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Predicting pest issues from common and curious pests of crop seedlings in south-eastern Australian

Submitted by

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Submitted in total fulfilment of the requirements of the degree of Doctor of Philosophy

June 2019

Pest Environmental Adaptation Research Group (PEARG)

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Abstract

Over forty species of invertebrate pests can be pests of emerging crop seedlings in south-eastern Australia. Some species are a common and widespread issue for farmers, while others only sporadically cause damage. The inability to predict potential pest problems is a key reason why insecticides are commonly applied to fields prior to sowing (applied as seed treatments or bare-earth sprays) and/or post-sowing (foliar sprays) in a prophylactic manner. This thesis investigates factors which predict the risk of pest problems arising from two common pests and two emerging sporadic pests of crop seedlings. The common pests are the earth mites, the redlegged earth mite, *Halotydeus destructor* and the blue oat mite, *Penthaleus destructor*. The emerging pests are the Portuguese millipede, *Ommatoiulus moreleti* and the common pillbug, *Armadillidium vulgare*. These species are a curious problem as they generally feed on decaying plant matter and have only recently been recognised as emerging pests.

Sampling was conducted to assess the capacity to predict the abundance of the damaging autumn generation of the major pest mites, *H. destructor* and *P. major*, at a field level. Across the fields sampled, abundances of both mites showed limited association with a range of common agronomic and environmental field variables of known biological importance. The abundance of *H. destructor* could however be divided into risk categories when fields were grouped into categories of field type. These categories were valid across two years and two farming regions. The abundance of *P. major*, by contrast showed a regional association.

The first pest step in understanding the pest status of *O. moreleti* and *A. vulgare* was to understand their ability to damage common crop seedlings. Under shadehouse conditions, this study found *O. moreleti* has a limited ability to feed on a range of crop seedlings. *A. vulgare* can damage a wide range of crops, although some crops are only susceptible in the first days of seedling establishment. This finding was supported by pest reports from fields across southern Australia over the past decade.

Lupinus angustinus was the most susceptible crop seedlings for *O. moreleti*. This appears to be due to the removal of deterrent chemicals in commercial breeding as well the thickness or strength of leaf tissue. The fitness benefits of feeding on seedlings appear to vary between crop seedlings. In controlled studies, the level damage was found to be influenced by the

lifestage and sex of the individual, while the presence of crop stubbles as alternate food sources were not found to influence the level of damage in controlled studies.

To understand the role of water stress on the pest status of *A. vulgare*, I conducted controlled trials on the influence of dry environmental conditions on the level of feeding damage *A. vulgare* caused to canola. I hypothesised water stress would result in individuals increasing their feeding on plants, as they searched for water. Conversely, I found individuals kept in environments with low ambient humidity caused less damage.

Field monitoring observations were made over the course of the thesis to provide the basis for further research on the population dynamics of *O. moreleti*. While stubble is known to be important in influencing population sizes, I also found evidence that soil-type has an important effect on *O. moreleti* numbers. Interestingly, abundances were found to vary greatly between years in some crop fields. This appears to be due to seasonal population dynamics, with *O. moreleti* leaving fields and, in some cases, not returning.

The research conducted in this thesis points to ways to guide monitoring and sustainable management practices. Farm managers should use the association of damaging abundances of *H. destructor* with field-type to predict pest risk at a farm level. The research on *A. vulgare* and *O. moreleti* helps to explain why these species are a sporadic pest risk, given they are limited in their ability to damage crop seedlings. This knowledge will assist in the development of pest management programs for *O. moreleti* and *A. vulgare*. The studies on the association of environmental and agronomic factors should be used to guide future research to understand the many remaining knowledge gaps.

Declaration

This is to certify that

- i. The thesis comprises only my original work towards the PhD except where indicated in the Preface,
- ii. due acknowledgement has been made in the text to all other material used,
- iii. the thesis is less than 100,000 words in length exclusive of tables, maps, bibliographies and appendices

Joshua Heath Douglas

May 2019

Preface

The following chapters of this thesis have either been published or been submitted under the following titles.

Chapter 2: Douglas, J., P. Umina, S. Macfadyen, and A. Hoffmann. 2018. Field associations of first generation densities of the pest mites *Halotydeus destructor* and *Penthaleus major* in pasture. *Experimental and Applied Acarology* 76: 487–506.

Douglas, J.

Developed study, undertook all the field work, sorted and counted all specimens, analysed data and wrote manuscript.

Umina, P.

Supervised development and design of study and edited draft manuscript.

Macfadyen, S.

Assisted development and design of study and edited draft manuscript.

Hoffmann, A.

Supervised development and design of study, assisted with data analysis and edited draft manuscript.

Chapter 3: Douglas, J., S. Macfadyen, A. Hoffmann, and P. Umina. 2017. Crop seedling susceptibility to *Armadillidium vulgare* (Isopoda: Armadillidiidae) and *Ommatoiulus moreletii* (Diplopoda: Iulidae). *Journal of Economic Entomology* 110: 2679–2685.

Douglas, J.

Developed study, undertook the shadehouse experiments, analysed data and wrote manuscript.

Macfadyen, S.

Assisted development and design of study and edited draft manuscript.

Hoffmann, A.

Assisted development and design of study and edited draft manuscript.

Umina, P.

Supervised development and design of study, assisted with data analysis and edited draft manuscript.

Chapter 4: Douglas, J., A. Hoffmann, P. Umina and S. Macfadyen. 2019. Factors influencing damage by the Portuguese millipede, *Ommatoiulus moreleti* (Julida: Julidae), to crop seedlings. Accepted for publication in the Journal of Economic Entomology.

Douglas, J.

Developed study, undertook the shadehouse and CT room experiments, analysed data and wrote manuscript.

Hoffmann, A.

Assisted development and design of study and edited draft manuscript.

Umina, P.

Assisted development and design of study and edited draft manuscript.

Macfadyen, S.

Supervised development and design of study, assisted with data analysis and edited draft manuscript.

We give consent for Josh Douglas to present these papers in his thesis for examination towards the degree of Doctor of Philosophy.

Paul Umina

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Sarina Macfadyen

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Chapter 1- General introduction

Invertebrate pests pose an ongoing problem for broadacre farmers in southern Australia, with over forty species known to damage establishing seedlings (Micic et al. 2008). Pest management strategies for these crop establishment pests largely revolve around the use of broad-spectrum pesticide applications. As the economic cost of these insecticides is minimal in relation to potential pest damage, insecticides are often applied without knowledge of the presence or density of pests, in order to be “safe rather than sorry” (Hoffmann et al. 2008, Nash 2012). However, their indiscriminate use is not only environmentally damaging but can lead to a range of negative outcomes, including pests developing chemical resistance and the removal of predatory organisms that can lead to secondary pest outbreaks (Hill et al. 2017).

Currently a range of factors are driving a push towards a more sustainable approach to pest management in the grains industry. These include pesticide resistance problems in major pest species (Endersby and Ridland 1997, Umina et al. 2018, de Little et al. 2017, Maino et al. 2018), export market demands for food with reduced pesticide residues and an increased awareness of the advantages of a more sustainable approach used in other farming systems (Horne et al. 2008). Among the challenges in affecting changing management practices is a lack of knowledge in the timing and intensity of pest outbreaks. This is particularly so in the case of sporadic and emerging pests, such as *Armadillidium vulgare* and *Ommatoiulus moreleti*, where very little research has been conducted. Research and extension support is required to provide farmers with the tools to confidently make pest management decisions in a time-effective manner.

This thesis aims to address two diverse challenges for Australian farmers. Firstly, I investigate the capacity to predict densities of the major pest mites, *Halotydeus destructor* and *Penthaleus major* in pasture fields. Secondly, I investigate factors that can help predict the pest status of two primarily detritivorous feeding species, *A. vulgare* and *O. moreleti*.

1.1 The importance of understanding species biology to create sustainable pest management practices for broadacre agriculture in Australia

1.1.1 Understanding and controlling pests

In most environments, invertebrate densities are regulated by a range of biotic and abiotic factors. However, when regulation is lost, outbreaks can occur. The behaviour and population ecology of invertebrates are highly influenced by changes in environmental conditions and the quality of resources. Changes in agricultural practices and environmental conditions greatly effect pest problems by changing plant susceptibility, the feeding behaviour of organisms and the environmental suitability for both pest and beneficial (predator and parasitoid) invertebrates. Pest species usually have life history strategies characterised by high fecundity and efficient and rapid searching mechanisms that are designed to find and overexploit available resources (Price et al. 2011).

The agricultural revolution of the post-war period introduced large changes in agricultural practices leading to major changes in the balance of predator/prey relationships. Cultivating crops in monoculture reduced the heterogeneity of habitats in the field, thereby reducing biodiversity. Increased trade has transported organisms beyond their previous boundaries, where they are free of many native beneficials. The continual use of broad-spectrum pesticides has reduced beneficial populations in fields and led to resistance in a wide variety of major pest species. The combination of these effects is herbivorous species provided with new environments where they have an abundance of resources with limited beneficial invertebrates.

The widespread development of pesticide resistance has led to an understanding that pesticides are not a silver bullet solution to controlling agricultural pests. This understanding led to the development of the concept of Integrated Pest Management (IPM) where a range of control strategies are used with pesticides as a last resort (Kogan 1998, Goodell 2009, Zalucki et al. 2009). IPM strategies focus on preventing pest outbreaks occurring by 1) ecologically engineering the field and surrounding landscape to promote natural protection from predation or parasites, 2) reducing crop susceptibility and suitability for pests, 3) using alternative control strategies (e.g. baits, traps, physical barriers or crushing), and 4) optimising efficacy of control strategies.

While “true IPM” is practiced by few farmers around the world, the development of resistance problems in major pest species has necessitated a variable approach in some farming environments (Kogan 1998, Horne and Page 2008). For instance, in America, resistance to a range of insecticides in the western corn rootworm, *Diabrotica virgifera virgifera* LeConte, led to the development of a crop-rotation strategy, while the subsequent resistance to crop-rotation led to the use of genetically modified crops (Miller et al. 2009). In horticultural systems around the world, insecticide resistance problems in the two-spotted mite, *Tetranychus urticae*, led to the widespread introduction of commercially bred predatory mite, *Phytoseiulus persimilis* and the use of soft chemicals aimed at maintaining a pest/predator equilibrium (Attia et al. 2013).

1.1.2 Problems in implementing IPM strategies in Australian broadacre agriculture

In Australian broadacre farming, pest management is still largely reliant on the use of broad-spectrum pesticides that are commonly applied without knowledge of the presence or abundance of pest species. However, the increasing threat of pest resistance in combination with increased appreciation of the protection provided by beneficial organisms are among the main drivers pushing a more sustainable approach towards pest control. A range of strategies currently employed to control other problems may have impacts on pest control, but these are not specifically targeted to pests. For instance, crop rotation is commonly employed to control weeds and maintain soil health, and also impacts on the control of establishment pests. Shelterbelts are planted for their benefits of shade and wind cover, but can also encourage beneficial invertebrates (Tsitsilas et al. 2006).

The key to successfully developing IPM practices is understanding pest biology (Geier 1966, Smith et al. 1976, Kogan 1998). Invertebrate species differ significantly in their biology, thus allowing a range of species to occupy similar niches. Closely related species can differ in their feeding, population dynamics, movement and resistance to chemicals. Thus, successful implementation of IPM strategies relies on properly identifying and understanding the biology of pest species. A lack of confidence in IPM can lead to farmers reverting to their previously trusted strategies (Horne et al. 2008). A lack of understanding of the biology of sporadic pests is a key problem in the adoption of IPM in Australia (Micic et al. 2008).

Over forty species of invertebrates cause damage to establishing grain crops in southern Australia (Micic et al. 2008). The major mite pests, *Halotydeus destructor* and *Penthaleus major* and the collembolan *Sminthurus viridis*, are common and have a widespread distribution. In contrast, the majority of pest species occur in a localised distribution or are sporadic pests. Widespread pests have received substantial attention in the research literature while sporadic pests have received far less research. While the sporadic pests may be causing damage at a low frequency and only localised, the intensity of such damage can be high when conditions are suitable for the pest. (Murray et al. 2013). Uncertainty surrounding sporadic pest problems is believed to be a key reason surrounding the lack of uptake in IPM in some parts of Australia (Micic et al. 2008).

1.1.3 The emergence of new sporadic pests

Pests that sporadically cause major damage require certain conditions for population densities to build and/or cause feeding damage. Thus they are only sporadically pests when these factors combine. Cutworm species, *Agrotis spp.*, are a good example. They are limited in their potential to damage seedlings by the growth stage. Thus, they are mostly only a major pest when present in large numbers at a time when the crop is emerging. For this to occur, they require a combination of seasonal rainfall and winds for population densities to build and facilitate migration into fields (Farrow and McDonald 1987).

