa that may be unique to *P. falcatus*. To further examine the specificity of the *P. falcatus* antibodies, affinity purification was performed. This involved separating water-soluble *P. falcatus* proteins by SDS-PAGE, transferring them to PVDF, and excising a broad region encompassing one of the bands. The region between 46 kDa and 54 kDa was used to affinity purify *P. falcatus* antibodies. Immunoblot analysis using this antiserum was then performed. Unfortunately, the affinity purified *P. falcatus* antibodies resulted in a similar banding pattern on immunoblots to that observed earlier using control *P. falcatus* antibodies (data not presented). Furthermore, cross-reactivity was seen in both *P. major* and *P. sp. x*, indicating that the antibodies purified may not be specific to *P. falcatus*.

5.4.7 Inhibition ELISAs

The specificity of antisera generated against the three *Penthaleus* species was further examined by inhibition ELISAs. This allowed the level of cross-reactivity between proteins of the species against which the polyclonal sera was raised and the proteins of other species to be analysed. If the homologous antigen gives a stronger inhibitory

effect than the heterologous antigen, this indicates the presence of some non-cross-reactive proteins and antibodies in the preparations. ELISAs were conducted for polyclonal antisera generated against all three *Penthaleus* spp. as well as pre-immune antisera (Figure 5.13). Data points are the average of three replicates and error bars are indicated.

The results showed some differences in the ELISA curves for the homologous and heterologous *Penthaleus* species. As expected, the highest amount of inhibition came from homologous antigen extracts, with the largest differences in absorbance observed for the inhibition ELISA using *P. falcatus* antibodies. This indicates that the antiserum raised against *P. falcatus* recognises more epitopes in *P. falcatus* than in *P. major* and *P. sp. x*. *Penthaleus falcatus* antigen was also the poorest inhibitor for the binding of *P. major* and *P. sp. x* antibody preparations. This further emphasises the differences between *P. falcatus* and the other two species. The similarities in the antigenic response of *P. major* and *P. sp. x* compared with *P. falcatus*, was also evident in the initial immunoblots carried out against each species (Figure 5.12).

5.4.8 Immuno-depletion of antibodies

Analysis using inhibition ELISA indicated some specificity of the polyclonal antibodies generated against *P. falcatus*. To examine these differences, immuno-depletion, which is a technique often used to enrich specific antibodies in a preparation of polyclonal antisera, was performed. Anti-*P. falcatus* serum was depleted of reactivity against *P. major* antigen by passing it over a column of *P. major* proteins covalently linked to a bead support. The specificity of the depleted *P. falcatus* antibodies was then tested by ELISA and immunoblot analysis. The ELISA titration

curves (Figure 5.14) indicated that following immuno-depletion, the majority of antibodies did not recognise *P. major*, and antibodies relatively specific to *P. falcatus* were retained. Immunoblot analysis using the depleted antibodies (Figure 5.15) confirmed this result, showing antibodies specific to *P. falcatus* were isolated from the polyclonal mixture. It is also worth noting, that the reactivity of the depleted antiserum was significantly reduced following this purification step. Additionally, probing the immunoblot with the bound antibody fraction resulted in a very similar banding pattern to the immunoblot using control 'un-purified' anti-*P. falcatus* serum. Therefore, the majority of antibodies in the original polyclonal pool were cross-reactive and not specific to *P. falcatus*. The similarities in immunoblot banding patterns and the immuno-depletion results may be explained by a proportion of the antibodies generated recognising different glycan epitopes on the same protein in each species.

Four bands that may represent candidate proteins specific to *P. falcatus* were revealed from immunoblot analysis using immuno-depleted antibodies. These bands were approximately 100 kDa, 90 kDa, 55 kDa and 40 kDa. There was also some high molecular weight material that was reactive, however, this appeared as a light smear and could be representative of protein aggregates or other contaminants. The band with an approximate molecular weight of 90 kDa was polydisperse and could represent more than one protein or a heavily glycosylated protein. The two bands with approximate molecular weights of 55 kDa and 40 kDa were also quite broad. The most promising band ran with an approximate molecular weight of 100 kDa. This band was very sharp and distinct, and is therefore, likely to represent a single protein. This could prove the best candidate to use in a diagnostic.

5.4.9 Monoclonal antibodies

The immuno-depletion experiments suggest that there are species-specific epitopes recognised by the polyclonal antisera raised against the *Pent haleus* species. This indicated the production of monoclonal antibodies was a feasible and practical next step. Monoclonal antibodies are raised by the fusion of B-lymphocytes of immunised mice with immortal cell cultures to produce hybridomas. Their specificity of binding, their homogeneity, and their ability to be produced in unlimited quantities, has been instrumental in the development of antibodies for diagnostic applications.

Attempts were made to generate hybridomas that produce antibodies to *P. major* antigens. Mice were injected three times with a total of approximately 700-800 µg water-soluble protein extract. After the final injection, eye bleeds were taken from each mouse and the immune response determined by ELISA. The spleen of the mouse with the highest titre was removed and used for hybridoma production. Once hybridomas were generated, they were selected on HAT medium and screened for antibody secretion by ELISA. In the first attempt, nine clones showed a weak antibody response (only slightly higher than the negative controls), while the remaining clones showed no positive antibody response. Another attempt to produce monoclonal antibodies was made using a second mouse spleen. However, no clones exhibiting a positive antibody response were detected using either ELISA or immunoblot screening methods (data not presented).

One potential problem was that mice were injected with a whole mixture of proteins, whereas normally only one or two proteins are targeted when producing monoclonal antibodies.

5.4.10 Gel filtration chromatography

Due to problems encountered in the generation of monoclonal antibodies, steps were taken to fractionate and simplify mite extracts. Gel filtration chromatography was used to separate proteins according to differences in their size. Water-soluble *P. major* proteins were dialyzed against PBS and subjected to chromatography. The elution profile (Figure 5.16a) was effectively a large polydisperse protein peak, therefore, 2-min fractions were collected and analysed. A smaller peak containing material with a molecular weight of > 300 kDa was also eluted in the void volume (around 16-20 min), but this probably represented protein aggregates. Another small peak was also detected in the 214 nm chromatograph, eluting around 45 min. This peak was not detected at 280 nm and is therefore, likely to be low molecular weight non-protein material (< 1 kDa), such as salts and other contaminants.

The 2-min fractions were separated using SDS-PAGE, transferred to PVDF and characterised by immunoblot analysis using anti-*P. major* serum (Figure 5.16b). The immunoblot showed that some fractionation was achieved by gel filtration, although further separation is needed to isolate fractions containing single protein/antigens. Also interesting to note is that the majority of fractionated immunoreactive bands were very broad and indistinctive. These findings suggest that the immunodominant proteins have a polydisperse molecular weight range consistent with carbohydrate epitopes present on several heterogeneous protein species.

5.4.11 Analysis of glycosylation in mite protein extracts

The lack of definite narrow bands in all immunoblots indicated glycosylation of some immunodominant proteins (Harlow and Lane 1988). An attempt was made to visualise glycoproteins in mite samples by separating *P. sp. x* water-soluble proteins and a control sample, erythrocyte membrane proteins, by SDS-PAGE and staining using periodic acid/Schiff's (PAS) procedure (Figure 5.17). This method, which stains vicinal diol groups found mainly on peripheral sugars and sialic acids, is used as a general glycoprotein stain. The periodic acid treatment oxidises glycols present in glycoproteins to aldehydes, which then react with Schiff's reagent to form a characteristic fuschia-coloured stain. Samples were also stained with Coomassie Blue to illustrate the amount of each sample separated by SDS-PAGE.

Three known glycoproteins were visualised after PAS staining of the erythrocyte membrane sample, demonstrating that the staining method was effective. These bands corresponded to glycophorin A, PAS-2 and PAS-3, with molecular weights of approximately 92 kDa, 44 kDa, and 25 kDa, respectively (Fairbanks *et al.* 1971; Zdebska *et al.* 2001). The results for *P. sp. x* also showed some areas stained with Schiff's reagent, indicating saccharides are bound to mite proteins. In particular, a broad region approximately 45 kDa was detected which may contain several glycosylated proteins. There was also some staining of high molecular weight material, although this appeared as a light smear and could represent high protein aggregates. Although PAS staining indicated some *P. sp. x* proteins have sialic acid-containing carbohydrates attached to them, immunoblot analysis suggested a greater degree of glycosylation in mite proteins. Moreover, the 45 kDa region in *P. sp. x* detected by PAS staining does not overlap with the broad fuzzy bands revealed by the various

immunoblots previously conducted. This may suggest some proteins are glycosylated with sugars that do not include sialic acid residues.

5.4.12 Analysis of *H. destructor* extracts

Due to the frequent misidentification of blue oat mites and the redlegged earth mite, the electrophoretic profile of *H. destructor* extracts were also examined. Water-soluble and water-insoluble extracts of *H. destructor* and the three *Penthaleus* spp. were separated by SDS-PAGE (4-12% NuPAGE®) and stained with Coomassie Blue (Figure 5.18). The protein profile of *H. destructor* extracts showed some similarities to *Penthaleus* species. As indicated by the arrows, several protein bands in both the water-soluble and water-insoluble extracts were common to all mite species. These bands may represent proteins that are relatively conserved and have an essential function, such as housekeeping proteins. A faint band with an approximate molecular weight of 55 kDa was seen in the water-insoluble extract of *H. destructor*. A band of this size was not apparent in any of the *Penthaleus* extracts, and may be unique to *H. destructor*.

Halotydeus destructor proteins were also examined and compared to *Penthaleus* spp. by immunoblot analysis using the anti-*P. major* serum (Figure 5.19). The banding pattern of *H. destructor* was similar to *P. major* and the other *Penthaleus* spp., although fewer proteins were recognised. The most immunogenic band observed in *H. destructor* (approximately 55 kDa) was identified in all three *Penthaleus* species. The other *H. destructor* bands (between 56 kDa - 100 kDa) were also common to either *P. major* or *P. sp. x*, or both. It is interesting to note that fewer bands were common to *H.*

destructor and *P. falcatus* compared to *H. destructor* and the other two *Penthaleus* species.

5.5 DISCUSSION

Using biochemical and protein electrophoretic techniques, this study examined the protein profiles of three *Penthaleus* spp. with the aim of identifying one or more species-specific proteins from each species. Characterisation and isolation of such proteins is essential for the development of a field-based diagnostic kit to distinguish the different species. Ideally, an unambiguous test that allows rapid identification of the species is needed. Diagnostic methods relying on molecular markers, such as PCR–RFLP (polymerase chain reaction-restriction fragment length polymorphism), are frequently used to separate morphologically indistinguishable insects and macroinvertebrates (Amstrong *et al.* 1997; Brown *et al.* 1999; Clark *et al.* 2001). These techniques are generally specific to the species level and have the added advantage of being relatively simple and quick to develop (Carew *et al.* in press). Unfortunately, these methods are only suitable for laboratory use; they have no benefit in field-based diagnostic kits. Immunoassays, on the other hand, are developed and routinely used in clinical and agricultural field diagnosis (Avrameas *et al.* 1992; Stephens 1993; Trowell *et al.* 2000). Immunoassay-based kits are very specific and generally rapid, low cost and simple to use. Therefore, in this study an experimental approach was undertaken with the view of identifying antibodies that recognise specific mite proteins.

The *Penthaleus* group is interesting, in that males are absent and reproduction occurs solely by thelytoky parthenogenesis (Weeks *et al.* 1995). This results in offspring genetically identical to their mothers except for mutations. This mode of asexual reproduction is generally accepted to result in slow evolutionary rates and limited genetic diversity (Tomiuk and Loeschcke 1992; Schön *et al.* 1998). Indeed, the longest

living asexual species known, *Darwinula stevensoni*, has very low genetic variability within and between individuals and populations (Schön and Martens 2003). However, many other asexual species, such as bdelloid rotifers, exhibit high morphological and genetic diversity (Welch and Meselson 2000). Prior to this study, no sequence information was available for any of the *Penthaleus* species. For this reason, the degree of diversity between the species was unclear, although they were known to differ markedly in their biology (Weeks and Hoffmann 1999; Chapter 1). This indicated that the three species were relatively divergent and detecting protein differences would be achievable.

This study provided important information about the complex nature of the *Penthaleus* group, the similarity between the three species, as well as identifying methods for isolating and characterising species-specific proteins. Because no biochemical or molecular work of this nature had been previously performed on these pests or any closely related species, the initial step was to examine the protein profiles of the three species. Preliminary SDS-PAGE results showed a complexity of separated proteins and a high degree of similarity between the species. Very few species-specific proteins were visible using 1-D gels, therefore, two-dimensional (2-D) electrophoresis was used to separate the mite samples. As mentioned previously, 2-D electrophoresis is a powerful and widely used method for the analysis of complex protein mixtures, although a major drawback is the poor resolution of alkaline proteins (Hanash 1998).

2-D electrophoretic analysis of mite extracts revealed that the majority of proteins were in fact very basic. The majority of protein spots were also not well resolved, indicating a number of possible problems during various steps of the 2-D process. For

instance, the sample may have been poorly soluble in the rehydration solution or the sample may have contained impurities that prevented steady state focusing in the first dimension. Glycosylation of proteins can also lead to problems in 2-D gels (Weistermeier and Naven 2002). Glycoproteins often run as diffuse smears or ‘families’ of spots of similar molecular weight, rather than discrete spots, when analysed by 2-D electrophoresis (Ledbetter *et al.* 1979). This heterogeneity is at least partly a reflection of their variable carbohydrate content (Ledbetter and Herzenberg 1979) and could in part explain the pattern observed. Osakabe and Sakagami (1993) found a large amount of streaking (horizontal and vertical) and poor resolution of 2-D gels when separating protein extracts of the spider mite, *Panonychus citri*.

N-terminal and internal amino acid sequence information was obtained for some bands that ran with different apparent molecular weights in the three species after separation by SDS-PAGE. Attempts were made to identify these polypeptides using the BLAST homology search algorithm. Homologies were identified using the search for short, nearly exact protein sequences, however, the matches obtained were not significant. Therefore, no positive identification of any sequence could be made. Similarly, no positive homology was detected using the FASTS search algorithm. These findings indicate that the mite proteins sequenced have not been identified previously and that there are no closely related proteins in the databases. This is not surprising, as very little work of this nature has been undertaken on acarids or similar species. It may also indicate that the proteins sequenced are not well conserved (i.e. probably not housekeeping proteins), as some homology would be expected to insect and other related groups represented in the databases.

As protein sequences could not be readily identified and no obvious candidate proteins were apparent from SDS-PAGE, antibodies were generated against a mixture of mite proteins rather than individual proteins. Polyclonal antisera against water-soluble proteins of the three *Penthaleus* spp. were raised in rabbits and tested by immunoblot analysis against soluble extracts of each species. For each serum, similar banding patterns were observed across all species, indicating a high amount of cross-reactivity between the three species. In particular, the banding pattern of *P. major* and *P. sp. x* were extremely similar in all three immunoblots while *P. falcatus* was somewhat different. The banding patterns on SDS-PAGE (after staining with either Coomassie Blue or silver) also showed closer similarities between *P. major* and *P. sp. x*. It is interesting to note that biologically, *P. falcatus* is also the most idiosyncratic species of the three (Umina and Hoffmann 1999; Chapters 3 & 4). Consequently, one could speculate that during the evolution of these species, *P. falcatus* diverged at an early stage. DNA sequencing and phylogenetic analysis could clarify this suggestion.

In all immunoblots that were performed there was a large amount of smearing and diffuse banding seen. This suggests that the dominant mite antigens were glycosylated and at least some of the antibodies generated recognised carbohydrate epitopes rather than protein epitopes. Glycosylated proteins are heterogeneous with respect to the chain length and degree of branching of the carbohydrate modification. Thus they do not have a uniform size or charge density and are visualised as polydisperse bands on SDS-polyacrylamide gels (Hempelman 1992). To investigate glycosylation in mite samples a common method for staining glycoproteins, periodic acid/Schiff's (PAS) procedure, was used. The results indicated saccharides were bound to mite proteins, although the extent of staining was quite low and the regions stained did not

correspond to the broad diffuse bands seen in immunoblots. One could speculate that other non-sialic acid-containing carbohydrates (not detected by PAS) are linked to various mite proteins. There are several methods that could be employed to provide more information on the nature of glycosylation in mite samples. One of these is the use of lectin affinity chromatography. Lectins are a group of proteins, which react reversibly with glycoproteins and polysaccharides, via specific sugar residues. They are routinely used to rapidly separate and analyse glycoproteins. To provide a suitable spectrum of separation parameters, a variety of lectins, such as wheat germ agglutinin, concanavalin A, and lentil lectin, could be used.

Treatment of nitrocellulose membranes with periodate prior to immunoblot analysis is another approach that could reveal the extent of glycosylation in mite extracts.

Periodate treatment works by preventing antibodies that recognise carbohydrate moieties binding to the membrane. Alternatively, mite proteins could be incubated with an endoglycosidase, which removes sugars from proteins under mild conditions while preserving the structure of the protein. PNGase F has the broadest specificity, cleaving most N-linked oligosaccharides (Gerard 1990), making it an ideal starting point. Immunoblot analysis could then be performed using deglycosylated samples. Deglycosylation of mite samples prior to rabbit or mouse immunisations may also dramatically alter the immune response and the number of species-specific antibodies produced. Time constraints did not allow these options to be pursued as part of this work.

During SDS-PAGE, the protein or antigen mixture is denatured prior to separation. Potentially, some epitopes may not refold correctly and regain their original

conformation following electrophoretic transfer to PVDF. As a result only those antibodies that recognise denaturation-resistant epitopes are certain to bind in immunoblots. Antibodies would not react in immunoblot analysis if SDS irreversibly altered the specific epitope conformation. Therefore, some antibodies, which could potentially recognise species-specific proteins, may not have been detected by immunoblot analysis. In theory, ELISA, which does not involve denaturation, is more sensitive and allows for the detection of more epitopes. Inhibition ELISAs were performed to compare the relative specificity and avidity of one antiserum for three antigens in the same system. Significant differences between species were revealed, particularly for *P. falcatus*, indicating species-specific epitopes were present in the antisera raised against the homologous mite species.

Immuno-depletion was performed to further enrich the species-specific reactivity of the antiserum generated against *P. falcatus*. This protocol was effective in removing all common or 'shared' antibodies, and generating a reagent that was specific to *P. falcatus*. Four candidate bands specific to *P. falcatus* were also identified following immunoblot analysis. These could be used to make specific diagnostic antibodies, although further analysis is needed to characterise the protein bands. At this stage, it is unclear whether the purified or 'depleted' antibodies recognise specific *P. falcatus* protein epitopes or whether some or all of the epitopes are carbohydrate in nature. A potential method of isolating these proteins for further analysis is antibody affinity chromatography using a column prepared by covalently linking the immunoaffinity purified serum to a bead support. *Penthaeus falcatus* protein extract could then be passed through the column and the bound fraction, containing the species-specific antigens, eluted. These antigens could then be separated by gel-filtration

chromatography or SDS-PAGE and potentially used to generate antibodies. This protocol could also be effective in the isolation of proteins and subsequent generation of antibodies specific to *P. major* and *P. sp. x*.

Monoclonal antibodies are preferable for use in field-based diagnostic kits due to their specificity, potentially inexhaustible supply and standardised procedures for manufacture. An attempt to generate monoclonal antibodies was made following the promising results obtained from the inhibition assays and immunoaffinity depletion experiment. Unfortunately, two attempts to produce monoclonals failed. The specific reasons for this are unclear. A major potential shortcoming is that mice were injected with a mixture of proteins. Furthermore, the immune response (as determined by titres) obtained from mice was much lower than obtained from rabbits. This was surprising as the protein/antigen preparation and immunisation procedures were essentially the same for mice injections as they were for rabbits when generating polyclonal antibodies. In order to increase the immune response and improve the chances of producing monoclonal antibodies, mite extracts could be purified such that only a few proteins are targeted. Using different strains of mice can also elicit different immune responses (R.F. Anders, personal communication).

Due to problems encountered in the generation of monoclonal antibodies and the complex nature of the mite extracts, steps were taken to fractionate and simplify mite samples using gel filtration chromatography. The preliminary results were somewhat encouraging, although additional separation steps are needed to obtain efficient separation of proteins of the three *Penthaless* species. For example, cation exchange chromatography, which separates proteins on the basis of their net charge, could be

used to further purify the gel-filtration fractions. In this technique, proteins are bound to negatively charged beads at low ionic strength and then eluted differentially with increasing salt concentrations.

In addition to problems distinguishing the *Penthaleus* spp., many farmers often have trouble differentiating between blue oat mites and the redlegged earth mite, *H. destructor*. In part, this is due to their small size and the fact they occur sympatrically within Australia (see Chapter 1). Confusion between these mite species could be responsible for ineffective control in crops and pastures (Umina *et al.* 2001).

Therefore, some preliminary experiments were conducted to analyse protein extracts of *H. destructor* and determine the feasibility of developing a diagnostic reagent for this species. The protein profiles of *H. destructor* were quite similar to *Penthaleus* spp., however, there was a polypeptide band appearing unique to *H. destructor*. Further work is needed to establish the specificity of this candidate protein, although it could potentially be used in the development of a diagnostic reagent for this species.

Immunoblot analysis of the *Penthaleus* spp. and *H. destructor* was also conducted and revealed close similarities between the four species. Bands that are common to all species probably represent well-conserved proteins, which are not likely to be useful in a diagnostic kit. In future experiments, antibodies directly against *H. destructor* proteins should be produced. These antibodies could be employed in immunological studies with the hope of identifying protein bands specific to *H. destructor*.

Alternatively, immunoaffinity purification of antiserum, similar to the method used to identify and isolate *P. falcatus* proteins from *P. major*, could be performed to isolate candidate *H. destructor* proteins.

In this chapter, various techniques to identify and isolate species-specific mite proteins have been investigated. Although diagnostic reagents have not yet been produced, a significant amount of information on the protein make-up of *Penthaleus* spp. has been obtained. A major obstacle was the lack of homologous sequences available in public databases, which meant no positive identification of any mite proteins could be made. This also restricts the opportunities for work at the DNA level. Protein analysis also indicated that the three species are remarkably similar, possibly a consequence of the ancestral lineage of this group which may have evolved from a single clonal genotype. Moreover, several results indicated a proportion of mite proteins were glycosylated with various carbohydrate moieties. These features complicated attempts to identify and characterise unique proteins suitable for generating specific antibodies. Nonetheless, antibodies specific to *P. falcatus* were isolated and four candidate polypeptide bands were identified in this species. Expanding on the techniques used, would be worthwhile, not only for the isolation of these proteins, but to also identify candidate proteins for *P. major* and *P. sp. x*. Working with a selected few proteins would dramatically improve the chances of generating monoclonal antibodies, which, in theory, would result in an inexhaustible supply of antibody reagents. This is preferable for the development of a commercially based diagnostic kit.

5.6 REFERENCES CITED

- Allen G. 1989. Laboratory techniques in biochemistry and molecular biology, Volume 9: sequencing of proteins and peptides. Elsevier Science Publishers, Amsterdam, The Netherlands.
- Altschul S. F., Madden T. L., Schäffer A. A., Zhang J., Zhang Z., Miller W. and Lipman D. J. 1997. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Research* 25 (17): 3389-3402.
- Armstrong K. F., Cameron C. M. and Frampton, E. R. 1997. Fruit fly (Ciptera: Tephritidae) species identification: a rapid molecular diagnostic technique for quarantine application. *Bulletin of Entomological Research* 87: 111-118.
- Annels A. J. 1994. The reproductive biology and mating behaviour of redlegged earth mite: an overview. *In: McDonald G. and A.A. Hoffmann (eds.), Proceedings of the Second National Workshop on Redlegged Earth Mite, Lucerne Flea and Blue Oat Mite.* Department of Agriculture, Rutherglen, Australia, pp. 29-31.
- Avrameas S., Nakane P. K., Papamichail M. and Pesce A. J. 1992. 25 Years of Immunoenzymatic techniques. *Journal of Immunological Methods* 150: 3-4.
- Brown B., Emberson R. M. and Paterson A. M. 1999. Mitochondrial COI and II provide useful markers for *Wiseana* (Lepidoptera: Hepialidae) species identification. *Bulletin of Entomological Research* 89: 287-293.

Carew M. E., Pettigrove V. and Hoffmann, A. A. in press. Identifying chironomids (Diptera: Chironomidae) for biological monitoring with PCR-RFLP. Bulletin of Entomological Research.

Chambers J., Dunn J. A. and Thind B. B. 1999. A rapid, sensitive, user-friendly method for detecting storage mites. HGCA Project Report No. 208. Home Grown Cereals Authority, London, UK.

Clark T. L., Meinke L. J. and Foster J. E. 2001. PCR-RFLP of the mitochondrial cytochrome oxidase (subunit I) gene provides diagnostic markers for selected *Diabrotica* species (Coleoptera: Chrysomelidae). Bulletin of Entomological Research 91: 419-427.

Córdoba O. L., Linskens S. B., Dacci E. and Santomé J. A. (1997). 'In gel' cleavage with cyanogen bromide for protein internal sequencing. Journal of Biochemical and Biophysical Methods 35: 1-10.

Dodge J. T., Mitchell C. and Hanahan D. J. (1963). The preparation and chemical characteristics of hemoglobin-free ghosts of human erythrocytes. Archives of Biochemistry and Biophysics 110: 119-130.

Fairbanks G., Steck T. L. and Wallach D. F. H. 1971. Electrophoretic analysis of the major polypeptides of the human erythrocyte membrane. Biochemistry 10 (13): 2606-2616.

Galfre G., Howe S. C., Milstein C., Butcher G. W. and Howard J. C. 1977. Antibodies to major histocompatibility antigens produced by hybrid cell lines. *Nature* 266: 550-552.

Gerard G. 1990. Purification of glycoproteins. *In*: Deutscher M.P. (ed.), *Methods in Enzymology: Guide to Protein Purification*. Academic Press, San Diego, America, pp 529-539.

Gorg A. 1999. IPG-Dalt of very alkaline proteins. *In*: Link A.J. (ed.), *Methods in Molecular Biology: 2D Proteome Analysis Protocols*. Humana Press, New Jersey, America, pp. 197-209.

Hanash S. M. 1998. Two-dimensional gel electrophoresis. *In*: Hames B.D. (ed.), *Gel Electrophoresis of Proteins: A Practical Approach*, 3rd Edition. Oxford University Press, Oxford, UK, pp. 189-211.

Harlow E. and Lane D. 1988. *Antibodies: a laboratory manual*. Cold Spring Harbor Laboratory Press. Cold Spring Harbor, America.

Hempelman E. 1992. Analytical SDS polyacrylamide gel electrophoresis (SDS-PAGE). *In*: Peters J.H. and H. Baumgarten (eds.), *Monoclonal Antibodies*. Springer-Verlag, Heidelberg, Germany, pp. 430-434.

Hewick R. M., Hunkapiller M. W., Hood L. E. and Dreyer W. J. (1981). A gas-liquid solid phase peptide and protein sequenator. *The Journal of Biological Chemistry* 256 (15): 7990-7997.

Jeppson L. R., Keifer H. H. and Baker E. W. 1975. *Mites Injurious to Economic Plants*. University of California Press, Berkeley, America.

Laemmli U. K. 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. *Nature* 227: 680-685.

Ledbetter J. A. and Herzenberg L. A. 1979. Xenogeneic monoclonal antibodies to mouse lymphoid differentiation antigens. *Immunological Reviews* 47: 63-90.

Ledbetter J. A., Goding J. W., Tsu T. T. and Herzenberg L. A. 1979. A new mouse lymphoid alloantigen (Lgp100) recognised by a monoclonal rat antibody. *Immunogenetics* 8: 347-360.

Luczynska C. M., Li Y., Chapman M. D. and Platts-Mills T. A. E. 1989. A two-site monoclonal antibody ELISA for quantification of the major *Dermatophagoides* spp. allergens Der p 1 and Der f 1. *Journal of Immunological Methods* 118: 227-235.

Narayan D. S. 1962. Morphological, biological and ecological studies on the winter grain mite, *Penthaleus major* (Dugés), Penthaleidae; Acarina Part 1. *Journal of Zoological Society of India* 14: 45-63.

Osakabe M. and Sakagami Y. 1993. Protein differences detected by two-dimensional electrophoresis among local populations of *Panonychus citri* (McGregor) (Acari: Tetranychidae) in Japan. *Applied Entomology and Zoology* 28 (4): 497-502.

Osakabe M. H., Saito Y. and Sakagami Y. 1993. Protein differences detected by two-dimensional electrophoresis in the species complex of *Schizotetranychus celarius* (Banks)¹. *Experimental and Applied Acarology* 17: 757-764.

Packer N. H. and Keatinge L. 2001. Glycobiology and proteomics. *In*: Pennington S.R. and M.J. Dunn (eds.), *Proteomics: from protein sequence to function*. Springer-Verlag, New York, America, pp. 257-275.

Qin T. K. and Halliday R. B. 1996. The Australian species of *Chromotydeus* Berlese and *Penthaleus* Koch, C.L. (Acarina, Penthaleidae). *Journal of Natural History* 30: 1833-1848.

Ridsdill-Smith T. J. 1997. Biology and control of *Halotydeus destructor* (Tucker) (Acarina: Penthaleidae) – a review. *Experimental and Applied Acarology* 21: 195-224.

Ridsdill-Smith T. J. and Pavri C. 1998. Spring spraying for redlegged earth mites. *Western Focus (Australian Grain)*, October-November: 1-4.

Robinson M. R. and Hoffmann A. A. 2001. The pest status and distribution of three cryptic blue oat mite species (*Penthaleus* spp.) and the redlegged earth mite

(*Halotydeus destructor*) in southeastern Australia. Experimental and Applied Acarology 25: 699-716.

Rose R. L., Barbhuiya L., Roe R. M., Rock G. C. and Hodgson E. 1995. Cytochrome P450-associated insecticide resistance and the development of biochemical diagnostic assays in *Heliothis virescens*. Pesticide Biochemistry and Physiology 51: 178-191.

Schön I. and Martens K. 2003. No slave to sex. Proceedings of the Royal Society of London (Series B) 270: 827-833.

Schön I., Butlin R. K., Griffiths H. I. and Martens K. 1998. Slow molecular evolution in an ancient asexual ostracod. Proceedings of the Royal Society of London (Series B) 265: 235-242.

Stephens D. 1993. Bunch Rot has nowhere to hide. Fruit Grower, August: 26-27.

Tomiuk J. and Loeschcke V. 1992. Evolution of parthenogenesis in the *Otiiorhynchus scaber* complex. Heredity 68: 391-397.

Trowell S. C., Forrester N. W., Garsia K. A., Lang G. A., Bird L. J., Hill A. S., Skerritt J. H. and Daly J. C. 2000. Rapid antibody-based field test to distinguish between *Helicoverpa armigera* (Lepidoptera: Noctuidae) and *Helicoverpa punctigera* (Lepidoptera: Noctuidae). Journal of Economic Entomology 93 (3): 878-891.