The relative impact of different pests is changing (Hoffmann et al. 2008). Increased surveillance of pests and better identification may account for an increased awareness of some pest problems. *Penthaleus* mites have only recently been recognised as major pests due to them previously being misidentified as *H. destructor*, given the similarity in morphology, life-cycle and distribution of these mites (Robinson and Hoffmann 2001, Umina et al. 2004). *A. vulgare* and *O. moreleti* have only emerged as pests within the past decade. While these species were not included in a list of establishment pests in 2008 (Micic et al. 2008), earlier reports of pest problems from these species were likely attributed to other organisms as they were not thought to be capable of causing widespread damage to crop seedlings (personal observation after discussion with farmers).

A range of agricultural practices and environmental factors are known to be changing the relative importance of pests problems (Hoffmann et al. 2008). Pesticide usage can influence

biotic interactions between invertebrates by lowering predation (Hill et al. 2017) or competition (Gower et al. 2008) for resources. The variable tolerance to different chemical groups found in different species means pesticides targeted at one pest may lead to increased problems from a competing species (Umina and Hoffmann 2005, Gower et al. 2008, Umina et al. 2010). The emergence of the Balaustium mite, *Balaustium medicagoense* is at least partly due to its higher pesticide tolerance (Arthur et al. 2008, 2013).

Climate change is also thought to be changing the importance of different pest species, both directly, as well as indirectly, through resource availability and interactions with predatory or parasitic organisms (Thomson et al. 2010, Castex et al. 2018). For different species this will change their distribution, population dynamics and the timing and intensity of outbreaks (Adamo et al. 2012, Macfadyen et al. 2018). For the terrestrial isopod species, *Australiodillo bifrons*, climate change is thought to be a cause of increased pest issues which result from their sporadic migratory behaviour which can lead to swarms attacking cereal seedlings in northern New South Wales (Paoletti et al. 2008).

Variable rainfall patterns associated with climate change have led to an increase in the practice of retaining crop stubble post-harvest to retain soil-moisture (Ugalde et al. 2007). The increased proportion of farmers adopting stubble retention practices (either no-till or minimum till practices) has led to changes in invertebrate abundance in fields (Barratt et al. 1994, Holland 2004). No-till (fields that are never tilled) or minimum till practices (fields that are only tilled every few years) minimize soil disturbance and are typically used in fields that still have the previous year's stubble. This practice reduces soil temperature, thereby increasing the retention of moisture and reducing the erosion of topsoil. The stubble then breaks down and returns nutrients back in to the soil (Arshad et al. 1990, Stinner and House 1990). A stubble retained/no-till environment provides a moist habitat for species of arthropods and molluscs that are susceptible to desiccation. This change has improved the suitability of fields for both pest and beneficial organisms (Barratt et al. 1994, Holland 2004, Nash et al. 2008).

The increased adoption of stubble retention practices is thought to have led to species of slugs and snails becoming major pests (Barratt et al. 1994, Nash, Thomson, and Hoffmann 2008). Substantial research has therefore now been conducted on slugs and snails (Baker 2008, 2012, Nash et al. 2008). Millipedes and slaters (terrestrial isopods) are increasingly being found as emerging pests; it is now appreciated they pose a sporadic but increasing issue,

particularly as no pesticides are currently registered against them. The pest status of these organisms is not well understood. Agronomists and farmers commonly report they can be present in fields without causing damage, while at other times they can cause widespread damage, leading to a crop (or parts of a crop) needing to be re-sown (cesar 2016, SARDI 2016).

1.1.4 Outline of thesis and general introduction

This thesis aims to understand the likelihood of pest problems arising from four diverse pest species. Two of these are common pests and two are emerging sporadic pests of crop seedlings in southern Australia. As these species have received vastly different degrees of research so far, I have separated this thesis in two sections.

1) For the common mite pests, *H. destructor* and *P. major*, a substantial amount of research has been conducted, but the extent to which these species become pests across seasons is still unclear. Chapter 2 investigates the capacity to predict densities of these major establishment pests in fields within the context of known information on these two species.

2) For the emerging sporadic pests, *O. moreleti* and *A. vulgare*, little or no research has been conducted on these species so far as pests of crop seedlings. The majority of this thesis (Chapters 3-6) focuses on understanding the factors dictating the sporadic pest status of these organisms. Chapter 3 focuses on understanding the susceptibility of crop seedlings to damage by both species. Chapter 4 uses the information obtained from chapter 3 to understand how a range of biotic factors affect the sporadic feeding behaviour of *O. moreleti*. Chapter 5 uses the information obtained from chapter 3 to investigate abiotic factors (humidity and soil moisture) affecting the sporadic feeding behaviour of *A. vulgare*. Chapter 6 investigates factors determining the abundance of *O. moreleti* in crop fields.

In the current chapter:

I firstly discuss previous research on the earth mites to provide background information. This section is followed by a discussion of research on modelling the densities of pest species and models specific to these earth-mite species.

As little or no research has been conducted on *O. moreleti* and *A. vulgare* as pests of crop seedlings, I firstly provide background research on the reasons why these species may have become sporadic pests and secondly relate this to research on these species. I discuss research on millipedes and slaters in an ecological and agricultural context as both pest and beneficial species. I then discuss causes of sporadic feeding behaviour in relation to detritivorous and generalist feeding organisms. Finally I discuss research on *O. moreleti* and *A. vulgare*. This includes detailed information on lifecycle, abundance, control and feeding behaviour in order to guide the direction of work detailed in this thesis.

1.2 Can first generation field densities of the major establishment pest mites, *Halotydeus destructor* and *Penthaleus major* be predicted?

1.2.1 The earth-mites, Halotydeus destructor and Penthaleus major

Distribution

H. destructor is an invasive mite, native to South Africa (Hill et al. 2016), that was first found in Western Australia in 1917 (Swan 1934). This species spread throughout southern Australia and is now found in climates with a hot dry summer and cool wet winter (Wallace and Mahon 1971). In contrast to its native South Africa, no population genetic structure exists (Weeks et al. 1995, Hill et al. 2016) and is thought to be the result of a single introduction event or multiple invasions from a single location (Hill et al. 2016).

P. major is also invasive and has been found in circumpolar parts of the world, including in the United States and in Iceland, where it has a winter diapause (Umina et al. 2004). Recently it has been found in the tropical environment of Brazil (Pereira et al. 2017). Like *H. destructor*, it is found across southern Australia, but is also found in northern NSW, where its inland distribution may be limited by the presence of grasses (Robinson and Hoffmann 2001).

Lifecycle

H. destructor is a sexual species (Weeks et al. 1995) that in Australia, is present in fields from April or May until October or November. During this time there are three generations. Development from eggs to adult occurs in 27-31 days under natural conditions, with adult mites living for a further 26-56 days (Ridsdill-Smith 1997). Mites grow to adults of about 1mm through five life-stages. During spring, *H. destructor* responds to cues of temperature, photoperiod and dryness and begins producing diapause eggs (Wallace 1970a, Ridsdill-Smith et al. 2005, Cheng et al. 2018). These eggs are not laid but remain within the carcass of the mite over summer. Diapause eggs differ in morphology from winter eggs, with a far thicker chorion, and they can survive extreme temperatures when dry but will die within 24 hours at 45°C when wet (Wallace 1970a). During autumn, rainfall and temperature trigger the development and hatch of diapause eggs (Wallace 1970b, McDonald et al. 2015).

P. major reproduces asexually through thelytokous parthenogenesis and produce clonal groups that differ in their ecological fitness traits (Robinson et al. 2002). The lifecycle of *P. major* is similar to *H. destructor*, although it shows greater intraspecific variability in its diapause, with individuals forming diapause eggs earlier in the year (Umina and Hoffmann 2003).

Population ecology

The population ecology of *H. destructor* has received substantial research in the laboratory as well as some research in the field (see Table 2.1). For *P. major*, research on population ecology is limited to competition with *H. destructor*. Competition between these two species in pasture fields varies depending on the host plant (Umina and Hoffmann 2004) and rainfall, with *P. major* outcompeting *H. destructor* in dry years and *H. destructor* outcompeting *P. major* in wet years (Weeks and Hoffmann 2000).

H. destructor can quickly utilise suitable conditions, with field densities building up to the carrying capacity of a field within a single season (Gower et al. 2008). Egg development is faster at increased temperatures which can allow mites to maximise the abundance of plant resources in spring, leading to exponential increases in population densities. Population expansion is limited by increased mortality of eggs and juveniles at temperatures over 25°C

particularly at low humidity. Grazing a pasture short during spring has been shown to reduce mite numbers (Grimm et al. 1994), presumably as it reduces the number of humid microenvironments and thus causes mite desiccation.

Within pasture fields, high densities of *H. destructor* are spatially aggregated and move in waves throughout the season (Ridsdill-Smith et al. 2013, Nansen et al. 2015). Food quality and available oviposition sites may regulate density-dependent effects (Ridsdill-Smith and Annells 1997, Ridsdill-Smith et al. 2013). The vegetative characteristics of pasture will influence mite densities through the availability of food resources and suitably humid microclimates that are essential for oviposition sites and mite survival (Ridsdill-Smith 1997). While *H. destructor* is highly polyphagous, the nutrition provided by different plant species is highly variable (Annells and Ridsdill-Smith 1994, Ridsdill-Smith and Gaull 1995, Gaull and Ridsdill-Smith 1996), although *H. destructor* can adapt to host plants across generations (Cheng et al. 2018b). *H. destructor* can complete their development by feeding on microflora and this food source may be vital for larval feeding or when food quality is poor (Maclennan et al. 1998).

Variation in rainfall will also effect *H. destructor* densities (Ridsdill-Smith 1997), either directly or indirectly by plant-growth. The survival of diapause eggs is unclear especially on poorly drained soils (James and O'Malley 1991a). A range of predatory organisms feed on this earth mite (Michael et al. 1991, James et al. 1995, Michael 1995, Weiss and McDonald 1998, Halliday 2005) but are not thought to regulate densities. The presence and control provided by these predatory organisms are determined by a range of vegetative variables, agronomic practices and landscape characteristics (James et al. 1995, Tsitsilas et al. 2006).

Economic importance

In Australia, *H. destructor* is regarded as a major establishment pests of both pasture and crop seedlings. In pasture, mite densities can be greater than 10,000 mites per m² and mites feed in aggregations on sub-clover seedlings (Gaull and Ridsdill-Smith 1996, Jiang et al. 1997). In crop fields, they can damage establishing seedlings of all major broadacre crops except for chickpeas (Merton et al. 1995), but they are particularly damaging to canola (Gu et al. 2007).

P. major is the most common of four pest mites in the *Penthaleus* species complex found in Australia. These include *Penthaleus minor*, *Penthaleus falcatus* and *Penthaleus tectus* which can be distinguished by their setae formation (Umina et al. 2004). The economic impact of *P. major* has only recently been understood, owing to frequent misidentification with *H. destructor*, given they occur together in fields, share a similar life-cycle and morphology. Thus, while *P. major* is now also recognised as among the most important crop pests in the Australian grains industry (Micic et al. 2008, Murray et al. 2013), it has received far less research than *H. destructor*.

Earth mite pest control strategies

Over the past decades, control has largely relied on broad-spectrum pesticides (Ridsdill-Smith et al. 2008). A range of chemicals provide good control, although higher doses are required for *P. major* (Umina and Hoffmann 1999, Arthur et al. 2013). The efficacy of pesticide applications is highly dependent on timing (Umina and Hoffmann 2003, Ridsdill-Smith et al. 2005).

For *H. destructor*, the advent of pesticide resistance against multiple classes of chemicals complicates their ongoing use, with resistance present in different parts of Australia (Maino et al. 2018). A range of biological and cultural control options have been evaluated. These include the introduction of a predatory mite, cultural control in pasture through grazing in spring (Grimm et al. 1994) and the development of resistance in sub-clover cultivars (Jiang et al. 1996, Ridsdill-Smith 1997).

1.2.2 Predicting pest invertebrate abundance

The abundance of any pest herbivore species is determined by a range of biotic and abiotic factors that affect population ecological processes such as fecundity, survivorship, mortality, dispersal and species interactions (Barbosa et al. 2012). As invertebrates are particularly sensitive to climate, models primarily use climatic associations to predict population dynamics over time and space. To be useful for pest management, their outputs must be

converted into a tool that can assist in decision-making processes (McCown 2002, Tonnang et al. 2017).