Umina P. A. and Hoffmann A. A. 1999. Tolerance of cryptic species of blue oat mites (*Penthaleus* spp.) and the redlegged earth mite (*Halotydeus destructor*) to pesticides. Australian Journal of Experimental Agriculture 39: 621-628.

Umina P., Robinson M. and Hoffmann A. 2001. Correct identification critical for mite control. Farming Ahead 116: 37-38.

Weeks A. R. and Hoffmann A. A. 1999. The biology of *Penthaleus* species in southeastern Australia. Entomologia Experimentalis et Applicata 92: 179-189.

Weeks A. R. and Hoffmann A. A. 2000. Competitive interactions between two pest species of earth mites, *Halotydeus destructor* and *Penthaleus major* (Acarina: Penthaleidae). Journal of Economic Entomology 93: 1183-1191.

Weeks A. R., Fripp Y. J. and Hoffmann A. A. 1995. Genetic structure of *Halotydeus destructor* and *Penthaleus major* populations in Victoria (Acari: Penthaleidae). Experimental and Applied Acarology 19: 633-646.

Weistermeier R. and Naven T. 2002. Proteomics in Practice: a laboratory manual of proteome analysis. Wiley-VCH, Darmstadt, Germany.

Welch D. M. and Meselson M. 2000. Evidence for the evolution of bdelloid rotifers without sexual reproduction or genetic exchange. Science 288: 1211-1214.

Zdebska E., Musielak M., Jaeken J. and Kościelak J. 2001. Band 3 glycoprotein and glycophorin A from erythrocytes of children with congenital disorder of glycosylation type-Ia are underglycosylated. *Proteomics* 1: 269-274.

5.7 TABLES AND FIGURES

Table 5.1: N-terminal sequence data following Edman degradation of proteins

appearing species-specific. For *P. major*, *P. falcatus* and *P. sp. x*, the polypeptides sequenced had approximate molecular weights of 66 kDa, 77 kDa and 64 kDa,

respectively. None of the sequences were found to have significant similarity to any known proteins using the standard protein-protein BLAST search. Weak homologies were identified using the search for short, nearly exact protein sequences algorithm.

The closest homologies are given along with the genus of the organism from which the protein sequence was obtained, and the statistical similarity scores (E values). Refer to Appendix 5.1 for BLAST sequence homology alignments.

<i>Species</i>	<i>N-terminal sequence data</i>	<i>Closest homologues</i>	<i>E value</i>
<i>P. major</i> (66 kDa)	AKARAAQQRAIVNA	hypothetical protein [Azotobacter]	5.2
		probable aminotransferase [Pseudomonas]	23
<i>P. falcatus</i> (77 kDa)	NRPGKPVQFAPGQ	hypothetical protein [Chloroflexus]	13
		env glycoprotein [HIV type 1]	55
<i>P. sp. x</i> (64 kDa)	NNTNIAPSKTARN	7 transmembrane receptor [Caenorhabditis]	74
		G-protein coupled receptor [Caenorhabditis]	74

Table 5.2: Sequence information of RP-HPLC-separated tryptic peptides of a *P. major* 66 kDa protein. A search for homologues within a non-redundant sequence database was made using NCBI's BLAST program. Peak numbers correspond to the peptide profile in Figure 5.9. For the peptide eluant corresponding to Peak 8, a tentative assignment of valine, alanine or serine was obtained for the first amino acid. All possibilities were examined for homologues to known sequences using the search for short, nearly exact protein sequences and the blastp algorithm. Unlike the standard protein BLAST search (blastp), some homology was identified using the search for short, nearly exact protein sequences. The closest homologue and the genus of the organism from which the protein sequence was obtained are given, along with the statistical similarity scores (E values). Refer to Appendix 5.2 for BLAST sequence homology alignments.

<i>Peak Number</i>	<i>Peptide Sequence</i>	<i>Closest Homologue</i>	<i>E value</i>
5	GPTETAVAVG	hypothetical protein [Lactobacillus]	41
8	(V)EAQPVPAPD	hypothetical protein [Pseudomonas]	2.9
	(A)EAQPVPAPD	LD24920p [Drosophila]	13
	(S)EAQPVPAPD	LD24920p [Drosophila]	13

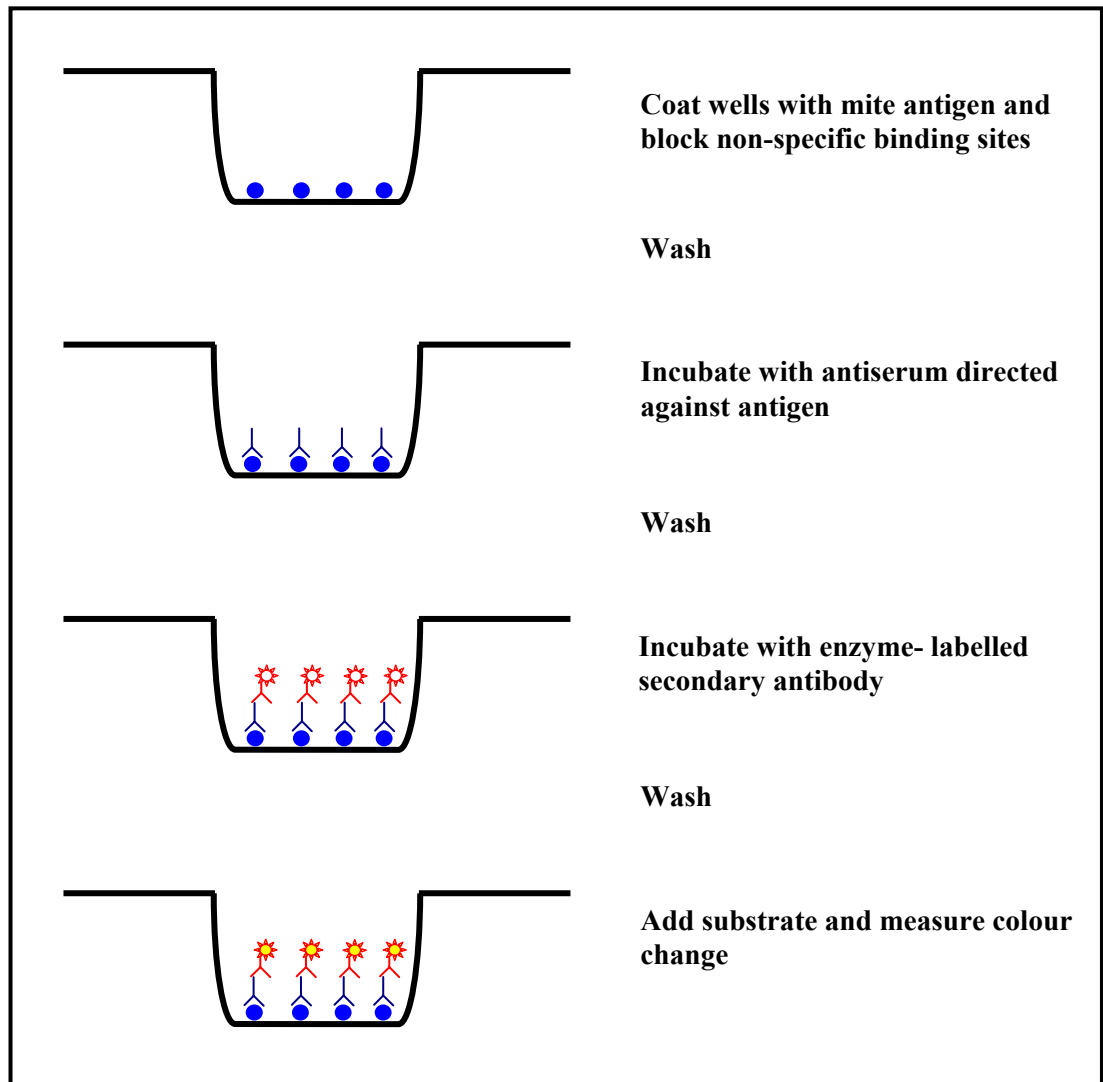


Figure 5.1: Schematic diagram of an enzyme-linked immunosorbent assay.

Antibodies react with antigen attached to the solid phase of microtitre plate wells. Any bound antibodies are detected by the addition of enzyme-labelled secondary antiserum and substrate.

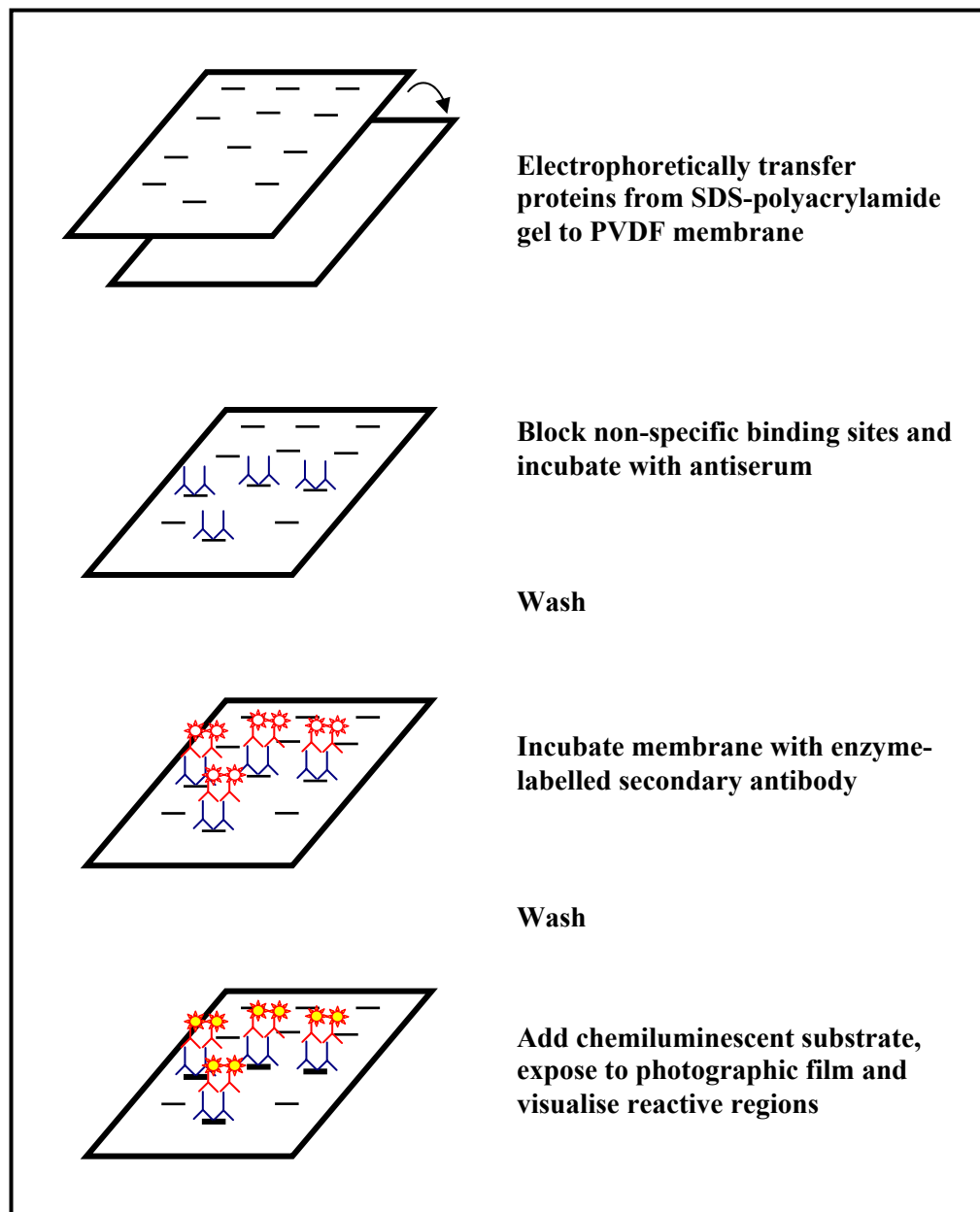


Figure 5.2: Schematic diagram of immunoblot analysis. Protein samples are separated by SDS-PAGE and transferred to PVDF. The membrane is then incubated with primary antiserum and enzyme-labelled secondary antiserum before detection by the addition of a chemiluminescent substrate and exposure to film.

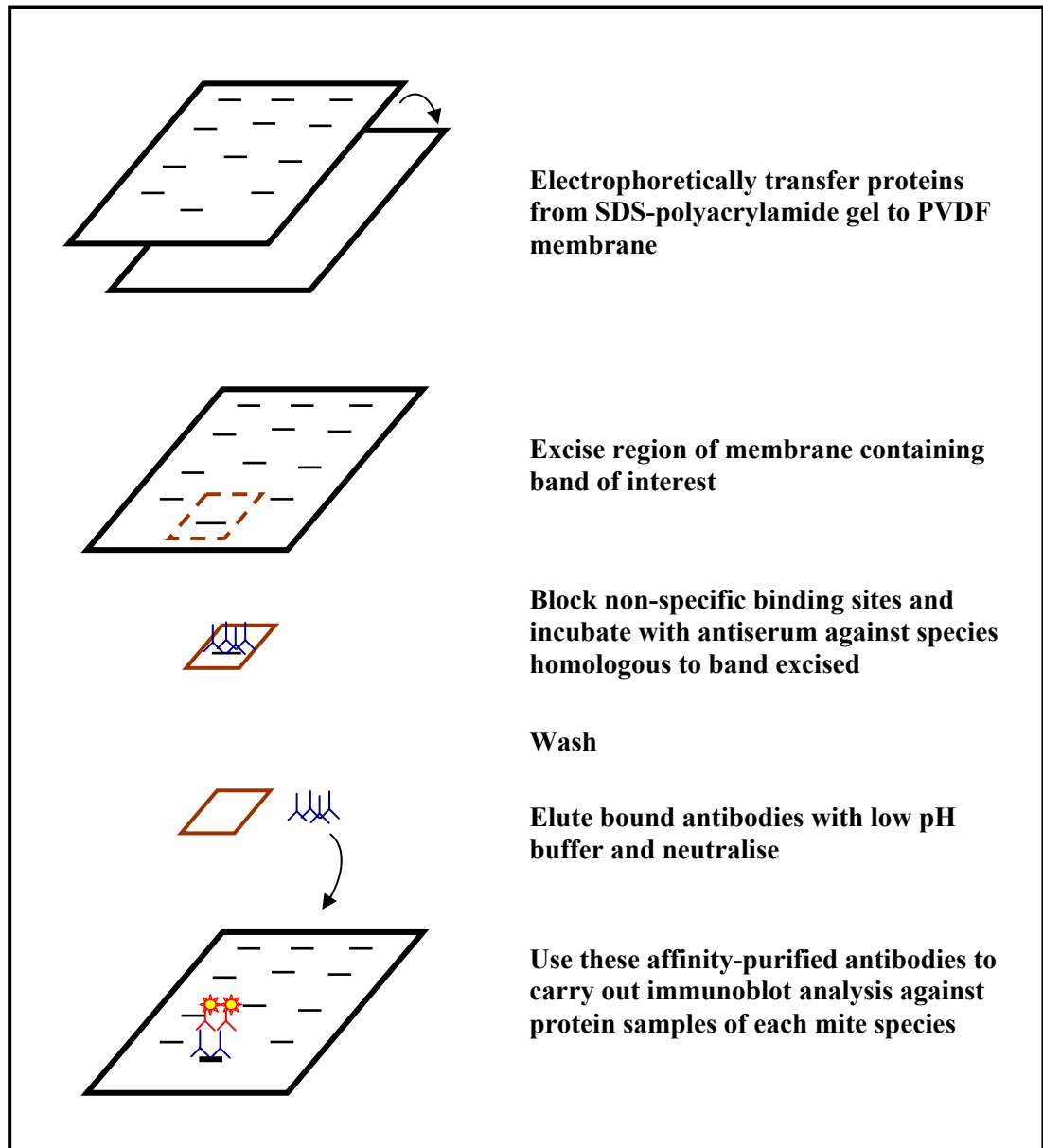


Figure 5.3: Schematic diagram of affinity purification of antibodies to a specific antigen immobilised on PVDF. Region of interest is excised from PVDF membrane and incubated with antiserum against homologous mite species. These captured species-specific antibodies are then eluted, neutralised and used in future immunoblot analyses.

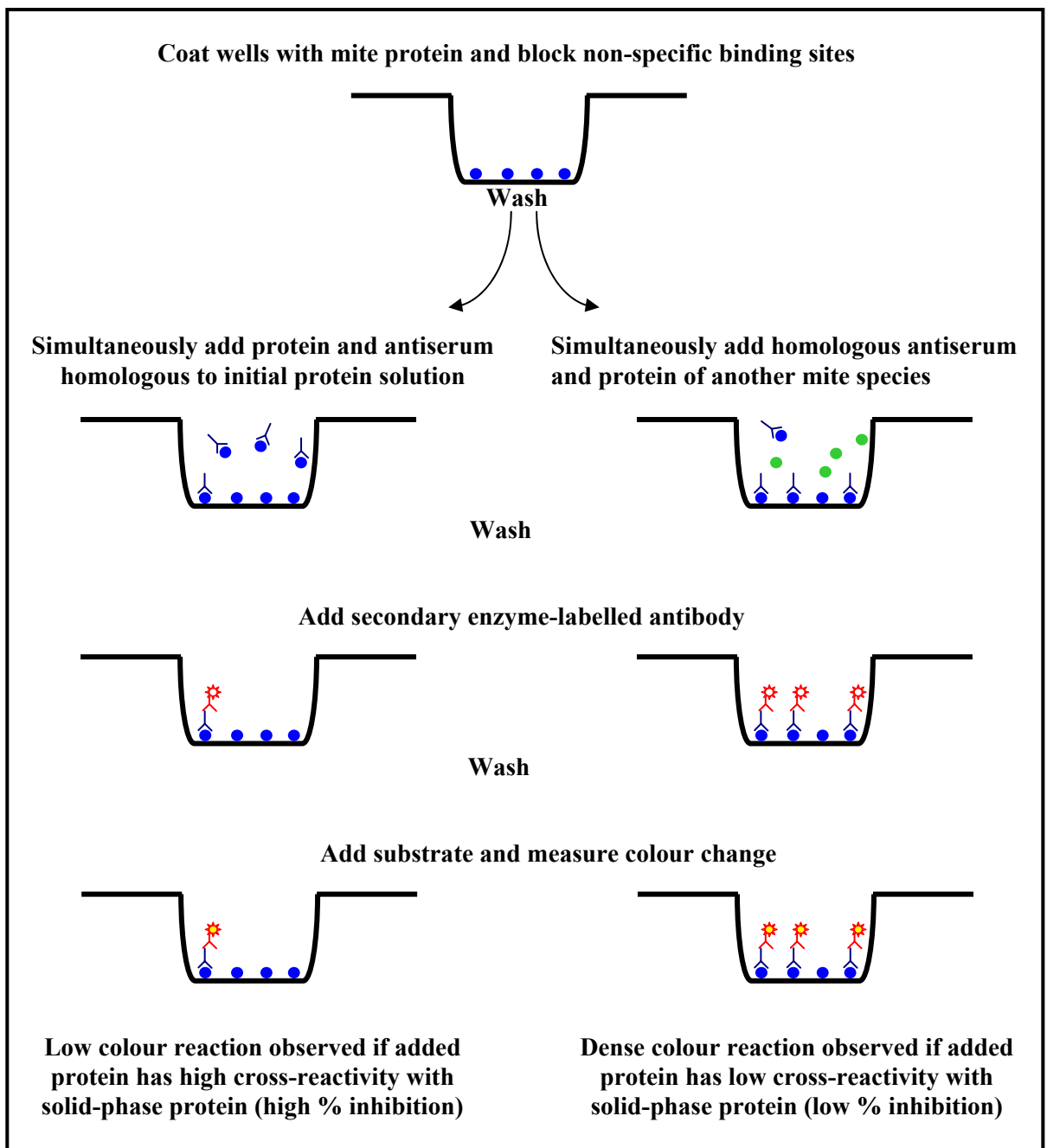


Figure 5.4: Schematic diagram of inhibition enzyme-linked immunosorbent assay. Protein extracts (homologous or heterologous) and primary antiserum are added simultaneously to wells with immobilised protein. Bound antibodies are quantified by the addition of secondary enzyme-labelled antiserum. If the proteins are cross-reactive and contain the same antigenic sites, the binding of the antiserum to the solid-phase protein will be blocked, resulting in a low colour reaction.

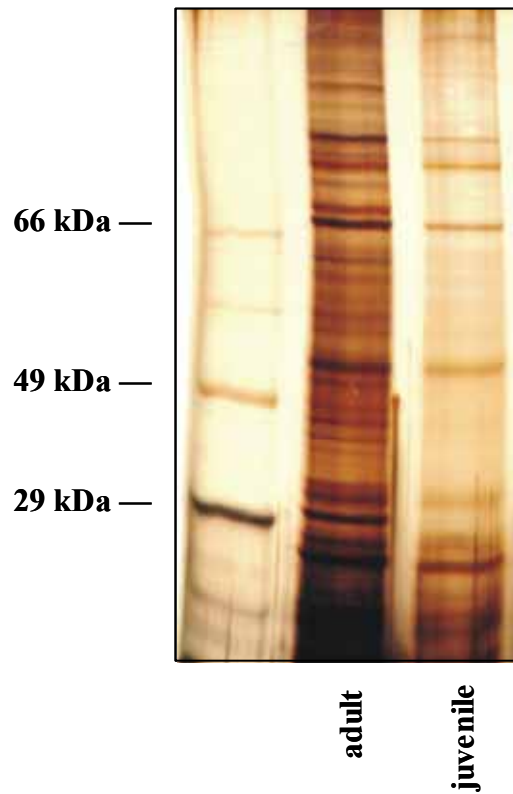


Figure 5.5: Characterisation of adult and juvenile mite extracts. Adult and juvenile *P. major* were crushed in SDS sample buffer and separated by SDS-PAGE (10% [v/v] acrylamide) along with silver stain SDS low molecular mass markers (Sigma). Proteins were visualised by silver staining.

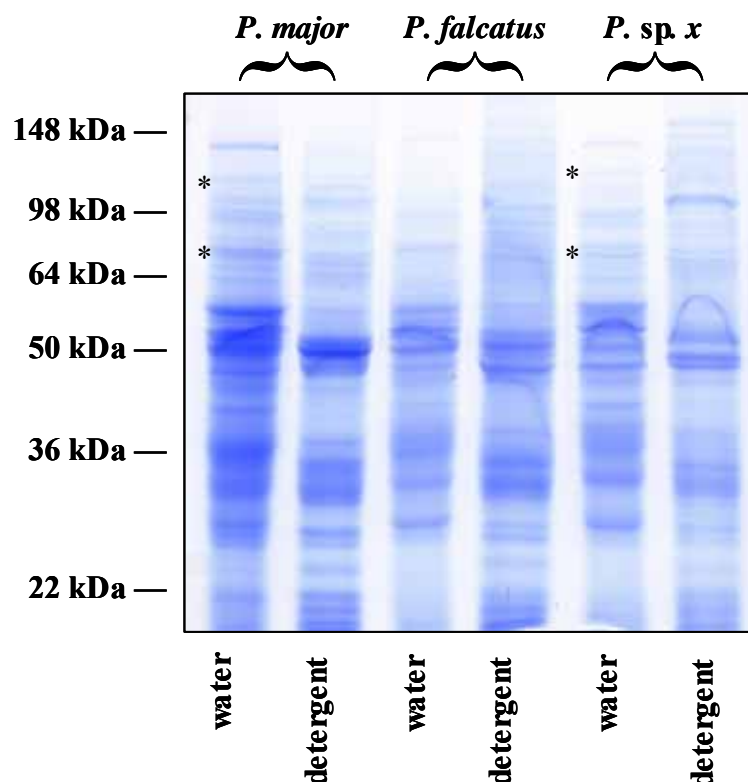


Figure 5.6: Characterisation of *Penthaleus* species extracts. Adult mites of each species were ground up in milliQ water, sonicated and centrifuged at 100 000g for 30 min. The supernatants (water-soluble proteins) were removed, while the water-insoluble proteins (pellets) were resuspended in SDS sample buffer. Detergent-soluble proteins were separated from the suspensions following centrifugation at 100 000g for 10 min. Water-soluble and water-insoluble/detergent-soluble extracts were then separated by SDS-PAGE (10% [v/v] acrylamide) along with SeeBlue[®] Plus2 prestained markers (Invitrogen Co.). Proteins were visualised after staining with Coomassie Blue. Asterisks represent two protein bands that are present in *P. major* and *P. sp. x* water-soluble fractions but not apparent in *P. falcatus*.

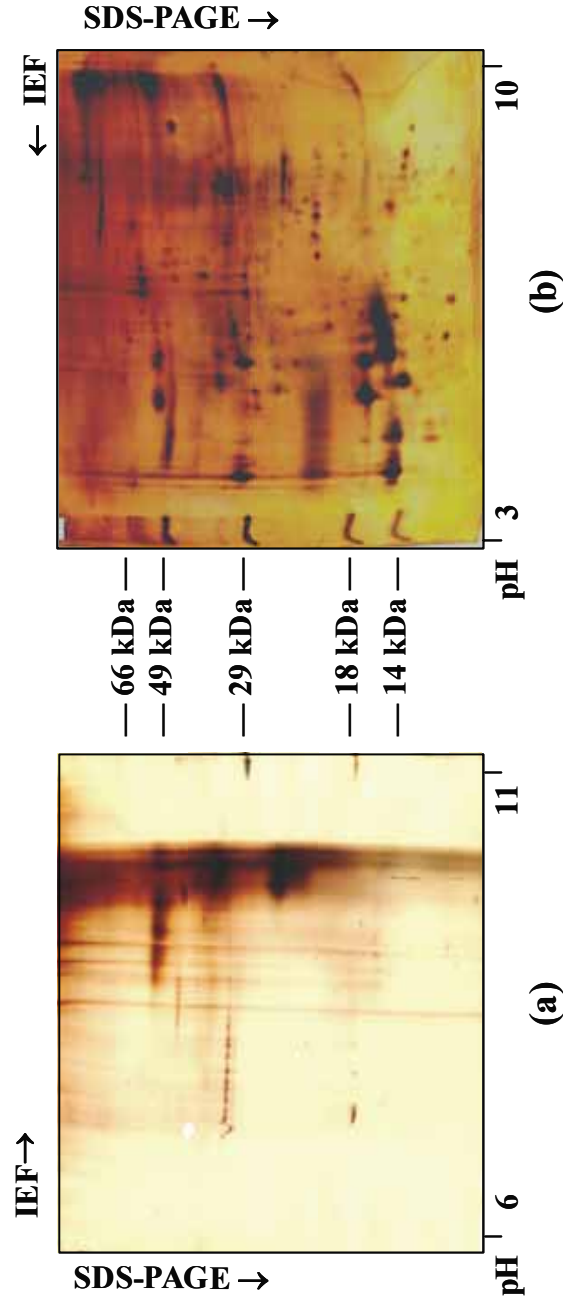


Figure 5.7: Two-dimensional SDS-PAGE of (a) water-soluble *P. major* extract and (b) *P. falciparum* (strain HB3) extract. Extracts were solubilised in 8M urea, 0.5% (v/v) IPG Buffer, 4% (w/v) CHAPS, 100mM DTT, and 10% (v/v) Triton X-100. A pH 6-11 gradient was used for the first dimension separation of *P. major* proteins (pH 3-10 used for *P. falciparum* sample). Samples were run simultaneously, with a total focusing of 62,540 Vhrs in the first dimension, and separated by SDS-PAGE (10% acrylamide) (along with SDS low molecular mass markers [Sigma]) and stained with silver.

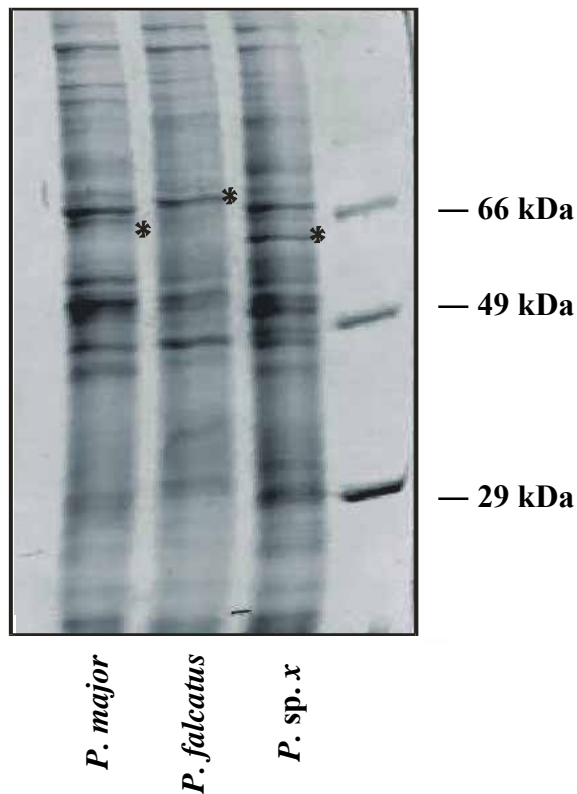


Figure 5.8: Characterisation and identification of protein bands unique to *Penthaleus* species. Detergent extracts of whole mites were separated by SDS-PAGE (7.5% [v/v] acrylamide) along with low molecular mass markers (Sigma). Proteins were visualised after staining with Coomassie Blue. Asterisks represent bands that may represent unique proteins. These bands were excised and sequenced using a gas-phase sequencer (Applied Biosystems Inc.).

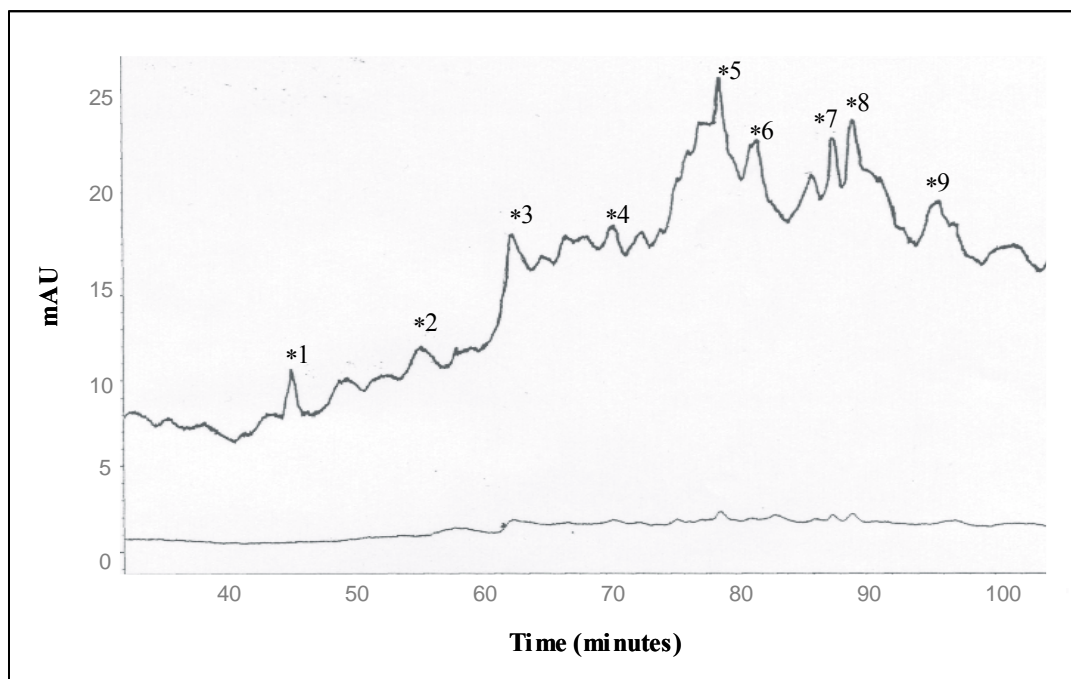


Figure 5.9: Reverse-phase HPLC profile of *P. major* tryptic peptides. A 66 kDa *P. major* polypeptide separated by SDS-PAGE was subjected to ‘in-gel’ digestion with trypsin. The resulting peptides were then separated by RP-HPLC. Asterisks represent peaks that were collected and subjected to Edman degradation to obtain N-terminal sequence information.