Distribution models

For pests, species distribution models have been used as tools to predict the risk of spread in to new environments, either for invasive species or increasingly to predict the spread of endemic pests under climate change (Sutherst 2014). These models are constructed by hypothesising which environmental and biotic elements are required for survival and reproduction to model the species distribution boundaries (Elith and Leathwick 2009). Correlative models, such as environmental niche models, fit binary presence/absence occurrence data to spatial environmental data using regressions, such as general linear models (GLM), boosted regression trees (BRT), a principle components analysis (PCA) or through maximum entropy (MAXENT) (Elith et al. 2006, 2008, 2011). Mechanistic models use biophysical data to describe spatial distribution based on the organism's physiological constraints (Kearney et al. 2010). Mechanistic models have been less commonly used than correlative models as they lack the ability to characterise the various interactions present in nature (Kearney et al. 2010), but can incorporate adaptation of the organism in new environments (Kearney et al. 2008). One of the most common distribution modelling tools is CLIMEX (Hill et al. 2012, Zhenyu Li et al. 2012, Avila et al. 2019). CLIMEX uses an integrative semi-mechanistic approach to describe a variety of climatic variables as a single score of environmental suitability (Sutherst 2014).

Phenology

Phenological models are derived from laboratory studies on biological processes under different climate conditions which are used to construct life table data (Tonnang et al. 2017). These models are primarily based on degree days (Mohandass and Zalucki 2004) and have been used as tools to predict the timing of population peaks and life-cycle events, such as migration and diapause to guide pest monitoring and management practices (Mohandass and Zalucki 2004, Ridsdill-Smith et al. 2008).

Abundances

The abundance of a species is regulated by environmental suitability for survival and reproduction (Warren et al. 2003, VanDerWal et al. 2009). Predicting abundance is more complicated than distribution as abundance is regulated by abiotic factors associated with resource fluctuations and interspecific interactions that have variable effects for different life-stages and climatic conditions (Price et al. 2011, Barbosa et al. 2012). Predicting the abundance of migratory species requires identifying the source of population abundance and understanding the factors leading to migration (Simpson et al. 2001, Campos et al. 2004, 2006, Veran et al. 2015). The factors that regulate abundances can therefore vary in different regions and in different years. For instance, cyclical fluctuations in the forest abundance of the larch bud-moth (*Zeiraphera diniana*) may be driven by variation in host-pathogen dynamics (Anderson and May 1980), food quality (Fischlin and Baltensweiler 1979), or food quality and parasitism (Turchin et al. 2003) in different locations.

Problems arise with either taking a correlative or mechanistic approach to predict field abundance. Correlative models can be considered as estimations as they are unable to capture the true complexity, while mechanistic models can incorporate complexity that does not hold true in natural systems (McCown 2002). Within the Australian broadacre environment, little is known about many of the species, let alone their interactions (Macfadyen et al. 2019). For a few species of particular economic importance, models have been created to predict fluctuations in severity in different years and in different regions (Zalucki and Furlong 2005, Veran et al. 2015, Li et al. 2016). These models have been used as tools to assess the efficacy of area-wide pest management practices at a temporal and spatial scale (Carriere et al. 2003, Baker and Tann 2017) or to assess regional pest abundance (Maelzer and Zalucki 2000, Zalucki and Furlong 2005, Li et al. 2016, Zhu et al. 2018).

For the migratory pest moths *Helicoverpa* spp. (*Helicoverpa armigera* and *Helicoverpa punctigera*), models have focused on predicting early season abundance in crop fields in eastern Australia using the climatic variable of rainfall. As early season abundance is the result of migrants from inland Australia, the size of this generation is dependent on the survival of moths on flowers in inland locations over winter. Predictive models have used a correlative approach to link long-term trapping data on early season abundance to winter rainfall, either at putative migrant source locations (Oertel et al. 1999, Zalucki and Furlong 2005) or using climatic indexes (Southern Oscillation Index (SOI) and Sea Surface

Temperature (SST)) that predict seasonal rainfall (Maelzer and Zalucki 2000). These models have shown that rainfall in putative source populations can provide useful predictions 4-5 months in advance (Zalucki and Furlong 2005). Using climatic indices, forecasts can be provided between 6-15 months, with abundance fitted to 25%, although this varies between regions of collection (Maelzer and Zalucki 2000). Subsequent generations, which are the result of both migrants and movement within and between fields (Fitt and Daly 1990), can be predicted with great accuracy using trapping data of the first generation, with a positive association with winter rainfall and a negative association with spring rainfall (Maelzer and Zalucki 1999).

For the diamond back, *Plutella xylostella* in China, Li et al (2016) created a mechanistic model to predict the relative effects of climate, agricultural practices and biological control. This model used DYMEX, which enables the building of mechanistic process-based models which focus on the species lifecycle, thus allowing factors to be directly linked to variable effects on different life-stages (Whish et al. 2015). This approach used a weekly growth index based on laboratory derived data on developmental, mortality and reproductive functions to predict age-structured population dynamics in a cohort based discrete time-model. As crop management and the availability of Brassica crops vary regionally across China (Li et al. 2016, Zhu et al. 2018), this index was combined with variable agronomic (crop and pest management practices) and biotic factors (parasitoid abundance). To validate this model, long-term trap data in different regions was fitted to explain seasonal population dynamics. This model has been used as the basis of crop simulation models used as tools to predict the optimal management strategy to maintain *P. xylostella* populations below a threshold level in different regions, years and under different crop management practices (Li et al. 2018, Zhu et al. 2018).

1.2.3 Predictive models for earth-mites

Distribution

The distribution of *H. destructor* in southern Australia was initially thought to be limited to areas with less than 225mm summer rainfall (Wallace and Mahon 1971). Greater summer rainfall leads to significant mortality of diapausing eggs. This distribution appears to be expanding to localities further inland, facilitated by changing agriculture practices and

climate change (Hill et al. 2013).

The worldwide distribution of *P. major* is facilitated by variable phenology, including a winter rather than summer diapause (Umina et al. 2004). In Australia, it is found throughout southern Australia and extends its distribution into northern New South Wales where its inland distribution is best predicted by stress indices of the heat and dryness of the environment (Robinson and Hoffmann 2001).

Phenology

Models which predict the timing of the production and hatch of diapause eggs have been developed for *H. destructor* but not for *P. major*. *P. major* is known to have a similar life-cycle but greater intra-specific variation in diapause (Umina and Hoffmann 2003). The induction of the production of diapause eggs in *H. destructor* during spring has been demonstrated to be a plastic response. Eggs of variable levels of diapause intensity are induced by multiple environmental cues of temperature, soil moisture and photoperiod (Cheng et al. 2018, 2019). The complexity of this response likely reflects a bet-hedging strategy that has helped this mite to survive under variable environmental conditions.

Despite this complexity, Ridsdill-Smith et al (2005) found the single variable of photoperiod could predict 80% of variation in the timing of the diapause response in pasture fields in Western Australia. As photoperiod varies between sites but not between years, this model was used to devise a calendar-based optimal spray schedule which targets mites prior to the production of diapause eggs (“Timerite - Wool.com - Australian Wool Innovation” 2018). Within Western Australia, spraying on this date has been found to reduce up to 99% of mites the following autumn. Within Victoria, where the commencement of stressful seasonal conditions in spring is more variable, this model and approach has been less effective, but still reduces abundance by up to 70% in some regions (Gower et al. 2008).

McDonald et al (2015) modelled the peak hatch of diapause eggs in autumn. Phenological variables (degree days, CLIMEX) were created using triggers for egg development and the subsequent rate of egg development based on field and laboratory data (Wallace 1970a, b). Using CLIMEX to fit field data, peak emergence was modelled as occurring when 5mm rainfall precedes a 10-day period of mean day temperatures less than 16°C in Victoria and 20.5°C in Western Australia.

Population dynamics

Earth-mites are largely immobile (Weeks et al. 2000). Population abundances are therefore dictated by the suitability of the field environment for reproduction and survival. Given appropriate conditions are known to rapidly expand to the carrying capacity of a field within a single field season (Gower et al. 2008). For *H. destructor*, the availability of suitable oviposition sites and food quality are factors known to regulate abundance and population growth, while survival is limited by the availability of suitably humid microhabitats. A range of agronomic and climate factors are known to dictate seasonal and in-field abundance patterns, but no models have been created to understand how these factors regulate abundances across years, in different fields and in different regions.

In lieu of a proper understanding of the factors that dictate population abundance, farm managers use their own proxy models to predict when *H. destructor* will be a problem. A modelling tool which predicts abundance would be particularly useful when used in combination with life-cycle models (Ridsdill-Smith et al. 2005, McDonald et al. 2015), optimal monitoring strategies (Arthur et al. 2014a, Nansen et al. 2015) and growth-stage specific thresholds which exist for canola (Arthur 2013, Arthur et al. 2014b).

1.3 Understanding the sporadic feeding behaviour of *O. moreleti* and *A. vulgare* on crop seedlings

1.3.1 Millipedes and slaters - pests and ecological engineers

Millipedes and terrestrial isopods are soil-dwelling invertebrates that exist in the interface between the soil and decaying leaf litter. In forest environments millipedes and terrestrial isopods ingest between 20-100% of leaf litter (Tajovsky et al. 1992). They play an important part in recycling nutrients back into the soil by digesting and degrading plant matter for further microbial decomposition (Vos et al. 2011), with similar efficiency as earthworms (Ambarish and Sridhar 2013). Thus in stubble-retained agricultural fields they should provide similarly beneficial roles in degrading crop stubble. Decomposing the crop stubble is a vital part of these systems, as otherwise stubble can build up and become problematic for farm machinery.

Millipedes and slaters are generally described as detritivores, as they primarily feed on decaying plant matter (Sutton 1980, Blower 1985). However, they can also be described as scavengers, as they feed on a wide range of food sources. These include fungi, carrion, as well as living plant tissue such as seeds (Saska 2008, Koprdoová et al. 2010), fruit and vegetables. Thus, they are considered occasional pests in gardens and horticultural crops (Sutton 1980, Blower 1985, Warburg 1993, Sierwald and Bond 2007). While they are known to occasionally feed on seedlings (Paris 1963, Francisco and Fontanetti 2015), this is thought to be limited by their ability to digest cellulose or detoxify deterrent plant compounds. Thus the first reports of millipedes and terrestrial isopods causing widespread damage to crop seedlings were met with skepticism by the grains industry (Henry K. personal comment). However, reports of both millipedes and slaters causing damage to crop seedlings have been increasing over the past decade (cesar 2016, SARDI 2016). The terrestrial isopod, *Armadillidium vulgare* is now recognised as a major problem of soybean in parts of the Americas (Faberi et al. 2011, 2014, Johnson et al. 2012, 2013).

Modern crop breeding, aiming for increased yield, may have influenced the susceptibility of seedlings to invertebrate pests (Chen et al. 2015). In contrast to natural selection promoting plant defensive traits, crop seedlings have been selected for traits such as 1) adaptation to environment conditions, 2) yield, 3) altered nutritional content, and 4) taste of the crop (Hanson et al. 1981, Björkman et al. 2011, Adhikari et al. 2012). Major Australian crops, including canola, *Brassica napus*, and lupin, *Lupinus angustinus*, are specifically bred to remove the major secondary plant defences (glucosinolates and alkaloids respectively), due to the effects these compounds have on the harvested seed crop. Barley is also known to have reduced levels of its major secondary defensive compound, gramine, due to inbreeding (Hanson et al. 1981). Breeding has led to increased suitability of these plants for a range of herbivorous arthropods, thereby leading to increased susceptibility, particularly as emerging seedlings (Adhikari et al. 2012).

Understanding the reasons why millipedes and terrestrial isopods feed on living plants is complicated by a lack of understanding on the feeding of these organisms. If viewed as detritivores, they may only feed on living plant matter when physiologically stressed, while as generalist feeders they may feed on living plant matter as part of a wider feeding strategy. Furthermore, given the wide range of environments in which these organisms exist, it is likely these species have variable feeding strategies in different locations, environments and

years (Sutton 1984). Variable microbiomes are suggested to facilitate the ability of millipedes and terrestrial isopods to adapt to available food sources (Taylor 1982, Kukor and Martin 1986), and have facilitated a variety of organisms to feed on food sources that are otherwise undigestible or toxic (Jones and Megarrity 1986, Chu et al. 2013, Ludwick et al. 2018). During different seasons, fluctuations in climatic conditions and resource availability will also change feeding behaviour. For the terrestrial isopod, *A. bifrons*, climate conditions were suggested to influence movement and feeding behaviour that leads to this species becoming a sporadic pest (Paoletti et al. 2008). Understanding both the ability of invertebrates to damage plants and how this varies in different environments is vital to helping predict the damage these species will cause.

1.3.2 Feeding variation in generalist feeding species

A generalist feeding strategy is an advantage when food quality and availability is unpredictable. Organisms must, however, adapt their food choice, feeding rates or feeding behaviour, depending on the suitability of environmental conditions to search for food and the availability of different food sources (Bernays and Minkenbergh 1997). In agricultural environments, this variability in feeding behaviour can determine the amount of damage a species causes or whether it behaves as a beneficial or pest species (Coll and Guershon 2002).