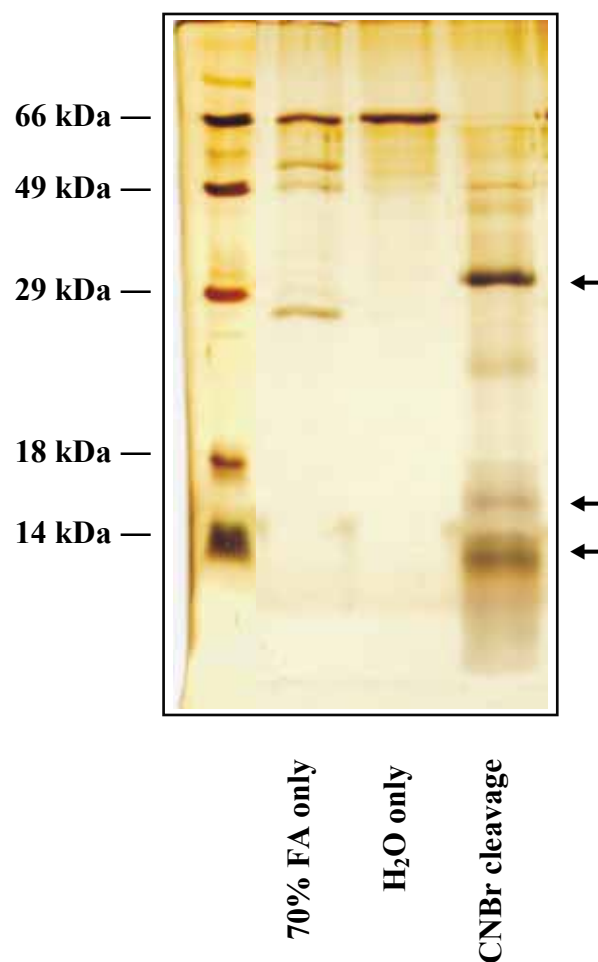


Figure 5.10: SDS-polyacrylamide gel of cleavage fragments following CNBr cleavage of a *P. major* protein. A protein band with an approximate molecular weight of 66 kDa, was excised from a gel matrix and cleaved with cyanogen bromide. Resulting fragments and controls were separated by secondary SDS-PAGE (10% [v/v] acrylamide) along with silver stain SDS low molecular mass markers (Sigma). The gel was then stained with silver. Arrows represent the cleavage fragments generated.

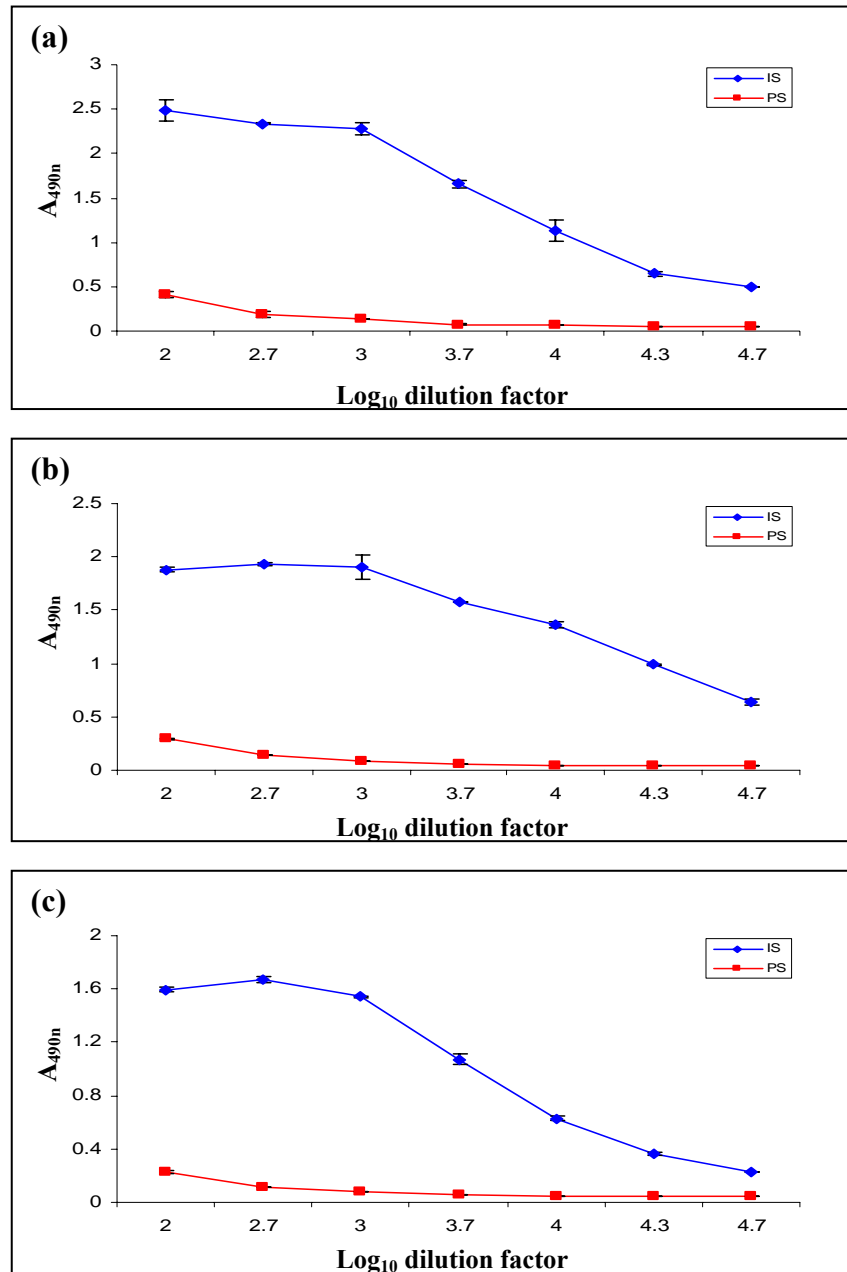


Figure 5.11: ELISA screening of anti-sera raised against (a) *P. major*, (b) *P. falcatus* and (c) *P. sp. x* in rabbit. Microtitre wells were coated with water-soluble extracts and reacted with homologous immune sera (IS). Binding of the antisera was detected with the addition of an enzyme-labelled secondary antibody, followed by OPD. Pre-immune rabbit sera (PS) was also assayed as a control and showed minimal background signal at all dilutions tested. Error bars represent 1 standard deviation.

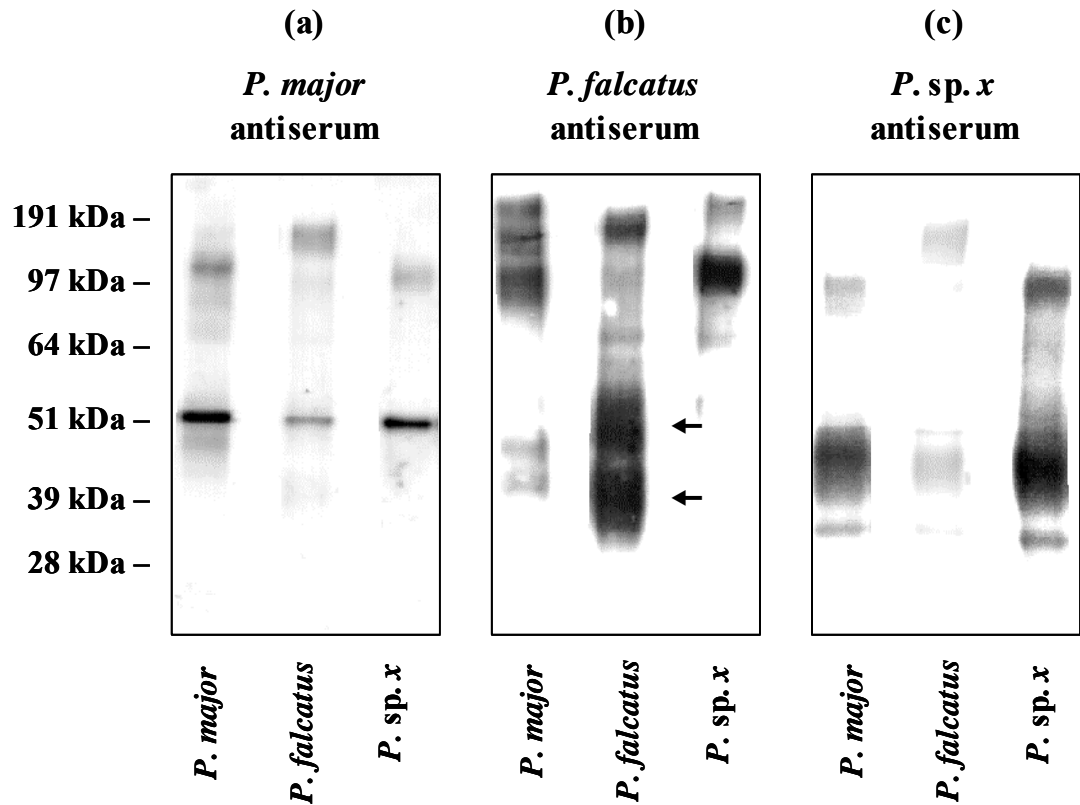


Figure 5.12: Immunoblot analysis of water-soluble extracts from *Penthaleus* species. Water-soluble extracts of each species were subjected to SDS-PAGE (4-12% NuPAGE[®]) along with SeeBlue[®] Plus2 prestained molecular mass markers (Invitrogen Co.). Proteins were then transferred to PVDF, incubated with blotto and probed with rabbit antisera generated against the three *Penthaleus* species (1/2000 dilution). Membranes were then probed with an enzyme-labelled secondary antibody, followed by chemiluminescent substrate and exposure to photographic film. Arrows indicate proteins that may be species-specific.

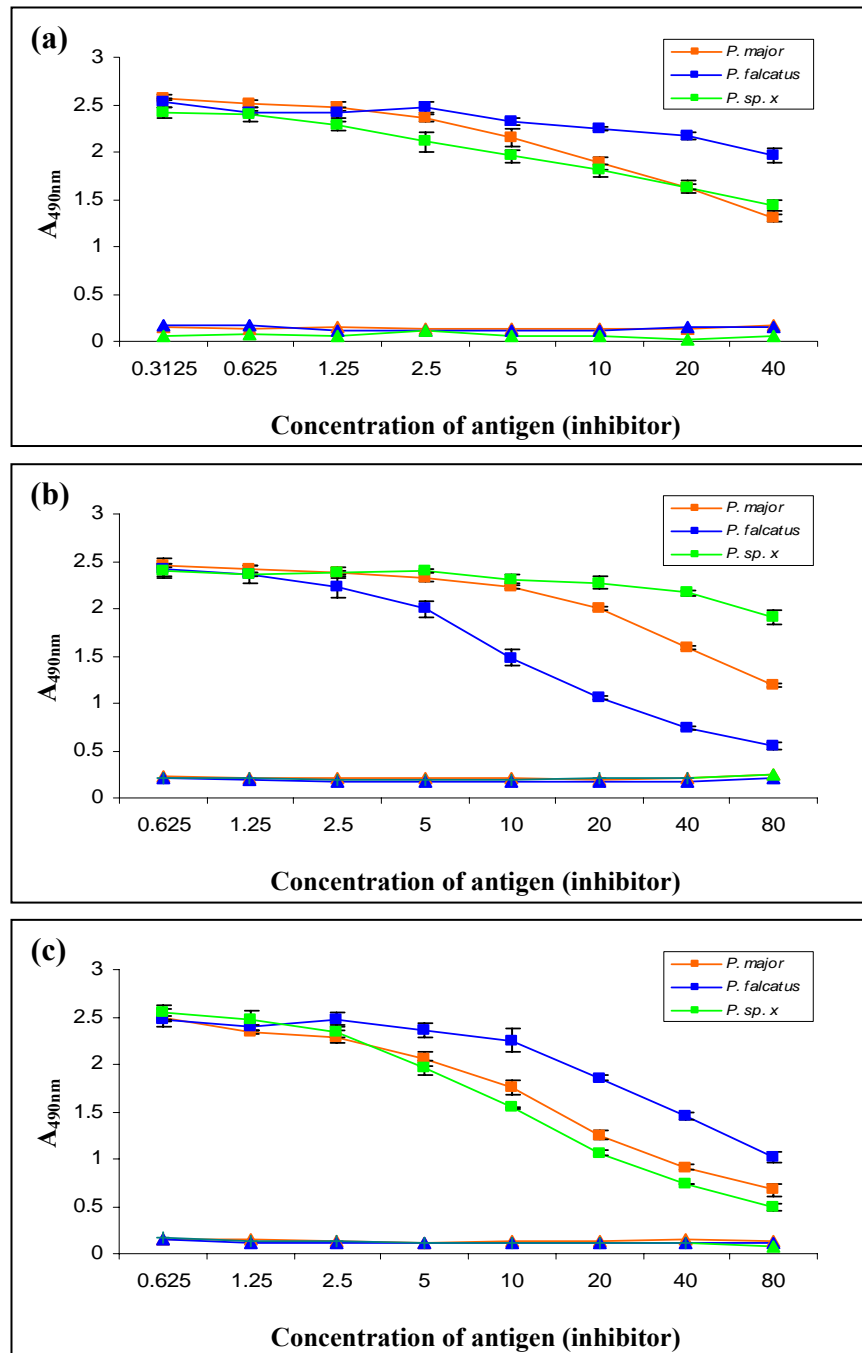


Figure 5.13: Inhibition ELISA of antisera raised against (a) *P. major*, (b) *P. falcatus* and (c) *P. sp. x*. For each assay, microtitre plates were coated with water-soluble extract of one mite species. The plates were probed with immune serum (■) against the species homologous to the initial protein coating solution, along with protein dilutions (inhibitor) of all three species. Pre-immune serum (▲) was also included as a control. Error bars represent 1 standard deviation.