Some species switch from feeding on arthropods to feeding on plant tissue depending on relative food quality and availability (Agrawal et al. 1999, Agrawal and Klein 2000, Eubanks and Denno 2000). The omnivorous western flower thrip, *Frankliniella occidentalis*, is known as a major pest in horticultural fields, as it feeds on flowers and fruit when these quality plant parts are in abundance. However, it will feed primarily on mite eggs when less preferred plant sources are available (Agrawal et al. 1999, Agrawal and Klein 2000). For species that feed on living and dead plant matter, both food sources are generally highly abundant and highly variable in quality. The proportion of living leaf matter plant consumed by primarily detritivorous feeding terrestrial gastropods can be higher when deterrent plant chemicals are lower (Speiser and Rowell-Rahier 1991) or when dead plant matter is dry (Mensink and Henry 2011).

The susceptibility of a crop seedling is also dependent on the nutritive benefits it provides, which can vary depending on the physiological requirements of an organism (Behmer et al. 2002, Behmer 2008). A range of invertebrates have been shown to adjust their feeding based on balancing their requirements for nutrients (Behmer et al. 2003, Raubenheimer and Jones 2006, Raubenheimer and Simpson 2018). Nitrogen is a limiting nutrient for herbivores (Price et al. 2011), with generalist feeders showing a greater focus on acquiring protein (Raubenheimer and Simpson 2003, Lee et al. 2006). Nitrogen may be even more limiting for detritivores (Cross et al. 2003), although little is known about the nutritional requirements of millipedes and slaters and their ability to obtain nutrition from different food sources (Bignell 1989, Zimmer 2002). As they lack the waxy cuticle of insects, the physiological requirement to maintain water balance (Edney 1949, 1951) is suggested to strongly influence their feeding behaviour.

While environmental effects may potentially increase feeding damage, by increasing an organism's physiological requirement for water, this is dependent on the way organisms adapt to different environments. The level of damage slugs will cause is positively correlated with soil moisture (Barker 2002). Environmental conditions are known to affect the food searching behaviour of both of these organisms (Bailey and Kovaliski 1993, Dias et al. 2012).

1.3.3 *The Portuguese millipede, Ommatoiulus moreleti*

The Portuguese millipede, *Ommatoiulus moreleti* (Julida : Julidae) is a black millipede that grows to approximately 3cm. *O. moreleti* is native to the Iberian peninsula and arrived in Australia either from a population near Lisbon or from the invasive South African population (Baker 1984). Of the 25 species of *Ommatoiulus* millipedes found in Spain and Portugal (Vicente 1985), it is the only one that has become invasive and is also found in some islands in the Atlantic as well as South Africa (Bailey and de Mendonça 1990). Within its native range it is found in comparatively small numbers, but high numbers are found in invasive populations in Madeira and Azores (Baker 1985a).

In Australia it was first found in 1953 near Adelaide (Baker 1978) and spread east and west across southern Australia (Baker et al. 2013) at a rate of approximately 200 metres a year (Baker 1978). Numbers in the invasive front were so large that their carcasses on train-tracks caused traction problems for trains. The high densities found in the invasive front

subsequently declined, likely due to density dependent effects under depleted resources (McKillup et al. 1988, Bailey 1997).

O. moreleti exhibits extreme variation in seasonal movement, being active in autumn and spring and largely inactive in winter and summer (Baker 1979). *O. moreleti* is well known to be an urban nuisance as it is positively attracted to light; it enters houses at night (McKillup 1988). It is also well noted as a pest of fruit both in gardens and horticultural crops. It may have a negative impact on native biota, although this aspect has received little research (Griffin and Bull 1995).

Life-cycle

The life-cycle of this millipede begins when females oviposit eggs in a hole in the soil. In Australia the reproductive component of the life cycle (at least primarily) occurs in autumn. In late winter or early spring, legless pupoids hatch which moult to a hexapod larva. During each subsequent moult, additional body segments are added. Maturation to the adult stage occurs between the eighth and twelfth stadium. This takes at least a year, so individuals only engage in mating during their second year, appearing to live 2-3 years (Baker 1978). It is unknown whether females produce multiple broods. In other millipede species, breeding occurs in cohorts as a bet hedging strategy against environmental fluctuations (David et al. 2003, David and Geoffroy 2011). Upon maturation, moulting is only twice a year, at the end of winter and at the end of summer. During spring males moult into an un-reproductive form. Summer is spent in a state of quiescence (Bailey and Kovaliski 1993), in a cell which the animal excavates a few centimetres below the soil surface (Baker 1985a). Upon emergence it moults, with males regaining their reproductive form. Rainfall, soil moisture and temperature are factors that trigger activity (Baker 1979, Bailey and Kovaliski 1993).

Distribution and abundance

A recent survey found *Ommatoiulus moreleti* across almost all of southern Australia (Baker et al. 2013). This distribution correlates closely to a predicted distribution from 1985 which suggested the spread of *O. moreleti* would extend to all locations with mean annual rainfall of

300–2400 mm (excluding areas where maximum rainfall occurs in summer), mean daily minimum air temperatures in winter of 0–15°C, and mean daily maximum air temperatures in summer of 18–33°C (Baker 1985a). Within this distribution it is found in a variety of habitats from dunes to forests to agricultural fields, where it is in higher densities in fields with a history of pesticide use (Nash et al. 2010). This wide range of habitats suggests it can adapt to a range of food sources and soil-types.

O. moreleti survives desiccating conditions during summer through a range of adaptations which are likely a reason for the high relative abundance of this species (O’Neill 1969). Physiological adaptations include adult males moulting to an unproductive form (Baker 1978), decreasing cuticular permeability, increasing water content (Baker 1980) and spending summer in a state of quiescence. *O. moreleti* does not appear to be particularly good at burrowing but finds refuge from adverse conditions in the litter layer or at the base of plants, aggregating in wetter patches (Bailey and Mendonça, 1990, Griffin and Bull 1995). Soil hardness and litter depth may be important determinants of the local distribution of *O. moreleti* (Bailey and de Mendonça 1990).

Millipede feeding

The specific nutritive requirements of millipedes are unknown (Blower 1985) and are thought to be obtained by feeding on a wide range of food sources (David and Célérier 1997). Nitrogen is suggested to be the limiting nutrient in growth and millipedes are predominantly found feeding on leaves with a high nitrogen content (Loranger-Merciris et al. 2008). A source of calcium is also believed to be important due to the highly sclerotized exoskeleton. Fungal feeding is thought to provide an important source of calcium, among other nutrients (Farfan, 2010).

Food choice is made by probing with chemoreceptor and mechanoreceptor covered antennae (Fontanetti and Camargo-Mathias, 2004), assessing food with mouthparts, then either beginning ingesting or moving on to find a higher quality food (Ashwini and Sridhar 2005). When feeding on dead plant matter, millipedes largely feed on leaf material in a high state of decay (Griffin and Bull 1995) and nutrition may be primarily provided by microbial rather than plant tissue (Bignell 1989). Food quality is dependent on food hardness and particle size,

particularly for juveniles (Farfan 2010). Moisture content of food strongly determines the ingestion rate (Wooten Jr and Crawford, 1975). Thus, feeding must vary seasonally as dry litter can become too hard (Bailey and de Mendonça 1990).

The ability of millipedes to utilise living plant material is unclear and may vary between species and in different environments (Taylor 1982). Millipedes have a native gut bacteria which include microbial symbionts that are amylase, cellulase, chitinase, xylanase and protease producers (Ramanathan and Alagesan 2012).

Pest control

No pesticides are currently registered for controlling *O. moreleti* in broadacre fields. In the native environment of the Iberian Peninsula, densities of this millipede are controlled by a range of parasites, while mammals such as hedgehogs are also a known predator (Baker 1984). Within Australia, while a range of invertebrates attack *O. moreleti* (Baker 1985b, Bailey 1989, McKillup 1988, Schulte 1989, Terrace and Baker 1994) they are not thought to regulate abundances in most environments (Baker et al. 2013). The nematode *Rhabditis necromena*, is thought to provide some control within South Australia (McKillup 1988b, Schulte 1989). The limited success of *Pelidnoptera nigripennis*, a fly parasite introduced from Portugal, is suspected to be due to the smaller size of Australian millipedes (Baker 1985b, Baker et al. 2013)

1.3.4 The common pillbug, Armadillidium vulgare

The common pillbug, *Armadillidium vulgare*, is one of only a few species of oniscid isopod that has achieved a largely cosmopolitan distribution (Warburg 1993), primarily found in Mediterranean type environments. In Australia, it has been found in all States and territories (Atlas of living Australia 2014).

A. vulgare can live for at least three years and takes two years to reproductive maturity. They produce sexually, although some populations may have feminising *Wolbachia* (Moreau et al. 2001). The timing of reproduction is influenced by temperature and photoperiod. Depending

on the population and the photoperiod regime, there are one to four generations per year (Souty-Grosset et al. 1998). Terrestrial isopods, including *A. vulgare*, possess a marsupium or brood-pouch, to which juveniles attach themselves. At this point they receive vital microbiomes from their mother, potentially including those with the capacity to break down cellulose (Zimmer 2002). Juveniles grow particularly fast as they are susceptible to environmental fluctuations at this stage. Moulting occurs approximately every 33 days in two stages, with the front section moulting first and then the back section with calcium sequestered in the front half during this process.

A. vulgare is described as a eurydynamic species. Populations in environments under conditions of resource abundance and microclimate instability produce more offspring than populations in less variable environments (Sutton 1984). Environmental factors such as extreme temperature or floods cause significant mortality, especially for juveniles. Food quality also impacts survival, growth and reproduction through nutritive, non-nutritive factors and the presence of microbes (Hassall and Rushton 1982, Rushton and Hassall 1983, Fadiel 1991, Zimmer and Topp 1997). In crop fields, the nitrogen content of different stubbles have been linked to population growth (Faberi et al. 2011).

Adaptations to environment

Terrestrial isopods have evolved from marine ancestors and include species found in terrestrial habitats from intertidal zones to the desert. Instead of gills, terrestrial isopods have pseudotracheae, or tree lungs on their abdominal region (Warburg 1993). Different species show variable levels of adaptations to survive water-limiting environments. Lungs range from a single infolding lung to covered tubular lungs opening into an atrium in isopod families that inhabit drier environments. Species that are found in dry environments, such as *A. vulgare*, have lower cuticular permeability (Warburg 1993) and greater ability to extract water from food (Kuenen 1959). *A. vulgare* further reduces water loss from their naked ventral surface by rolling into a ball (Warburg 1993). In doing so, they do not need to cling to a moist substrate like *Porcellio scaber*, a species with which they commonly cohabit in Australia.

Feeding

Until recently terrestrial isopods were regarded as complete generalists (Sutton 1980). Their feeding strategy, however, suggests they must be able to choose between different kinds of food according to their metabolic needs and nutritional status, and they have been found to make food choice using contact and distance chemoreception (Warburg 1993). They are known to have an ability to deal with a wide spectrum of foods and food limitations, by varying feeding rate as well as assimilation efficiency (Hassall and Rushton 1982, Rushton and Hassall 1983). They have low sensitivity to high concentrations of certain toxic substances (Faulkner and Lochmiller 2000). The vast quantities of copper in the midgut of terrestrial isopods likely play a role in detoxification (Zimmer 2002). Plant phenolic compounds are deterrents that decrease fecundity and reduce gut microbiota (Zimmer 1999). Similarly the presence of plant compounds, such as jasmonates, affect seedling susceptibility (Farmer and Dubugnon 2009).

When feeding on plant matter, they primarily choose matter in a state of high decay, although there are exceptions (Hassall and Rushton 1984). The ingestion of microbes plays a role in enzymatic break down of hard to digest plant chemicals such as cellulose (Zimmer and Topp 1998), lignin, as well as deterrent plant chemicals both prior and post ingestion (Zimmer 2002). Terrestrial isopods are thought to possess a native gut flora of cellulolytic microbes. The selection of microbially inoculated plant matter is therefore thought to be primarily due to the presence of the microbes as a label for digestible food, rather than a selection for cellulolytic microbes (Zimmer and Topp 1998, Ihnen and Zimmer 2008).

Little is known about the nutritional requirements of terrestrial isopods, as they have an amazing ability to cope with a nutritional deficit through digestive flexibility (Zimmer 2002) and through their gut microbiota (Carefoot 1984). An artificial diet developed for *Ligia pallasii* indicated no requirement for vitamins, fatty acids or cholesterol for growth or survival (Carefoot 1984). These nutrients are considered to be essential for growth in invertebrates and may therefore be provided by the gut microbiota. Calcium is not thought to be a limiting nutrient (Beeby 1978) as isopods can sequester calcium during the moulting process and efficiently extract it by ingesting their exuvia or feeding on dead individuals (Zimmer 2002).

Behaviour is highly dependent on the environment with speed of movement and duration of

resting periods related to humidity (Waloff 1941, Dias et al. 2012). During summer, *A. vulgare* burrow into the soil and moves on average 13m per day (Paris 1963). Terrestrial isopods are not known to undergo a period of metabolic arrest, although the rate of energy metabolism is profoundly affected by photoperiod (Wieser 1984). The swarming behaviour of *A. bifrons* has not been recorded in *A. vulgare* and only been recorded in a few species of terrestrial isopods (Sutton 1980).