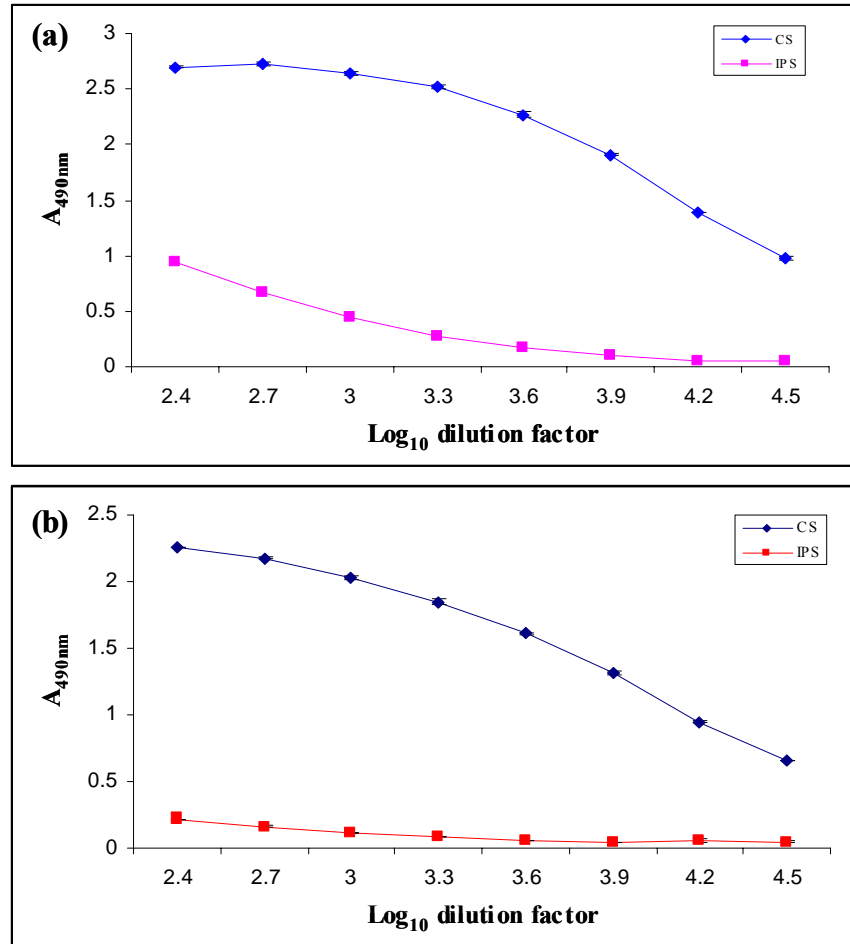


Figure 5.14: ELISA of anti-*P. falcatus* serum immuno-depleted against *P. major* antigen. Microtitre plates were coated with (a) *P. falcatus* and (b) *P. major* water-soluble proteins. Non-specific binding sites on wells were then blocked and dilutions of immuno-depleted anti-*P. falcatus* sera (IPS) added. After 1 hr incubation, the wells were washed and the bound antibodies detected using an enzyme-labelled secondary antibody and OPD substrate. Control anti-*P. falcatus* sera (prior to immuno-depletion) (CS) was also tested as a comparison. Error bars represent 1 standard deviation.

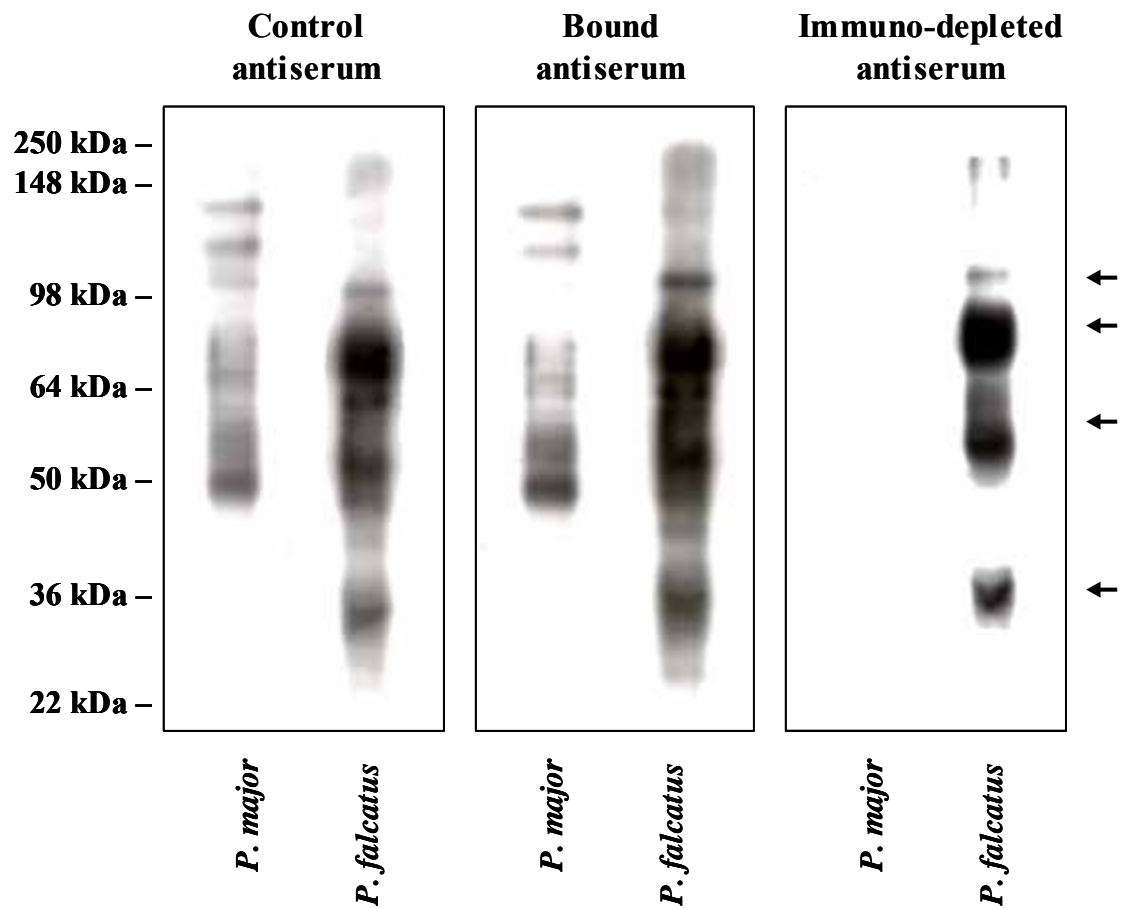


Figure 5.15: Immunoblot analysis of anti-*P. falcatus* antibodies immuno-depleted against *P. major* antigen. *Penthaleus major* and *P. falcatus* water-soluble protein extracts were subjected to SDS-PAGE (10% [v/v] acrylamide), transferred to PVDF and probed with either control anti-*P. falcatus* serum, bound 'shared' antibody fraction, or immuno-depleted *P. falcatus* antiserum. Enzyme-labelled secondary antibody and OPD substrate were then added and the blots exposed to photographic film. SeeBlue[®] Plus2 prestained markers (Invitrogen Co.) were used. Arrows indicate protein bands that are specific to *P. falcatus*.

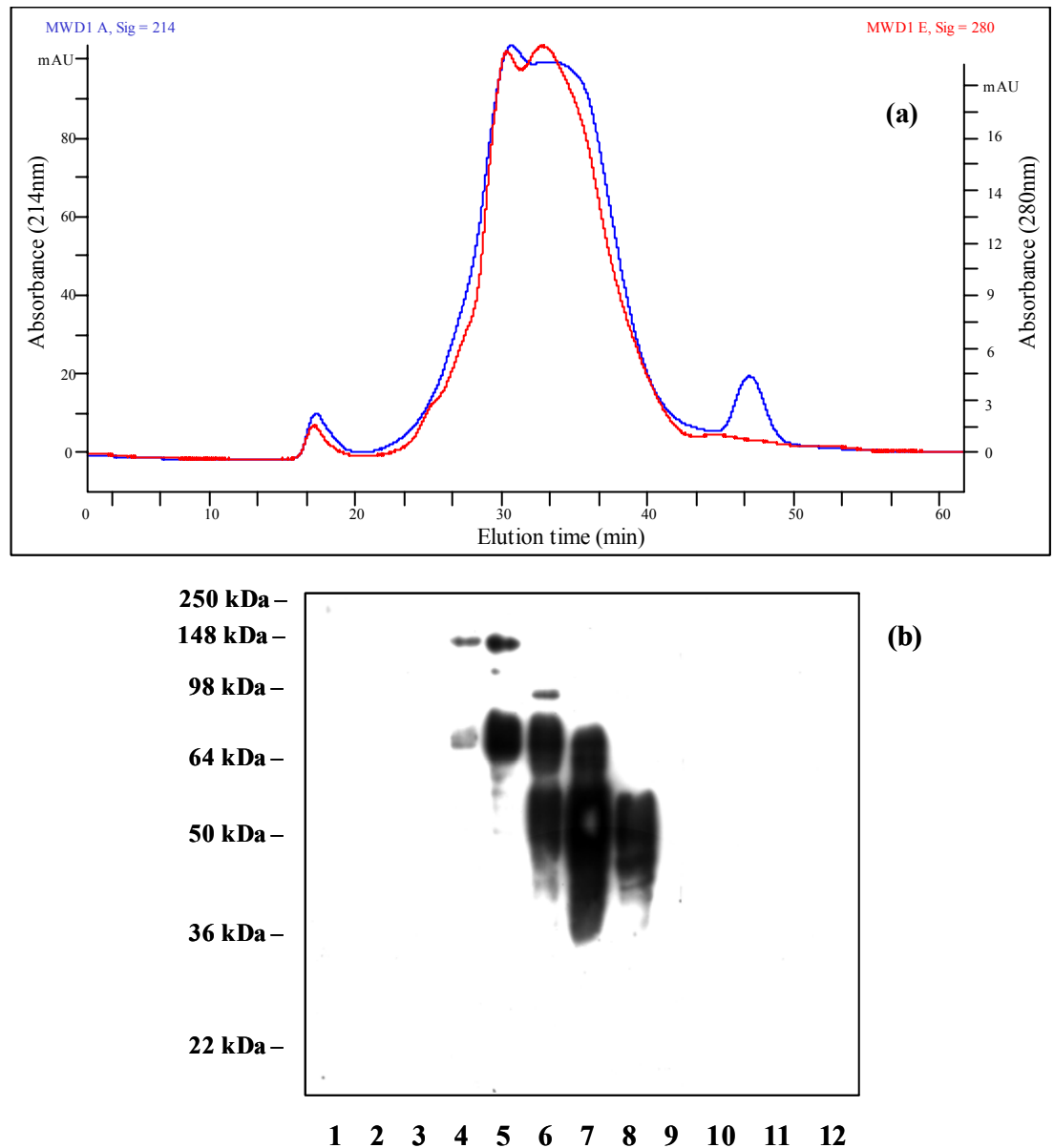


Figure 5.16: Analysis of *P. major* water-soluble proteins subjected to gel filtration.

A chromatographic profile (a) was generated after 100 μg *P. major* sample was passed through a Superose 12 (Pharmacia) gel filtration column. 2-min fractions were collected, concentrated and separated by SDS-PAGE (10% acrylamide) along with SeeBlue[®] Plus2 prestained markers (Invitrogen Co.). After transfer to PVDF, immunoblot analysis was performed with anti-*P. major* serum (b). [Lane 1=18-20 min, Lane 2=20-22 min, Lane 3=22-24 min, Lane 4=24-26 min, Lane 5=26-28 min, Lane 6=28-30 min, Lane 7= 30-32 min, Lane 8= 32-34 min, Lane 9= 34-36 min, Lane 10= 36-38 min, Lane 11= 38-40 min, and Lane 12=40-42 min fraction].

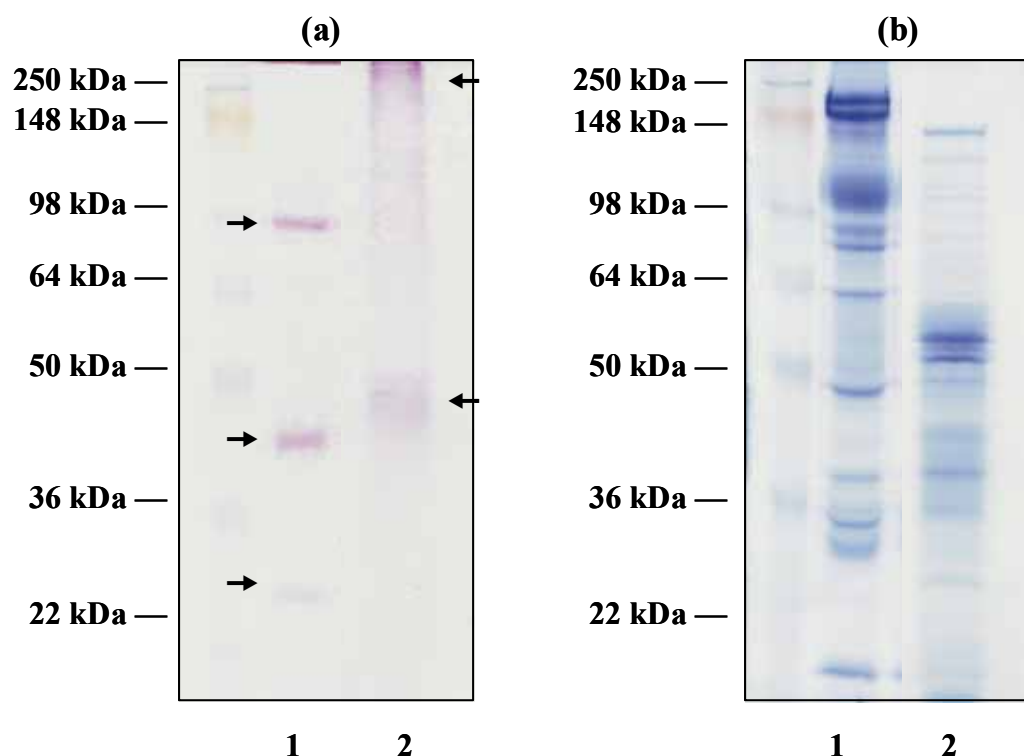


Figure 5.17: Analysis of glycosylation in mite protein extracts. Erythrocyte membrane proteins (Lane 1) and water-soluble *P. sp. x* protein extracts (Lane 2) were separated by SDS-PAGE (10% [v/v] acrylamide) and stained with (a) Periodic acid/Schiff's reagent, or (b) Coomassie Blue. The molecular weight standards used were the SeeBlue[®] Plus2 prestained markers (Invitrogen Co.). Arrows indicate protein bands visualised after staining with PAS reagent.

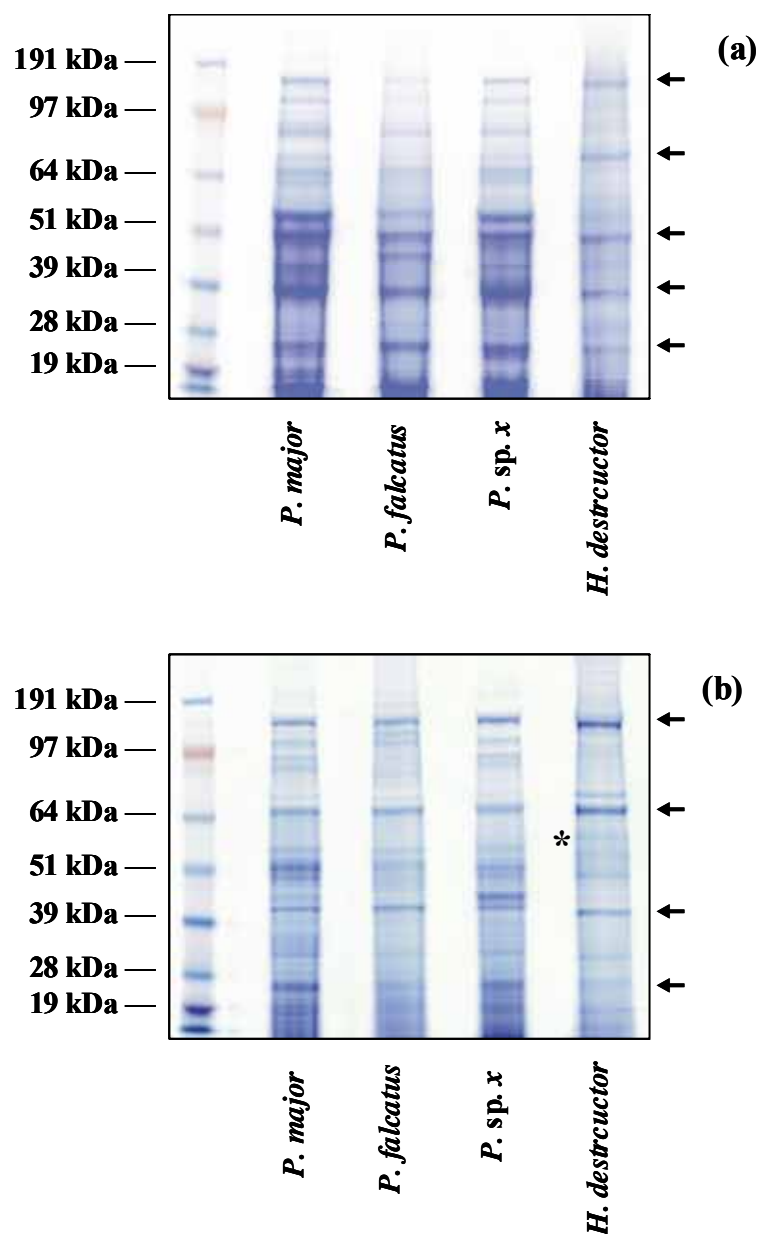


Figure 5.18: Characterisation of *H. destructor* and *Penthaleus* spp. protein extracts.

Water-soluble (a) and water-insoluble protein samples (b) were separated by SDS-PAGE (4-12% NuPAGE ®) along with SeeBlue® Plus2 prestained markers (Invitrogen Co.). Both gels were stained with Coomassie Blue. Arrows indicate abundant *H. destructor* proteins (proteins with similar molecular weights were also observed in *Penthaleus* species). The asterisk indicates a protein band that appears unique to *H. destructor*.

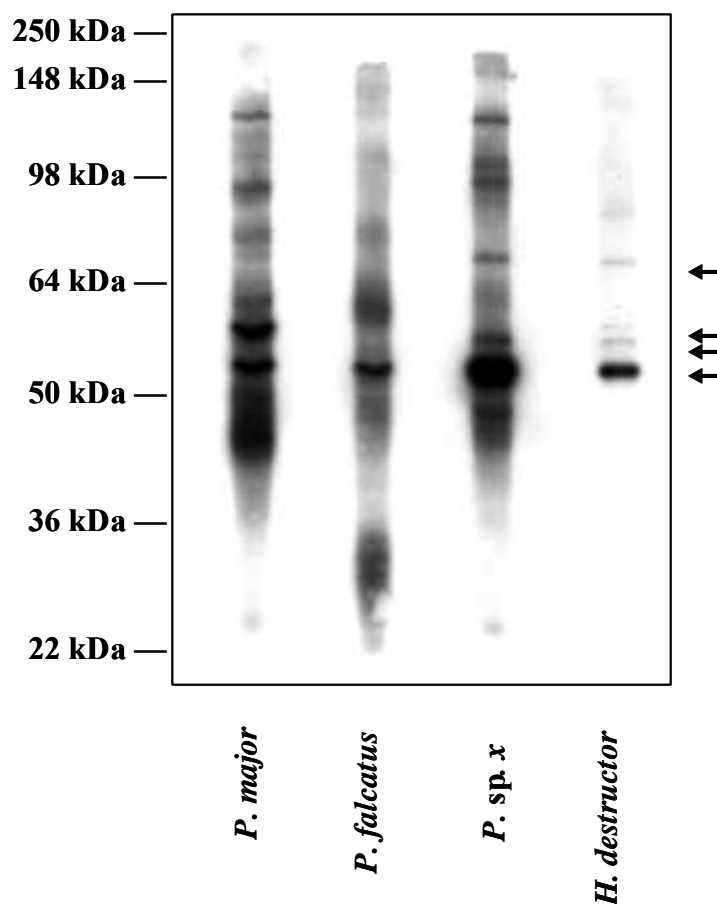


Figure 5.19: Characterisation of *H. destructor* extract by immunoblot analysis with anti-*P. major* serum. Water-soluble extracts of *Penthaleus* spp. and *H. destructor* were separated by SDS-PAGE (10% [v/v] acrylamide) along with SeeBlue[®] Plus2 prestained molecular mass markers (Invitrogen Co.). Proteins were transferred to PVDF and incubated with anti-*P. major* serum (1/1000 dilution). Enzyme-labelled secondary antibody and OPD substrate were then added and the blot exposed to photographic film. Arrows indicate major immunoreactive bands revealed in *H. destructor*.

5.8 APPENDICES

Appendix 5.1: Homology BLAST results for N-terminal amino acid sequences

P. major and *Azotobacter vinelandii* - hypothetical protein (E value = 5.2)

Query: A K A R A A Q Q R A I V N A
Match: A K A R A A + Q R A
Subject: A K A R A A E Q R A R L A E

P. major and *Pseudomonas aeruginosa* - probable aminotransferase (E value = 23)

Query: A K A R A A Q Q R A I V N A
Match: A K A R A A Q R A
Subject: A K A R A A M Q R A A A L C

P. falcatus and *Chloroflexus aurantiacus* - hypothetical protein (E value = 13)

Query: N R P G K P V Q - - F A
Match: N R P G K P + Q F A
Subject: N R P G K P I Q I I F A

P. falcatus and HIV type 1 - env glycoprotein (E value = 55)

Query: N R P G - - - - K P V Q F A P G Q
Match: R P G K + F A P G Q
Subject: S R P G N N T R K S I H F A P G Q

P. sp. x and *Caenorhabditis elegans* - 7 transmembrane receptor (E value = 74)

Query: N N T N I A P S K T A R N
Match: N N T I P S K T A
Subject: N N T T I - P S K T A T D

P. sp. x and *Caenorhabditis elegans* - G-protein coupled receptor (E value = 74)

Query: N N T N I A P S K T A R N
Match: N N T I P S K T A
Subject: N N T T I - P S K T A T D

Appendix 5.2: Homology BLAST results for internal amino acid sequences of 66

kDa *P. major* polypeptide

Chromatographic Peak 5 and *Lactobacillus gasseri* - hypothetical protein
(E value = 41)

Query: G P T E T A V A V G
Match: G P T E T V A V
Subject: G P T E T T V A V T

Chromatographic Peak 8 (with first residue valine) and *Pseudomonas fluorescens* -
hypothetical protein (E value = 2.9)

Query: V E A Q P V P A P D
Match: V E A Q P V P A D
Subject: V E A Q P V P A S D

Chromatographic Peak 8 (with first residue alanine) and *Drosophila melanogaster* -
LD24920p (E value = 13)

Query: A E A Q P V P A P D
Match: E A Q P V P A P
Subject: N E A Q P V P A P R

Chromatographic Peak 8 (with first residue serine) and *Drosophila melanogaster* -
LD24920p (E value = 13)

Query: S E A Q P V P A P D
Match: E A Q P V P A P
Subject: N E A Q P V P A P R

CHAPTER 6.

Overview and future directions

6.1 OVERVIEW

The objective of this study was to expand the knowledge of earth mites in southern Australia, in particular the *Penthaleus* spp., which are important agricultural pests causing substantial losses to the wool, meat, dairy and grain industries. Previously, little attention had been directed towards the *Penthaleus* spp. because of the misconception that they are of relatively minor economic importance within Australia (Robinson and Hoffmann 2001). A summary of the four areas of experimental work undertaken during this study is presented below. Implications for future research and questions raised during this study are also discussed.

Developing the most effective pest management programs for the control of earth mites will most likely result from the integration of a number of strategies and advances in our understanding of mite biology and ecology. The initiation of diapause egg production in earth mites was examined in Chapter 2 using field and shade-house experiments. This was stimulated in part by the recent development and implementation of Timerite[®], an effective control method based on the optimum spring spraying date for *H. destructor*. This approach is based on years of research and the development of a model that predicts the date in spring when *H. destructor* start to produce diapause eggs (Ridsdill-Smith and Pavri 2000a). By examining the diapause characteristics of all earth mite species, I was able to determine the effectiveness of a carefully timed spring spraying regime for the control of each species. Experiments were conducted across a number of years and at several locations to account for temporal and spatial environmental variation.

The results indicated that each species differed in their timing of diapause. In agreement with previous findings, *H. destructor* mainly produced diapausing eggs towards the end of the active mite season in spring (Annells and Ridsdill-Smith 1991; James and O'Malley 1993), although small numbers were also produced in winter. In contrast, *P. major* produced diapause eggs almost immediately after emergence in autumn and continued producing these eggs throughout the season. *Penthaleus falcatus* also produced diapause eggs in early winter, although the first appearance of these eggs occurred somewhat later in the season than for *P. major*. The diapause response of *P. sp. x* was also later than in *P. major* and *P. falcatus*, but earlier than in *H. destructor*. This indicates that Timerite[®] will not be effective in the control of *Penthaleus* spp., providing further support for the argument that identifying mite species before the implementation of control strategies is critical (Umina *et al.* 2001). Spraying *Penthaleus* spp. within two to three weeks of emergence in autumn could be the most effective means of reducing the impact of these pests, although further research is needed to assess this approach. The production of diapause eggs throughout the mite season is likely to reduce population growth, and may be responsible for the relative abundance of *Penthaleus* spp. increasing in early spring to a lesser extent than *H. destructor* (Weeks and Hoffmann 2000). Differences in diapause strategies may also contribute to the different distributions of earth mite species within southeastern Australia (Robinson and Hoffmann 2001).

Although this study focused on the diapause strategies of the different species, there is also evidence for diapause variation within species. Electrophoresis of *P. major* samples emerging from shade-house tubs indicated that clones may differ in the timing

of diapause egg production. This provides another potential selective factor contributing to the maintenance of clonal diversity within and among populations (Weeks and Hoffmann 1998; Umina and Hoffmann 1999). To clarify the potential effect of variation on diapause differences, experiments should ideally be performed for pure cultures of the clones. Weeks and Hoffmann (1998) provided evidence of spatial and temporal differences in the clonal distribution of *P. major*. The data collected here provide additional evidence for marked differences in clonal distributions.

Different pesticide tolerance levels in *Penthaleus* spp. and evidence of resistance in some *H. destructor* populations (Hoffmann *et al.* 1997; Umina and Hoffmann 1999) indicate the need for alternative control practices that do not rely solely on chemicals. In Chapter 3, I investigated the effects of different plant hosts on the persistence and reproduction of earth mites, which could be important for the development of environmentally friendly and sustainable control strategies, such as crop rotations. I considered canola, wheat and oats, pasture, lentils, and the common weed ‘bristly ox-tongue’ under shade-house and field conditions. All species survived and reproduced from one mite season to the next when confined to pasture. In contrast, lentils were an unsuitable host plant for all mite species. Canola and bristly ox-tongue were suitable hosts for *H. destructor* and *P. falcatus*, whereas *P. sp. x* and *P. major* failed to persist on these plants. *Penthaleus sp. x* and *H. destructor* persisted on a mixture of wheat and oats, while the data for *P. falcatus* and *P. major* were inconclusive. My results suggest that wheat and oats may not successfully sustain *P. falcatus* or *P. major*, although under field conditions the presence of microflora and weeds may permit long-term survival on these crops (MacIennan *et al.* 1998; Ridsdill-Smith and Pavri 2000b). The

presence of a fourth cryptic species closely resembling *P. falcatus* (see Chapter 1) may have also complicated previous interpretations.

Information was also obtained regarding the plant host preferences of mite species throughout Victoria and southern New South Wales. Field samples were randomly taken from crops, pasture paddocks and along roadsides, and later identified to the species level. There were significant differences in the plants attacked among mite species and these generally corresponded to the plant host data collected from the shade-house and field experiments. For example, *P. falcatus* and *H. destructor* survived on canola and ox-tongue in the plant host experiments and were also the most predominant species in collections from these plants in the field survey. In two-thirds of all collections made, *Penthaleus* spp. were the most predominant earth mite species. This, combined with pest status information collected over a number of seasons by Robinson and Hoffmann (2001), highlights the importance of *Penthaleus* spp. as agricultural pests and argues against the common belief that *H. destructor* is the most abundant and economically significant earth mite species in southeastern Australia.

Crop rotations have been used for years to suppress a variety of agricultural pests throughout the world. In Europe, typical rotations that involve grasses, legume and root crops have proven successful in the control of wireworms, chafers and leatherjackets (Coaker 1987). Information collected in this study suggests preliminary recommendations for the control of the different mite species, although without a simple method of distinguishing *Penthaleus* species, the number of ‘workable pest management strategies’ is limited. For example, if blue oat mites are recognised as the main pests on canola (indicating *P. falcatus*) farmers should consider rotating crops

with lentils and remain diligent about weed control. When blue oat mites are found attacking wheat or oats (indicating *P. sp. x*), rotations that involve any non-cereal crops are recommended. If blue oat mites (and/or *H. destructor*) are present in high numbers within pastures (suggesting *P. major*) it is advisable to rotate the paddock with canola, lentils, lupins, or chickpeas, while pastures containing a high clover content should be carefully monitored and rotated with crops in regions where *H. destructor* is problematic. There are various other crops not examined in this study that may be useful in rotation programs and better suited to particular regions or cropping situations.

There is now strong evidence demonstrating marked differences among mite species with regards to diapause strategies, plant hosts, distributions and response to pesticides (Chapters 2 & 3; Umina and Hoffmann 1999; Robinson and Hoffmann 2001).

Therefore, control strategies based on one species may not be adequate for other species of mites. Moreover, if mite species compete with one another for resources, control attempts that suppress or eradicate one species may not necessarily lead to a reduction in pest pressure because another species would likely increase in abundance. To consider this scenario, I examined competitive interactions among the three *Penthaleus* spp. and *H. destructor*, using four different plant treatments: pasture; canola; bristly ox-tongue; and a mixture of wheat and oats.

Results showed that both intra- and interspecific competition occurs in all species. The intensity, frequency, and type of competition also varied with plant type and from year-to-year, providing strong evidence that competitive interactions influence the population dynamics of earth mites. Intraspecific effects were generally stronger than

interspecific effects, supporting the hypothesis of Denno *et al.* (1995) for phytophagous organisms. On pasture, the competitive advantage swayed between *P. major*, *H. destructor*, and *P. falcatus*. *Penthaleus* sp. *x* and *H. destructor* were the two strongest competitors in a mixture of wheat and oats, while in canola and bristly ox-tongue treatments, *P. falcatus* and *H. destructor* were the superior competitors. These results again highlight the importance of identifying mite species when considering control options and suggest that effective control recommendations need to be developed for individual species. When looking at larval competition in *Aphidius smithi* and *Praon pequodorum*, Chow and Mackauer (1984) showed competition could have long-term consequences on the size and structure of parasite guilds and, hence, the success of species as biological control agents. There are numerous other studies highlighting the importance of examining competitive interactions in agricultural pest species, such as the brown citrus aphid (Persad and Hoy 2003), the spruce beetle (Poland and Borden 1998), blowflies (Prinkkilä and Hanski 1995), and several aphid parasitoids (Völkl and Stadler 1991).

For the successful development and implementation of control strategies for each mite species, farmers require a method of identifying the different species. *Penthaleus* spp. and *H. destructor* can be distinguished in the field quite easily by differences in body colour and pattern. Unfortunately, these species are rarely differentiated, adding to the misconception that *Penthaleus* spp. are of less importance than *H. destructor* throughout southern Australia. The three *Penthaleus* spp., however, can only be distinguished morphologically by an experienced researcher or by genetic markers, which require skill and access to equipment not widely available. In this study, I

examined the protein profiles of *Penthaleus* spp. with the aim of identifying species-specific proteins for use in the development of a field-based species identification kit.