Pest control

No pesticides are currently registered for controlling *A. vulgare* in broadacre fields. Johnson et al (2013) assessed the efficacy of a range of IPM solutions in reducing damage to soybean stands in the US. These pest management practices were aimed at reducing pest abundance by burning stubble prior to sowing and reducing the susceptibility of the crop by applying seed treatments and varying the timing of sowing and seeding rates. Increasing the seeding rate was found to be the only control method to consistently increase stand survival (Johnson et al. 2013). Burning stubble in fields is a commonly used strategy for pest control in Kansas, however it is unclear whether this will lead to reduced damage, as while it causes mortality, it may also increase feeding from surviving individuals (Johnson et al. 2012). In Argentina, 30-100 individuals/m² have been found to be threshold levels where economic damage will be caused to sunflower and soybean seedlings (Faberi et al. 2014).

1.4 Thesis outline

Chapter 2 investigates the capacity to predict densities of the major establishment pest mites, *Halotydeus destructor* and *Pentthaleus major*, in pasture fields using field variables known to affect these mites. This study was conducted from field sampling over two years in two diverse farming regions in south-eastern Australia during the weeks following peak emergence of the first generation. This chapter has been published in *Experimental and Applied Acarology*.

Douglas, J., P. Umina, S. Macfadyen, and A. Hoffmann. 2018. Field associations of first generation densities of the pest mites *Halotydeus destructor* and *Penthaleus major* in pasture. *Experimental and Applied Acarology* 76: 487–506.

Chapters 3-6 investigate factors that influence the risk factors influencing when *Armadillidium vulgare* and *Ommatoiulus moreleti* will damage crop seedlings.

In Chapter 3 I aimed to understand the susceptibility of crop seedlings to feeding damage by these species. This study was conducted by introducing adults to commonly grown crops at one of three stages of emerging seedlings. The results obtained from this study were compared with reports of field damage to view the correlation of this shade-house study with field reports. This chapter has been published in the *Journal of Economic Entomology*.

Douglas, J., S. Macfadyen, A. Hoffmann, and P. Umina. 2017. Crop seedling susceptibility to *Armadillidium vulgare* (Isopoda: Armadillidiidae) and *Ommatoiulus moreletii* (Diplopoda: Iulidae). *Journal of Economic Entomology* 110: 2679–2685.

In Chapter 4 I focus on *O. moreleti*. Here I conducted experiments under controlled conditions to minimise the influence of environmental conditions. I focused on the effects of life-stage, crop-breeding, alternate food sources and fitness benefits of feeding on crop seedlings. This chapter has been accepted for publication to the *Journal of Economic Entomology*.

In Chapter 5 I focus on *A. vulgare*. In contrast to Chapter 4, here I vary the environmental conditions to conduct a preliminary study on the effect of ambient humidity on the level of damage *A. vulgare* causes to canola seedlings.

Chapter 6 is a preliminary study of factors determining the abundance of *Ommatoiulus moreleti* in crop fields.

Chapter 7 is an overview of the research conducted, a discussion of the practical implementations of the results and recommendations for future research.

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CHAPTER 2- Field associations of first generation densities of the pest mites *Halotydeus destructor* and *Penthaleus major* in pasture

2.1 Abstract

Halotydeus destructor and *Penthaleus major* are species of earth mite commonly found at high densities in agricultural fields in Australia and other parts of the world. These mites pose a risk to a range of winter crops and pastures when seedlings emerge in autumn. In order to predict likely mite pressure, we investigated whether autumn densities in pastures can be determined from agronomic and environmental field variables. For *H. destructor*, field densities showed little association with a range of vegetation variables but could largely be explained using the variable field type, with high densities present when fields had mixtures of grass, clover and weeds. For *P. major*, we found a regional effect. In the region where most data were available, *P. major* field densities were associated with grass abundance, while an association with field type was significant but different to that found for *H. destructor*. For both species, densities were not associated with rainfall, but there was a weak association with soil moisture capacity. We discuss how these results can help in managing these important pest mites.

2.2 Introduction

The earth mites, *Halotydeus destructor* (Tucker) (Acari: Penthaleidae) and *Penthaleus major* (Duges) (Acari: Penthaleidae), are key pests of a range of winter plants in fields across southern Australia (Ridsdill-Smith 1997, Robinson and Hoffmann 2001, Umina et al. Hoffmann 2004, Murray et al. 2013). They are present in fields throughout the winter-growing season, surviving the summer as diapause eggs that hatch the following autumn when conditions are suitable (Ridsdill-Smith 1997). This first generation of mites can cause severe damage to emerging seedlings of many crop and pasture species (Ridsdill-Smith 1997, Umina et al. 2004, Micic et al. 2008). Uncertainty regarding earth mite abundance in fields is a key reason why insecticides are commonly applied prior to sowing (applied as seed treatments or bare-earth sprays) and/or post-sowing (foliar sprays) in a prophylactic manner. A predictive framework for earth mite pressures would be useful in pasture fields being planted with susceptible crops, such as rape which is often grown in a rotation after pasture.

The lifecycle of *H. destructor* has been well studied, which has enabled the development of models that aid farmers in their attempts to reduce mite densities in autumn and reduce the risk to emerging seedlings (Ridsdill-Smith et al. 2005, McDonald et al. 2015). Spraying on a critical date associated with photoperiod when *H. destructor* females produce diapause eggs leads to a reduction of 70-99% of the *H. destructor* autumn generation (Ridsdill-Smith et al. 2005, 2013, Gower et al. 2008). This spray date is less effective for *P. major* which has a similar lifecycle but varies more in the production of diapause eggs (Umina and Hoffmann 2003). Autumn hatch of *H. destructor* can be predicted using a combination of data on rainfall and temperature (McDonald et al. 2015) although again this information is not available for *P. major*.

The population ecology of *H. destructor* in pasture fields has been investigated in some detail while less research has been conducted on *P. major* (Table 2.1). Field densities of *H. destructor* can build up within a single season (Gower et al. 2008) and reach densities as high as 29,000 mites per m² late in the growing season (Ridsdill-Smith and Annells 1997). Within fields, high densities are spatially aggregated and move in waves throughout the season (Ridsdill-Smith et al. 2013, Nansen et al. 2015). Food quality and available oviposition sites may regulate density-dependent effects (Ridsdill-Smith and Annells 1997, Ridsdill-Smith et al. 2013). *Halotydeus destructor* and *P. major* are polyphagous and thus not limited in the classical sense by the presence of host plants. The nutrition provided by different plant

species is highly variable (Annells and Ridsdill-Smith 1994, Gaull and Ridsdill-Smith 1996), although *H. destructor* can adapt to host plants across generations (Cheng et al. 2018b). The vegetative characteristics of pasture will also influence mite densities through the availability of food resources and suitably humid microclimates that are essential for oviposition sites and mite survival (Ridsdill-Smith 1997). Within fields, *H. destructor* densities can be positively associated with pasture height and negatively associated with patches of bare earth (Nansen et al. 2015). Similarly, *H. destructor* densities in spring pastures are negatively associated with the intensity of grazing practices (Grimm et al. 1995) (Table 2.1).

A range of biotic and abiotic factors may also be associated with mite densities in autumn. Interspecific interactions between *H. destructor* and *P. major* may be important, which depend in turn on the host plant and other factors (Weeks and Hoffmann 2000, Umina and Hoffmann 2005). A wide number of predatory organisms are known to feed on both mites (Table 2.1) although they are not thought to heavily regulate densities in Australia (Swan 1934, Michael 1995, Ridsdill-Smith and Annells 1997, Weeks et al. 2000). Chemical applications will have a substantial impact on mite numbers, but this is highly dependent on their timing (Umina and Hoffmann 2003, Ridsdill-Smith et al. 2005). Furthermore, the survival of *H. destructor* summer eggs may be low on poorly drained soils (James and O'Malley 1991).

However, given host plant and other factors influence the abundance of earth mites, it may be possible to identify attributes associated with local mite pressures in autumn. This study makes such an attempt, by evaluating the association between first generation densities of *H. destructor* and *P. major* in a range of pastures with differences in host plant composition and other factors. The research questions we address are: (1) can descriptors of pasture composition and other variables including rainfall and soil type be used to predict mite density? and (2) are descriptors consistent across species and across farming regions?

Table 2.1. Factors likely to influence the density of post-diapause generations of *H. destructor* and *P. major* in Australian fields.

Factor	<i>H. destructor</i>	<i>P. major</i>
Lifecycle	<i>H. destructor</i> sexually reproduces and typically has 3-4 generations a year between April and November (Ridsdill-Smith 1997). Diapause eggs are almost solely produced by the third (or spring) generation. The timing of the diapause egg production is induced by environmental cues of temperature, dryness and photoperiod (Wallace 1970, Ridsdill-Smith et al. 2005, Cheng et al. 2018a).	<i>P. major</i> reproduces asexually and typically has 2-3 generations a year between April and November (Umina et al. 2004). The timing of diapause egg production shows a high level of intraspecific variability with some individuals producing diapause eggs early in the winter-growing season (Umina and Hoffmann 2003).
Species distribution in Australia	Species distribution appears limited by rainfall and temperature (Wallace and Mahon 1971, Robinson and Hoffmann 2001, Hill et al. 2012b, Hill et al. 2013).	More widely distributed than <i>H. destructor</i> extending its range in to northern and inland New South Wales (Robinson and Hoffmann 2001, Hill et al. 2012a).
Population structure in Australia	A lack of population genetic structure (Weeks et al. 95, Qin 97, Hill et al. 2016).	Clonal group diversity varies with region (Robinson et al. 2002)
Movement	Adults typically move less than 30m in their lifetime although diapausing eggs may be passively blown much further (Weeks et al. 2000).	Adults typically move less than 30m in their lifetime although diapausing eggs may be passively blown much further (Weeks et al. 2000).
Effect of temperature and humidity	Prefers humidity close to saturation (Solomon 1937a). Nymphs and non-diapausing eggs are particularly susceptible to desiccation (Solomon 1937b). Diapausing eggs can survive	Clonal groups vary with climate (Robinson et al. 2002).

	extreme temperature unless wet (Wallace 1970).	
Soil	Soil clay content influences the survival of diapause eggs during summer with greater mortality on poorly drained clay soils (James and O'Malley 1991).	Soil water holding capacity found to be a limiting factor in species distribution (Robinson and Hoffmann 2001).
Food sources in pastures	Sub-clover (<i>Trifolium subterraneum</i>) is preferentially fed on (Ridsdill-Smith and Pavri 2000). During spring capeweed (<i>Arctotheca calendula</i>) flowers are a source of carbohydrate that provides a reproductive benefit (Annells and Ridsdill-Smith 1994, Gaull and Ridsdill-Smith 1996). Microflora may be vital for larvae (Ridsdill-Smith 1997, Maclennan et al. 1998).	Prefers to feed on grasses. Not considered an important pest of sub-clover and rarely observed feeding on broadleaf weeds (Umina et al. 2004).
Habitat use within pastures	Oviposits eggs on the underside of low lying leaves, particularly on sub-clover and capeweed (Ridsdill-Smith and Annells 1997). Often found resting in pasture with a tall canopy or under broadleaf weeds (Ridsdill-Smith and Pavri 2000).	Not studied
Insecticides	Susceptible to pyrethroids, organochlorines, organophosphates and neonicotinoids (Umina and Hoffmann 1999, Robinson and Hoffmann 2000, Umina et al. 2010, Arthur et al. 2013) but efficacy of the spray application is highly dependent on the timing (Umina and Hoffmann	Susceptible to pyrethroids, organophosphates and neonicotinoids (Umina and Hoffmann 1999, Robinson and Hoffmann 2000, Umina et al. 2010). Efficacy of spray application is highly dependent on the timing (Umina and Hoffmann 2003).

	2003, Ridsdill-Smith et al. 2005). Pesticide resistance a growing issue (Umina 2007, Umina et al. 2012, 2017, Maino et al. 2018a, b)	
Species competition	<i>H. destructor</i> outcompetes <i>P. major</i> in wet years and is a strong competitor in pastures and on broadleaf weeds (Weeks and Hoffmann 2000, Umina and Hoffmann 2005).	<i>P. major</i> is strong competitor in pastures and outcompetes <i>H. destructor</i> in dry years (Weeks and Hoffmann 2000, Umina and Hoffmann 2005).
Predation	Wide range of natural enemies but impact in Australia unclear or low (Michael et al. 1991, James et al. 1995, Michael 1995, Weiss and McDonald 1998, Halliday 2005).	Wide range of natural enemies but impact in Australia unclear or low (Michael et al. 1991, Halliday 2005).