Various techniques were employed to identify and isolate species-specific mite proteins. My studies provided information about the complex nature of the *Penthaleus* group and I found that the three species are remarkably similar, possibly a consequence of the ancestral lineage of this group which likely evolved from a single clonal genotype. Using immunological techniques, antibodies specific to *P. falcatus* were isolated and four candidate polypeptide bands were identified. It would be worthwhile expanding on these methods, not only for the isolation and characterisation of these proteins, but also to identify candidate proteins from *P. major* and *P. sp. x*. Some preliminary experiments were also performed analysing protein extracts of *H. destructor*. Surprisingly, they were not vastly different from *Penthaleus* spp. extracts, however, further analysis is needed, ideally using antibodies raised against *H. destructor* proteins. The commercialisation and widespread use of similar tests has improved management of many agricultural pests within Australia and the world (Trowell *et al.* 1992; Ng *et al.* 1998). Often the benefits are two-fold. There are immediate cost savings through more selective pesticide use, as well as the additional long-term advantage of being equipped to better manage pesticide resistance (Trowell *et al.* 2000).

6.2 FUTURE DIRECTIONS

This study has answered a number of questions relating to the biology and molecular analysis of earth mite species in southeastern Australia and provides a basis for developing integrated management practices. The finding that the three *Penthaleus* spp. have different plant hosts and diapause strategies, and that all species compete with one another for resources, highlights the importance of collecting adequate biological information about pest species. Without this type of information, it is difficult to develop adequate control strategies for the management of pests, particularly in the context of more sustainable and environmentally acceptable means of control.

There are several directions for future research. Because of the large-scale adoption of Timerite[®] throughout southern Australia, it would be useful to investigate the effects of spring spraying on other mites, including blue oat mites. A primary concern is that *Penthaleus* spp. and other pests could fill the niche left by the Timerite[®] strategy against *H. destructor*. In Western Australia, pests such as cockchafer, slugs and moth larvae appear to be increasing and causing damage to pasture environments (see Western Australian Agricultural pest reports 2003, www.agric.wa.gov.au/). To investigate these effects, simple field experiments could be conducted by spraying field plots according to Timerite[®] specifications and leaving others unsprayed. Monitoring sites where Timerite[®] application has occurred and comparing these with control sites would also provide invaluable information on the impact to non-target invertebrates that supply a functional role in pastoral ecosystems.

It would also be interesting to examine the effect of repeated years of spring spraying on *H. destructor*. My results suggest that continued exposure of *H. destructor* to Timerite[®] may result in populations evolving to alter their diapause strategy, more closely resembling that of the *Penthaleus* species. Given the known resistance problems in earth mites (Hoffmann *et al.* 1997), it would be worthwhile establishing a monitoring strategy in areas where there is widespread use of Timerite[®]. Field plots would enable these potential effects to be examined. Similarly, monitoring sites that have been using Timerite[®] since its inception and comparing these with control sites that have never used the Timerite[®] strategy is essential to detect any resistance and, if necessary, implement management strategies to prolong the effectiveness of this approach.

Additionally, knowledge on the diapause strategy of *P. sp. x* is currently lacking. Multiple sites across several years need to be examined for this species to gain a better understanding of the timing of diapause egg production. *Penthaleus sp. x* also emerges from diapause in autumn, considerably later than both *P. major* and *P. falcatus* (unpublished data). This has consequences for the population dynamics of *P. sp. x* and requires further investigation, as greater knowledge should assist in the development of improved management strategies. Moreover, information about *P. sp. x* from northern New South Wales, where there is a disjunct population of this species (Robinson and Hoffmann 2001), has yet to be obtained. Comparisons between these two areas could help to determine why this species has such restricted distributions.

My research suggests that clones of *P. major* differ in their diapausing strategies.

Weeks and Hoffmann (1998) showed *P. major* clones differ ecologically, while Umina

and Hoffmann (1999) found that clones of *P. major* and *P. falcatus* respond differently to pesticides. Clones from each *Penthaleus* species might also differ in their competitive abilities or performance on different plant hosts. Because of these clonal differences, the effectiveness of control strategies may depend on the clonal composition of a population as well as the species present. More data on the ecological differences among clones and how these differences will relate to control strategies would be beneficial.

Further research is also needed on plant host selection, with the aim of identifying additional crops unfavourable to all species for potential crop rotation practices. In this study, lentil was the only plant type that was a poor host against all species. In many farming situations, rotation practices involving this crop may not be suitable, therefore, alternative options need to be explored. Other pulse species, such as lupins, beans and chickpeas, have varying levels of mite feeding resistance (Liu and Ridsdill-Smith 2000) and should be examined for their suitability as hosts for each of the blue oat mite species. The fact that mite species differ considerably in their host plants suggests differences in their ability to overcome chemical and/or physical mechanisms of plant resistance. Therefore, screening programs aimed at developing resistant plant varieties to *H. destructor* (eg. Gillespie 1991; Gillespie 1993; Ridsdill-Smith *et al.* 1995; Weinman *et al.* 1995) may not necessarily reduce feeding damage by *Penthaleus* species. Future work in these areas should consider all mite species.

There is still some confusion over the host suitability of wheat and oats for earth mites. While *H. destructor* and *P. sp. x* can persist on these plant types, the results for *P. major* and *P. falcatus* are unclear. This may in part be related to the microclimate,

microflora on the soil surface, and/or the presence of weeds (MacIennan *et al.* 1998; Ridsdill-Smith and Pavri 2000b; Chapter 3). Field-based experiments are needed to determine the host suitability of oats and wheat separately, as well as quantifying the effects of weeds and microflora on the long-term persistence of mite species. A simple experiment might involve a similar experimental set-up to that at Yeungroon, involving field plots, but with half the plots sown with oats and half with wheat. By selectively applying herbicides and fungicides, some of the plots could be controlled for weeds, some for microflora, and some for weeds and microflora. There should also be some unsprayed 'control' plots. Mite sampling would ideally be conducted throughout the active mite season and at the beginning of the following season.

Experiments are also needed to confirm that results from the plant host experiments were not confounded by prior exposure of mites to certain plant types. A shortfall in the methodology used is that mites were collected from different hosts before release. This is an aspect of experimental work that plagues researchers attempts to use field collected material for trial work. It would be worthwhile examining whether there are any gross differences, most likely in the short-term survival, between mites collected from different hosts and then maintained under the same conditions in the shade-house. Improvements could also be made to the plant host and competition experiments by altering the sampling regime. My work showed that each mite species peak at different times of the year. Therefore, repeating the experiments, using this information so that samples are taken to accurately represent these periods (rather than a single point in time for all species), would augment the findings of this thesis.

My research has highlighted the importance of the three *Penthaleus* spp. in southeastern Australia and the need to consider each earth mite species separately. However, little is currently known about the composition and distribution of *Penthaleus* spp. in Western Australia. Only the presence of *P. major* has been confirmed for Western Australia (Qin and Halliday 1996). However, because *P. falcatus* occupies a similar distribution in southeastern Australia and are likely to be limited by the same environmental factors, this species may also occur in that state. Extensive sampling is needed to examine the distribution of *P. falcatus* and *P. sp. x*, particularly given the implications for control management as outlined in this study.

Lastly, molecular analysis of mite extracts identified several promising avenues that merit further investigation for the isolation and characterisation of potential candidate proteins for use in a diagnostic kit. Because most farmers consult their agronomists before they attempt to control each mite outbreak, more efficient control could be obtained at this point if the species responsible for the outbreak could be identified and the appropriate control strategy recommended. This would be particularly important when dealing with chemical control failures and devising pest management strategies. This is a realistic approach as the vast majority of mite outbreaks are dominated by a single species (Robinson and Hoffmann 2001).

Several key directions for the molecular work have been mentioned. These are mainly proteomics and immunological based approaches. To obtain more information on the nature of glycosylation in mite samples, lectin affinity chromatography could be used. This would allow the rapid separation and analysis of any glycoproteins. Alternatively, mite proteins could be incubated with an endoglycosidase and used to generate

polyclonal antiserum for future studies. Two-dimensional electrophoresis is another avenue that could be explored further, with the aim of identifying and sequencing specific protein spots. Immuno-depletion studies revealed several candidate proteins in *P. falcatus* and were used to generate a relatively species-specific polyclonal reagent. Expansion of these techniques should be pursued, not only for the isolation of these proteins, but for the identification and isolation of proteins specific to *P. major* and *P. sp. x*. Once identified, the production of recombinant peptides and monoclonal antibodies to those proteins found to be species-specific would be essential.

The development of a diagnostic kit, combined with a thorough understanding of mite biology and ecology, is necessary for maximum benefit to eventuate from this research and for enabling the most cost effective and sustainable control options to be implemented.

6.3 REFERENCES CITED

- Annels A. J. and Ridsdill-Smith T. J. 1991. The effect of moisture on aestivating eggs of *Halotydeus destructor* (Tucker) (Acari: Pentheleidae). *In*: Ridsdill-Smith T.J. (ed.), Proceedings of a National Workshop on Redlegged Earth Mite, Lucerne Flea and Blue Oat Mite. Department of Agriculture, Perth, Australia, pp. 7-9.
- Chow F. J. and Mackauer M. 1984. Inter- and intraspecific larval competition in *Aphidius smithi* and *Praon pequodorum* (Hymenoptera: Aphidiidae). *Canadian Entomologist* 116: 1097-1107.
- Coaker T. H. 1987. Cultural methods: the crop. *In*: Burn A.J., Coaker T.H. and P.C. Jepson (eds.), Integrated pest management. Academic Press, London, UK, pp. 69-88.
- Denno R. F., McClure M. S. and Ott J. R. 1995. Interspecific interactions in phytophagous insects: competition reexamined and resurrected. *Annual Review of Entomology* 40: 297-331.
- Gillespie D. J. 1991. Identification of resistance to redlegged earth mite *Halotydeus destructor* in pasture legumes. *Plant Protection Quarterly* 6: 170-171.
- Gillespie D. J. 1993. Redlegged earth mite (*Halotydeus destructor*) resistance in annual pasture legumes. *In*: Delfosse E. (ed.), Pests of pastures: weed, invertebrate and disease pests of Australian sheep pastures. CSIRO, Melbourne, Australia, pp. 211-213.

Hoffmann A. A., Porter S. and Kovacs I. 1997. The response of the major crop and pasture pest, the red-legged earth mite (*Halotydeus destructor*) to pesticides: dose-response curves and evidence for tolerance. *Experimental and Applied Acarology* 21: 151-162.

James D. G. and O'Malley K. J. 1993. Phenology of egg production and diapause in *Halotydeus destructor* Tucker and *Penthaleus major* Dugés (Acari: Penthaleidae) in southern New South Wales during 1988/89. *General and Applied Entomology* 24: 33-38.

Liu A. and Ridsdill-Smith T. J. 2000. Feeding by redlegged earth mite (*Halotydeus destructor*) on seedlings influences subsequent plant performance of different pulse crops. *Australian Journal of Experimental Agriculture* 40: 715-723.

MacLennan K. E., McDonald G. and Ward S. A. 1998. Soil microflora as hosts of redlegged earth mite (*Halotydeus destructor*). *Entomologia Experimentalis et Applicata* 86: 319-323.

Ng S. S., Cibulsky R. J., and Trowell S. C. 1998. LepTon HTK – a heliothine diagnostic kit: an update. *In*: Dugger P. and D. Richter (eds.), *Proceedings of the 1998 Beltwide Cotton Conference*. Cotton Council of America, Memphis, America, pp. 1040-1043.

Persad A. B. and Hoy M. A. 2003. Intra- and interspecific interactions between *Lysiphlebus testaceipes* and *Lipolexis scutellaris* (Hymenoptera: Aphidiidae) reared on

Toxoptera citricida (Homoptera: Aphididae). Journal of Economic Entomology 96: 564-569.

Poland T. M. and Borden J. H. 1998. Competitive exclusion of *Dendroctonus rufipennis* induced by pheromones of *Ips tridens* and *Dryocoetes affaber* (Coleoptera: Scolytidae). Journal of Economic Entomology 91: 1150-1161.

Prinkkilä M. and Hanski I. 1995. Complex competitive interactions in four species of *Lucilia* blowflies. Ecological Entomology 20: 261-272.

Qin T. K. and Halliday R. B. 1996. The Australian species of *Chromotydeus* Berlese and *Penthaleus* Koch, C.L. (Acarina, Penthaleidae). Journal of Natural History 30: 1833-1848.

Ridsdill-Smith T. J. and Pavri C. 2000a. Single spring spray protects pastures. Farming Ahead 103: 60-63.

Ridsdill-Smith T. J. and Pavri C.C. 2000b. Feeding life style of redlegged earth mite, *Halotydeus destructor* (Acari: Penthaleidae), in pastures and the role of broad-leaved weeds. Experimental and Applied Acarology 24: 397-414.

Ridsdill-Smith T. J., Jiang Y. and Ghisalberti E. L. 1995. A method to test compounds for feeding deterrence towards redlegged earth mites (Acarina: Penthaleidae). Annals of Applied Biology 127: 593-600.

Robinson M. R. and Hoffmann A. A. 2001. The pest status and distribution of three cryptic blue oat mite species (*Penthaleus* spp.) and the redlegged earth mite (*Halotydeus destructor*) in southeastern Australia. *Experimental and Applied Acarology* 25: 699-716.

Trowell S., Forrester N., Daly J., Garsia K., Bird L. and Lang G. 1992. Developmental trials of the *Heliothis* ID kit. *In*: Swallow D. (ed.), *Proceedings of the Sixth Australian Cotton Conference*. ACGRA, Wee Waa, Australia, pp. 209-216.

Trowell S. C., Forrester N. W., Garsia K. A., Lang G. A., Bird L. J., Hill A. S., Skerritt J. H. and Daly J. C. 2000. Rapid antibody-based field test to distinguish between *Helicoverpa armigera* (Lepidoptera: Noctuidae) and *Helicoverpa punctigera* (Lepidoptera: Noctuidae). *Journal of Economic Entomology* 93 (3): 878-891.

Umina P. A. and Hoffmann A. A. 1999. Tolerance of cryptic species of blue oat mites (*Penthaleus* spp.) and the redlegged earth mite (*Halotydeus destructor*) to pesticides. *Australian Journal of Experimental Agriculture* 39: 621-628.

Umina P., Robinson M. and Hoffmann A. 2001. Correct identification critical for mite control. *Farming Ahead* 116: 37-38.

Völkl W. and Stadler B. 1991. Interspecific larval competition between *Lysiphlebus testaceipes* and *Aphidius colemani* (Hym., Aphidiidae). *Journal of Applied Entomology* 111: 63-71.

Weeks A. R. and Hoffmann A. A. 1998. Intense selection of mite clones in a heterogeneous environment. *Evolution* 52: 1325-1333.

Weeks A. R. and Hoffmann A. A. 2000. Competitive interactions between two pest species of earth mites, *Halotydeus destructor* and *Penthaleus major* (Acarina: Penthaleidae). *Journal of Economic Entomology* 93: 1183-1191.

Weinman J. J., Djordjevic M. A., Creaser E. H., Lawson C. G. R., Broderick K., Mathesius U., Gärtner E., Pittock C., de Majnik J. and Rolfe B.G. 1995. Preparing subterranean clovers for future biotechnology: molecular analysis of genes and proteins involved in stress and defence reactions and the construction of transgenic plants. *Plant protection Quarterly* 10: 47-49.