2.3 Methods

2.3.1 Field sampling and laboratory analysis of mite densities

Sixty-two pasture fields were vacuum sampled at a single time-point during May and June in 2014 and 2015, respectively. Fields were located on 29 farms in Victoria and southern New South Wales, Australia (Figure 2.1). Thirteen fields were sampled from seven farms in 2014 and 49 fields were sampled from 26 farms in 2015. A maximum of three fields were sampled from a single farm. Fields were selected with help from local agronomists to cover a range of pasture types. All fields chosen represented pasture sown at least a year prior to sampling. All fields had a history of no/low insecticide applications, given chemical sprays are known to be toxic to mites unless resistance is present (Table 2.1). No fields were sprayed in the same year as the sampling event or during the TIMERITE period the previous spring.

We timed our sampling to capture the peak abundance of the first generation of pest mites emerging after summer diapause. This was guided by a model predicting the date of the peak-hatch of *H. destructor* summer diapause eggs (McDonald et al. 2015), which is also largely applicable to *P. major* in eastern Australia (see Supplementary material 1). Peak emergence

in fields was confirmed by assessing the life-stages of *H. destructor* and *P. major* found in vacuum samples of vegetation in the period leading up to the expected hatch date. After peak hatch had been confirmed, sampling was conducted within each field.

For each field, sampling was conducted at ten points, equally spaced in a circular arrangement around the middle of the field. As *H. destructor* has been shown to spatially aggregated within a pasture, each sampling point was separated by 30m and can be viewed as representing an area with its own population dynamics (Nansen et al. 2015). We expect the same to be true for *P. major* given the similarities in dispersal capability and lifecycle (Table 1). All samples were at least 30m away from the fence boundary and at least 15m away from trees/shelters within a field.

The vacuum sampler used was a petrol-driven BlowerVac (Stihl SH86) with a circular collection sieve of 9cm diameter attached. This sampling method is particularly efficient at collecting small invertebrates, such as earth mites, from the vegetation and soil surface (Gower et al. 2008, Doxon et al. 2011, Nansen et al. 2015). Vacuum sampling was conducted by pressing the collection sieve onto the soil surface for a period of 5 seconds. At each point we took three side-by-side vacuum samples, each covering a 15x60cm quadrat. The three samples were pooled in the field. After sampling, a visual assessment was made of the quadrat to ensure all mites had been collected. In all except three fields, the visual inspection confirmed that vacuum sampling had been successful. In three fields, however, loose litter and soil meant that not all mites were sampled with this technique which is not uncommon (see Arthur et al. 2015). In these instances, visual assessments were used to estimate numbers when coupled with the vacuum collections (c.f. Arthur et al. 2014). The visual assessment consisted of direct counting of the number of mites in the sieve compared to the number on the ground within the quadrat. In these fields around a quarter of the mites had been collected, thus vacuum sampled mite numbers were multiplied by four.

All vacuum samples were transferred to 70% ethanol for later investigation in the laboratory. Mite densities were counted under a binocular microscope. Mite counts were converted to densities of mites per m² based on the sampled area (0.27m²). *H. destructor* was distinguished from *Penthaleus* species by their colour, body shape and the relative position of the anal plate. Mites of the *Penthaleus* species complex were identified to species by visually analysing the arrangement of their dorsal setae (Umina et al. 2004) of at least ten individuals in each field. All *Penthaleus* mites were identified as *P. major*. Other pest mites, *Balaustium*

medicagoense and *Bryobia spp.*, as well as the collembolan, *Sminthurus viridis*, were also recorded, but were not considered in this study.

2.3.2 Assessment of environmental variables

To analyse regional effects, we divided the data into the two sampled areas, each encompassing approximately a 100km radius. These areas represent two farming regions, the Riverina (on the New South Wales and Victorian border) and the Western District (in central-west Victoria). A single farm from the Goulburn Valley region of Victoria was sampled in 2014; this is situated between the two regions and was grouped into the Riverina region due to climatic similarity (Figure 2.1). The Western District shows greater fluctuations in seasonal rainfall than the Riverina region, and can suffer from dry seasons to a greater extent, as was the case for the spring of 2014 when an average of 34mm was recorded in the period 16th August to 15th October, compared with 78mm in the Riverina.

Rainfall was assessed as total rainfall (mm) in the period 16th August to 15th October as a continuous variable. This two-month period is optimal for spring plant growth when mite densities can exponentially increase (Ridsdill-Smith and Annells 1997, Weeks and Hoffmann 1999) and is the period when the majority of diapause eggs are produced (Umina and Hoffmann 2003, Ridsdill-Smith et al. 2005). Rainfall data was accessed from the Australian Water Availability Project (AWAP) (Raupach et al. 2012) which provide archived data at 5km grids.

Soil information, consisting of clay content and soil water holding capacity (WHC), was accessed from the Australian Soil Resource Information System database (ASRIS 2011). We retained only the WHC, due to the fact that clay content is a subcomponent of this measure. The WHC of soil is based on an equation that predicts the soil-retention curve based on a range of properties including particle size, bulk density and clay content (Paydar and Cresswell 1996).

2.3.3 Assessment of field variables

The sampled fields were typical pastures of south-eastern Australia, primarily planted with rye-grass (*Lolium rigidum*) and sub-clover (*T. subterraneum*) or phalaris (*Phalaris aquatica*), rye-grass and sub-clover. Lucerne (*Medicago sativa*) fields were also common. Four fields were planted solely to sub-clover. Fields had different proportions of broadleaf weeds, capeweed (*A. calendula*), mallow (*Malva* spp.), dandelion (*Taxacum* spp.), Paterson's curse (*Echium plantagineum*), plantain (*Plantago* spp.), and thistles (*Onopordum* spp.) as well as the thin-leaf weeds, erodium (*Erodium* spp.) and nightshade (*Solanum* spp.). Fields varied in plant density and the amount of food available for grazing. Twelve fields had applied an insecticide in the previous year (but not in the year of sampling).

The high correlation between the variables measured in these fields makes it difficult to interpret the predictive value of individual variables. Those associated with the growth of different plant types in fields were particularly highly correlated. For example, phalaris shows strong competition with broadleaf weeds and thus the percent cover of these plant groups were (as expected) negatively correlated. Grazing pressure was also highly correlated with plant type. For example, many lucerne fields were left ungrazed to make hay, leading to a positive correlation between lucerne and the amount of food on offer in spring.

Because of correlations between variables, we analyzed field variable effects on mite density in two ways. Firstly, we analyzed individual pasture variables, including plant composition (as described by the percentage of plant groups), pasture thickness (as described by the proportion of bare earth in a field) and resource limitation in spring (as described by the amount of food on offer in spring). Secondly, we used the single descriptive variable "field-type" as a way of classifying fields into the representative classes that reflected agricultural practice. This approach proved more powerful than considering combinations of individual variables and we, therefore, present this analysis in the main document and include an analysis using individual predictive variables in the Supplementary material 2. Data on insecticide use was removed after initial consideration as uncommon applications were restricted to the previous winter (i.e. not at a time that would immediately impact mite populations) (see Supplementary material 2).

To construct the variable "field-type", we evaluated fields by plant composition in the year prior to sampling (of the same pasture) based on interviews with farmers and sampling

vegetation at the time of collections. The farmer interview provided information on when the pasture had been sown, the plant composition at the time of sowing, how plant composition varied throughout the year (and between years), grazing practices as well as insecticide and herbicide applications. For a direct measure, we recorded the composition and area occupied by each plant type and the amount of bare earth in a 10x10 grid (90cm x 90cm) at each of the 30 sampling points per field.

Six categories of fields were used in establishing this pasture variable (Table 2.2): (1) ryegrass/sub-clover, (2) phalaris/ryegrass/sub-clover, (3) sub-clover, (4) lucerne, (5) grassy, and (6) weedy. In most cases (69%), fields could be assigned based on the mix of plants used at sowing. However, a few fields had a high proportion of broadleaf weeds which emerged later and affected the structure of the pasture at the time of sampling, and therefore fields where a significant amount (>15% based on interviews) of broadleaf weeds persisted throughout the previous year were classified as weedy. By following this procedure, we avoided labelling fields which carried a low plant biomass as weedy; in such fields, weeds had typically predominated through adverse conditions in the year prior to sampling. Weedy fields typically contained the broadleaf weeds *A. calendula*, *Malva* spp., *Taxacum* spp., *E. plantagineum*, *Plantago* spp., and *Onopordum* spp.. Three fields with high levels of *Erodium* spp. and/or *Solanum* spp. were not classified as being weedy as these plants are not considered to represent suitable mite hosts (Ridsdill-Smith 1997). Ryegrass/sub-clover and phalaris/ryegrass/sub-clover fields contained at least 15% sub-clover. To determine the proportion of sub-clovers, we used direct assessment in combination with the farmer interviews on how the proportion of sub-clover content had changed throughout the course of the previous year. In cases where grasses persisted in the absence of a minimal amount of sub-clover (<15%), fields were categorized as grassy. The sub-clover category included four fields planted solely to clover and two lucerne fields that had been undersown with clover. These fields were grouped together, as sub-clover comprised >50% plant growth in each case. Of the remaining lucerne fields sampled, minimal amounts of sub-clover were present, and (with <15% broadleaf weeds in the previous year) these fields were categorized as lucerne.

Table 2.2. The six categories of field type and the composition of plant groups in these fields.

Field type	Description
Phalaris/ryegrass/ sub-clover (<i>n</i> =15)	Composed of thick-blade (14 fields were phalaris, one was kikuyu (<i>Pennisetum clandestinum</i>)) and thin-blade grasses (primarily ryegrass but also barley grass (<i>Hordeum spontaneum</i>), cocksfoot (<i>Dactylis glomerata</i>) and onion grass (<i>Allium vineale</i>)). 15-50% sub-clover content and < 15% broadleaf weeds. Fields were primarily planted as phalaris/ryegrass/sub-clover, except for one field that was planted as kikuyu/ryegrass/sub-clover.
Ryegrass/sub-clover (<i>n</i> =13)	Composed of thin-blade grasses (as described above), 15-50% sub-clover content and < 15% broadleaf weeds. Fields were planted with ryegrass (and in some cases other thin-blade grasses such as cocksfoot) and sub-clover.
Weedy (<i>n</i> =12)	Composition of broadleaf weeds in fields was > 15%. Broadleaf weeds were capeweed, mallow, dandelion, Patterson's curse, plantain and thistles. Other plants in these fields were thin-bladed grasses (primarily ryegrass), lucerne and sub-clover. No fields contained thick-blade (phalaris or kikuyu) grasses.
Lucerne (<i>n</i> =9)	Lucerne with no/low amounts of broadleaf weeds (<15%) and sub-clover (<15%). Other than lucerne, thin-blade grasses (as described above) were common. One field had >15% erodium and one field had >15% nightshade.
Grassy (<i>n</i> =7)	Composed of thin-blade grasses (primarily ryegrass, barley grass, cocksfoot, onion grass). Two fields also contained > 20% of the thick-blade grass, phalaris. These fields were planted as ryegrass/sub-clover or phalaris/ryegrass/sub-clover. The sub-clover had either completely died out over time or did not predominate and was < 15%. Broadleaf weeds were also < 15%.
Sub-clover (<i>n</i> =6)	Sub-clover composition > 50%. This included four fields sown solely to sub-clover and two fields where lucerne was undersown with sub-clover.

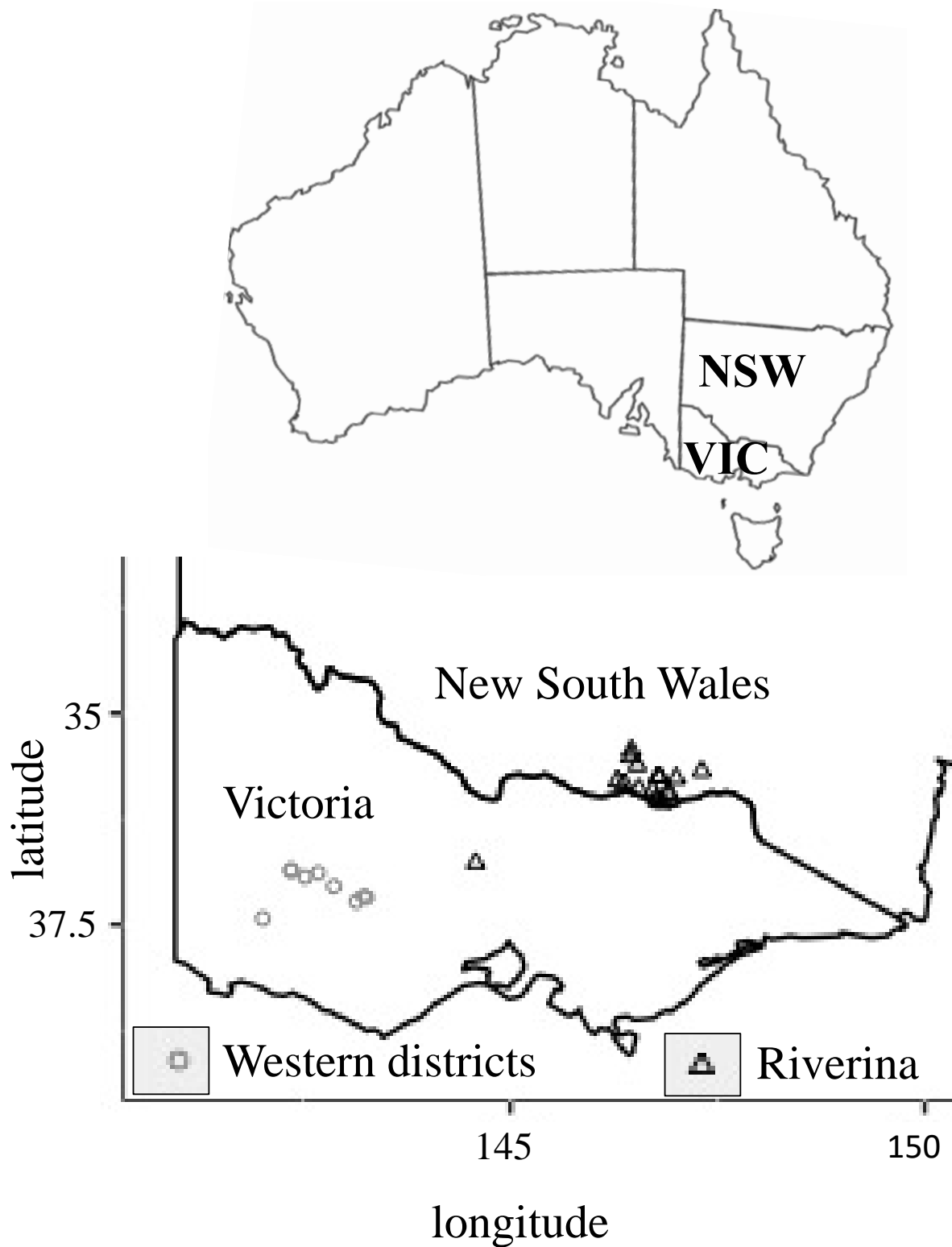


Figure 2.1. Map of Victoria (VIC) and southern New South Wales (NSW) showing the distribution of farms sampled. These farms fall into two distinct regions, the Western District (western Victoria) and the Riverina (south-eastern New South Wales and north-eastern Victoria) with the exception of one point in the Goulburn Valley which, due to climate similarity, was combined with Riverina for analysis.

2.3.4 Data analysis

We analysed data by considering the density of each mite species (\log_{10} transformed mites per m^2) and tested the predictive variables: field type, WHC, region, rainfall and the year sampled. All variables were treated as categorical except for rainfall which was treated as continuous. We conducted an initial analysis of the effect of mite densities using all individual variables in a single general linear model (categorical variables, logit link) or regressions (continuous variables) (R studio, stats package). Variables were considered significant with p -values < 0.05 . Boxplots and scatterplots were used to visualise the relationship between densities and predictive variables (R studio, ggplot2 package). A final model (incorporating all variables and treating each state of the categorical variable as a dummy variable) was then constructed through a process of forward regression. This analysis was only carried out when using the field type variable, rather than the co-correlated composition variables.

For *P. major*, we found a regional effect, thus pasture variables were assessed using regional datasets. We focus on the results from Riverina (the largest dataset and the region with the highest *P. major* densities). The relative abundance of *H. destructor* and *P. major* in fields was also assessed for the Riverina dataset. Due to competitive interactions that can exist between these two species, we also tested factors affecting the proportion of *H. destructor* in each field, tested against the total number of *H. destructor* + *P. major*.

2.4 Results

2.4.1 Preliminary analysis with vegetative variables (see Supplementary material 2)

For *H. destructor*, the abundance of broadleaf weeds was the only vegetative variable that was significantly associated with mite density across fields ($p < 0.05$). Plant-type (thin-blade grasses, thick-blade grasses, lucerne and sub-clover) and plant production variables (bare earth and food on offer) were not significantly associated (Supplementary material 2).

For *P. major*, densities varied with region ($p < 0.001$), with higher median densities in the Riverina than Western District. In fields in the Riverina, *P. major* densities were significantly associated with thin-blade ($p < 0.001$) and thick-blade grasses ($p < 0.01$). In fields in the

Western District, broadleaf weeds were associated with *P. major* densities. Other plant-types and plant production variables were not significantly associated with *P. major* densities in either region (Supplementary material 2).

2.4.2 *Halotydeus destructor* analysis based on field-type

The main factor contributing to *H. destructor* densities in pasture fields was field type ($p < 0.001$) (Table 2). High densities of *H. destructor* were found in phalaris/ryegrass/sub-clover ($2120 \text{ per m}^2 \pm 474 \text{ (SE)}$), ryegrass/sub-clover ($1733 \text{ per m}^2 \pm 666$) and weedy ($1931 \text{ per m}^2 \pm 462$) fields. Low densities were found in in lucerne ($85 \text{ per m}^2 \pm 30$), grassy ($40 \text{ per m}^2 \pm 22$) and sub-clover ($154 \text{ per m}^2 \pm 75$) fields This constitutes a 20-fold difference in the mean densities found in these field-types (Figure 2.2).

WHC was associated with *H. destructor* densities but to a much weaker extent. The effect of a high WHC varied between field types, with higher densities in phalaris/ryegrass/sub-clover fields ($F_{1,13}=6.90, p=0.02$) but no difference in ryegrass/sub-clover ($F_{1,13}=0.07, p=0.80$) or grassy fields ($F_{1,13}=0.20, p=0.67$) (Figure 3). However, in the GLM there was no significant interaction between WHC and field type ($p=0.104$). Other factors were also not significant, and in a multiple regression with forward variable selection only field type and WHC showed significance.

Table 2.3. The significance of selected variables explaining the density of *H. destructor* and *P. major* in pasture fields as assessed through linear models. The *p*-values < 0.05 are bolded. The significance of variables is shown using the total dataset for *H. destructor*. For *P. major*, data has been divided into regions. WHC = water holding capacity of the soil.

Species (region)	Variable	df 1	df 2	<i>F</i>	<i>P</i>
<i>H. destructor</i>	Year	1	52	0.5	0.48
	Region	1	52	0.42	0.52
	Field type	5	52	8.11	<0.001
	WHC	1	52	4.67	<0.05
	Rainfall	1	52	0.49	0.49
<i>P. major</i> (Riverina)	Year	1	32	1.05	0.31
	Field type	5	32	4.25	<0.01
	WHC	1	32	2.55	0.12
	Rainfall	1	32	0.01	0.93
<i>P. major</i> (Western District)	Year	1	12	0.24	0.63
	Field type	5	12	3.93	<0.05
	WHC	1	12	1.43	0.25
	Rainfall	1	12	3.88	0.07

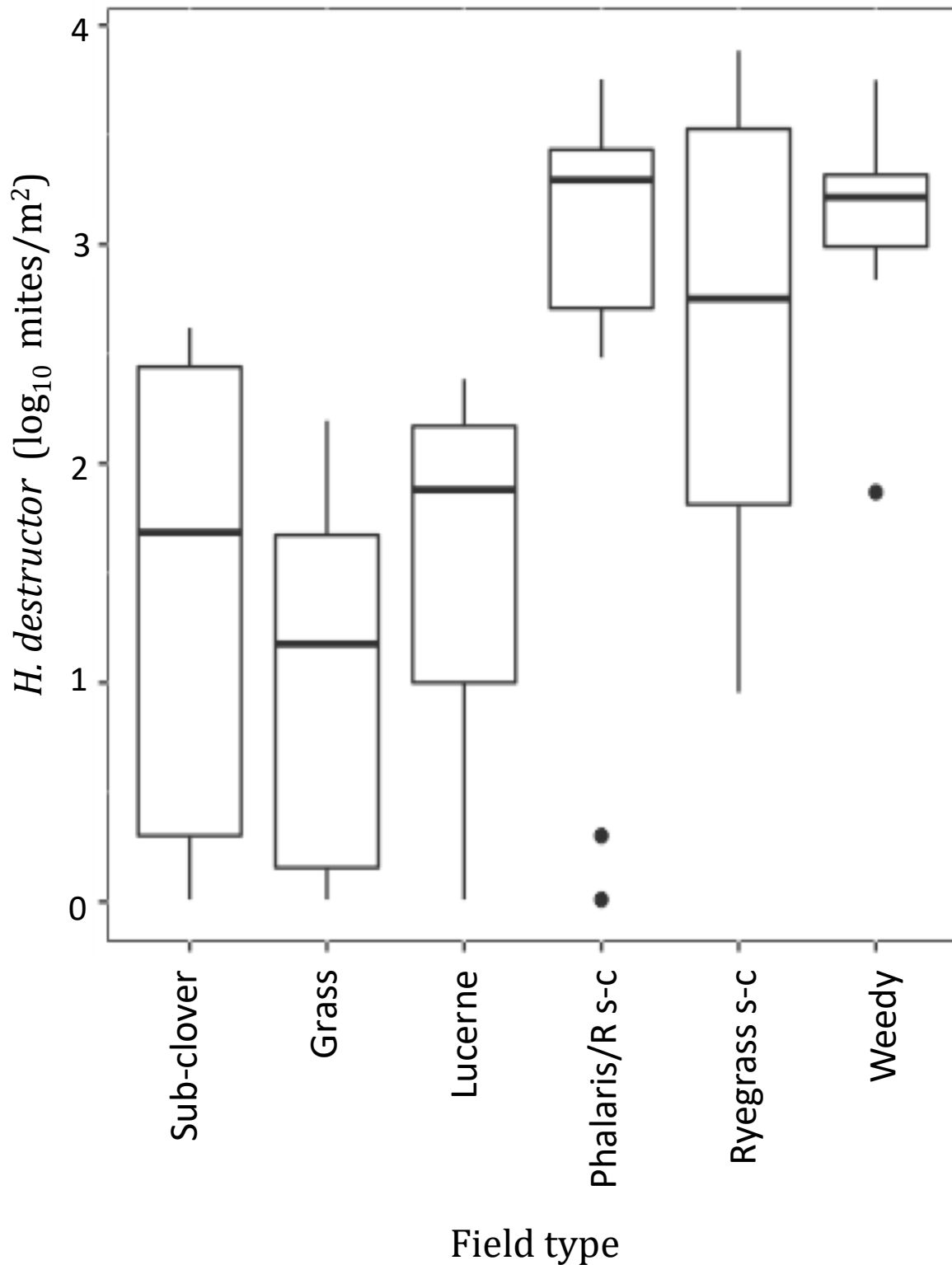


Figure 2.2. Boxplots of *H. destructor* log₁₀ transformed data (mites per m²) comparing densities across six field types. The horizontal line of the boxplot shows the median value, the edges show interquartile ranges, whiskers show the 95% confidence interval. Closed circles show outlier values outside of this interval. Phalaris/ryegrass/sub-clover is abbreviated to Phalaris/R s-c and Ryegrass/sub-clover to Ryegrass s-c.

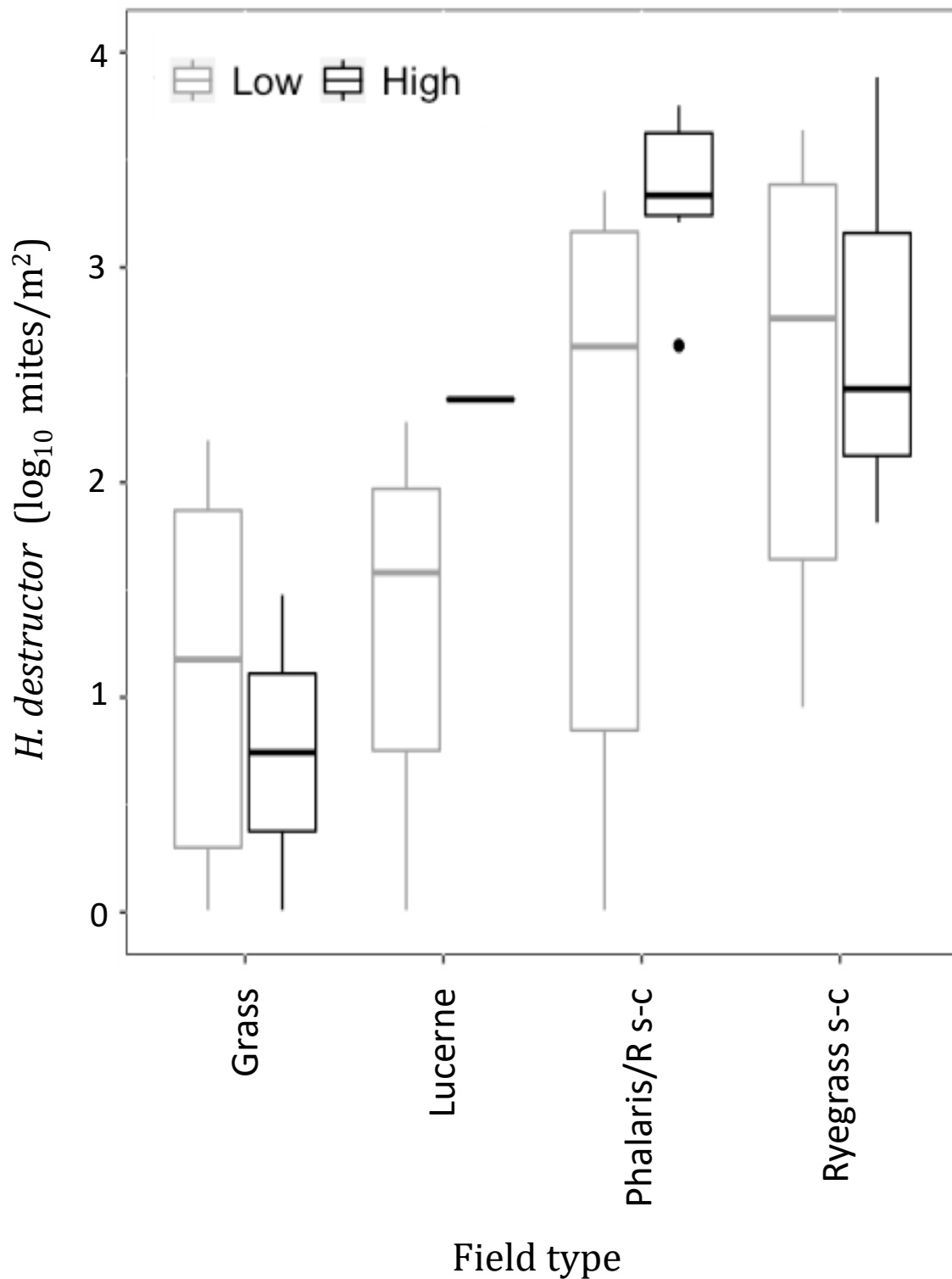


Figure 2.3. Boxplots of *H. destructor* \log_{10} transformed data (mites per m^2) in fields with low (50-80mm) and high (80-130mm) WHC. The horizontal line of the boxplot shows the median value, the edges show interquartile ranges, whiskers show the 95% confidence interval. Closed circles show outlier values outside of this interval. Phalaris/ryegrass/sub-clover is abbreviated to Phalaris/R s-c and Ryegrass/sub-clover to Ryegrass s-c. WHC = water holding capacity of the soil.

2.4.3 *Penthaeus major* analysis based on field-type

For *P. major*, a significant regional effect was evident ($p < 0.001$). Within each region, field type was the only factor positively associated with mite densities (Table 2.3). Within the Riverina dataset, high densities of *P. major* were found in fields classified as phalaris/ryegrass/sub-clover (680 per $m^2 \pm 255$), ryegrass/sub-clover (275 per $m^2 \pm 93$), weedy (644 per $m^2 \pm 190$), lucerne (253 per $m^2 \pm 78$) and grassy (402 per $m^2 \pm 184$). Mean densities of *P. major* in sub-clover fields were by contrast 5-15-fold smaller (47 per $m^2 \pm 27$) (Figure 2.4). Other factors (year, WHC and rainfall) did not show a significant association with mite densities and were also not significant in a multiple regression once field type had been included in the model. The association with WHC was marginally non-significant ($p = 0.12$, or $p = 0.08$ when only field-types containing fields with high WHC were considered). Relatively higher densities of *P. major* were found in all field-types with high WHC although this effect was non-significant when analysed within individual field types (grassy: $F_{1,5} = 4.40$, $p = 0.09$; phalaris/ryegrass/sub-clover $F_{1,13} = 0.03$, $p = 0.87$; ryegrass/sub-clover $F_{1,11} = 2.61$, $p = 0.13$) (Figure 2.5).

Within the Western District dataset, mite densities were significantly lower although an effect of field type was again identified ($F_{(5,12)} = 3.93$, $p = 0.02$). Mean densities greater than 100 mites per m^2 were only found in phalaris/ryegrass/sub-clover (271 per $m^2 \pm 108$) ($n = 2$) and in weedy fields (132 per $m^2 \pm 50$) ($n = 7$) (Figure 6). The amount of rainfall had a marginally non-significant effect, and no association was found with the year sampled or WHC (Table 2.3).

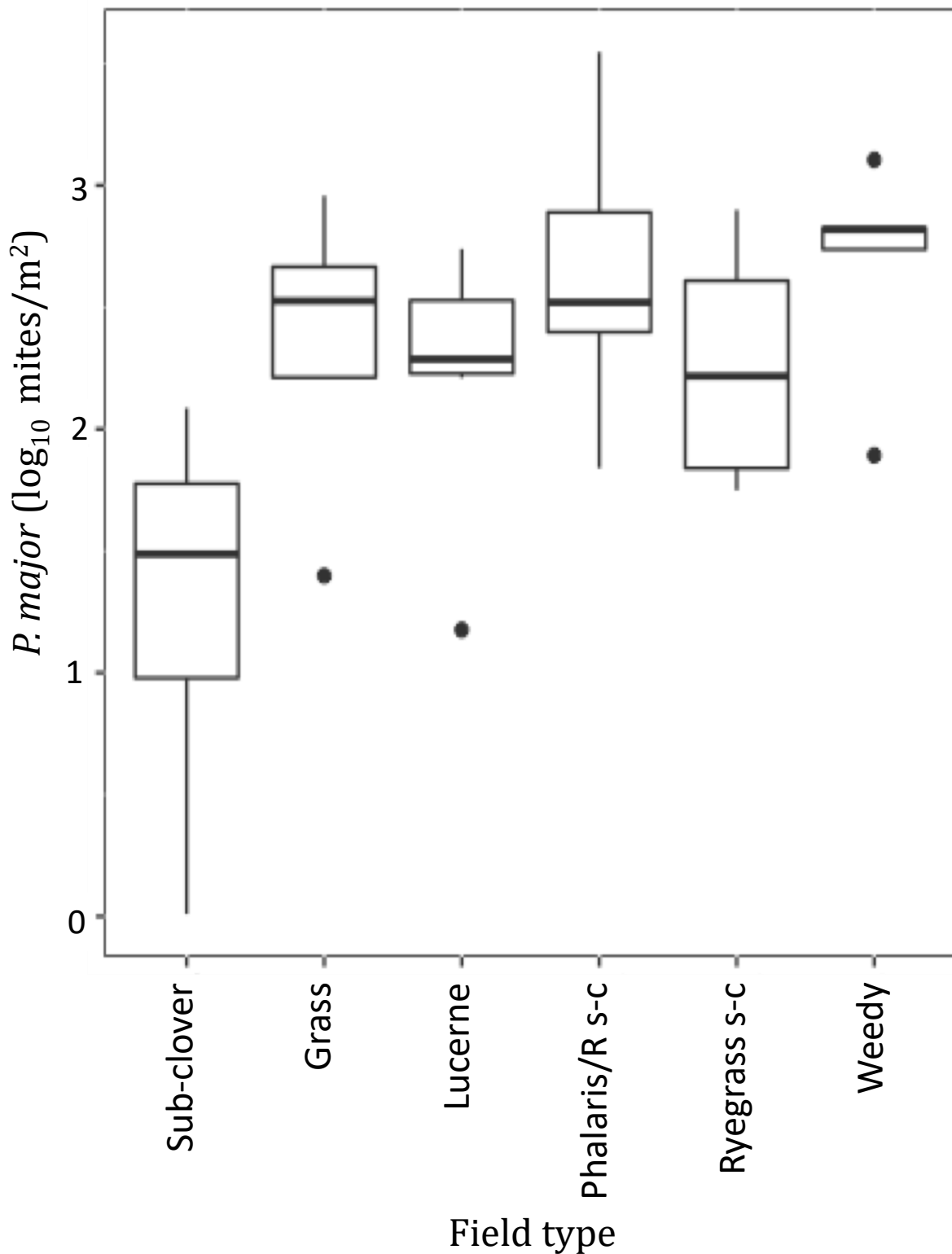


Figure 2.4. Boxplots of *P. major* log₁₀ transformed data (mites per m²) comparing densities across six field types in the Riverina. The horizontal line of the boxplot shows the median value, the edges show interquartile ranges, whiskers show the 95% confidence interval. Closed circles show outlier values outside of this interval. Phalaris/ryegrass/sub-clover is abbreviated to Phalaris/R s-c and Ryegrass/sub-clover to Ryegrass/s-c.

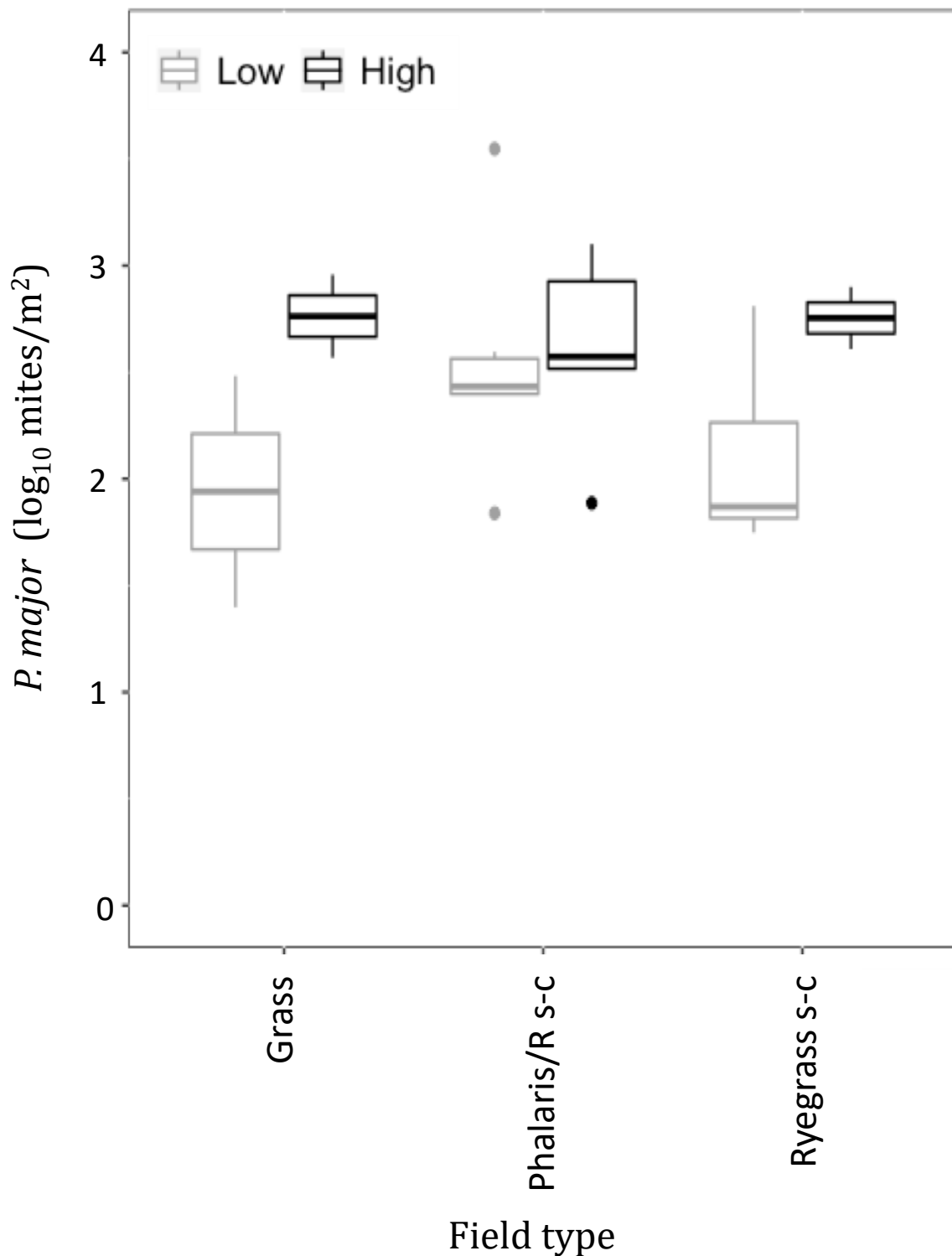


Figure 2.5. Boxplots of *P. major* log₁₀ transformed data (mites per m²) in fields with low (50-80mm) and high (80-130mm) WHC sampled in the Riverina. The horizontal line of the boxplot shows the median value, the edges show interquartile ranges, whiskers show the 95% confidence interval. Closed circles show outlier values outside of this interval.

Phalaris/ryegrass/sub-clover is abbreviated to Phalaris/R s-c. Ryegrass/sub-clover is abbreviated to Ryegrass/s-c. WHC = water holding capacity of the soil.

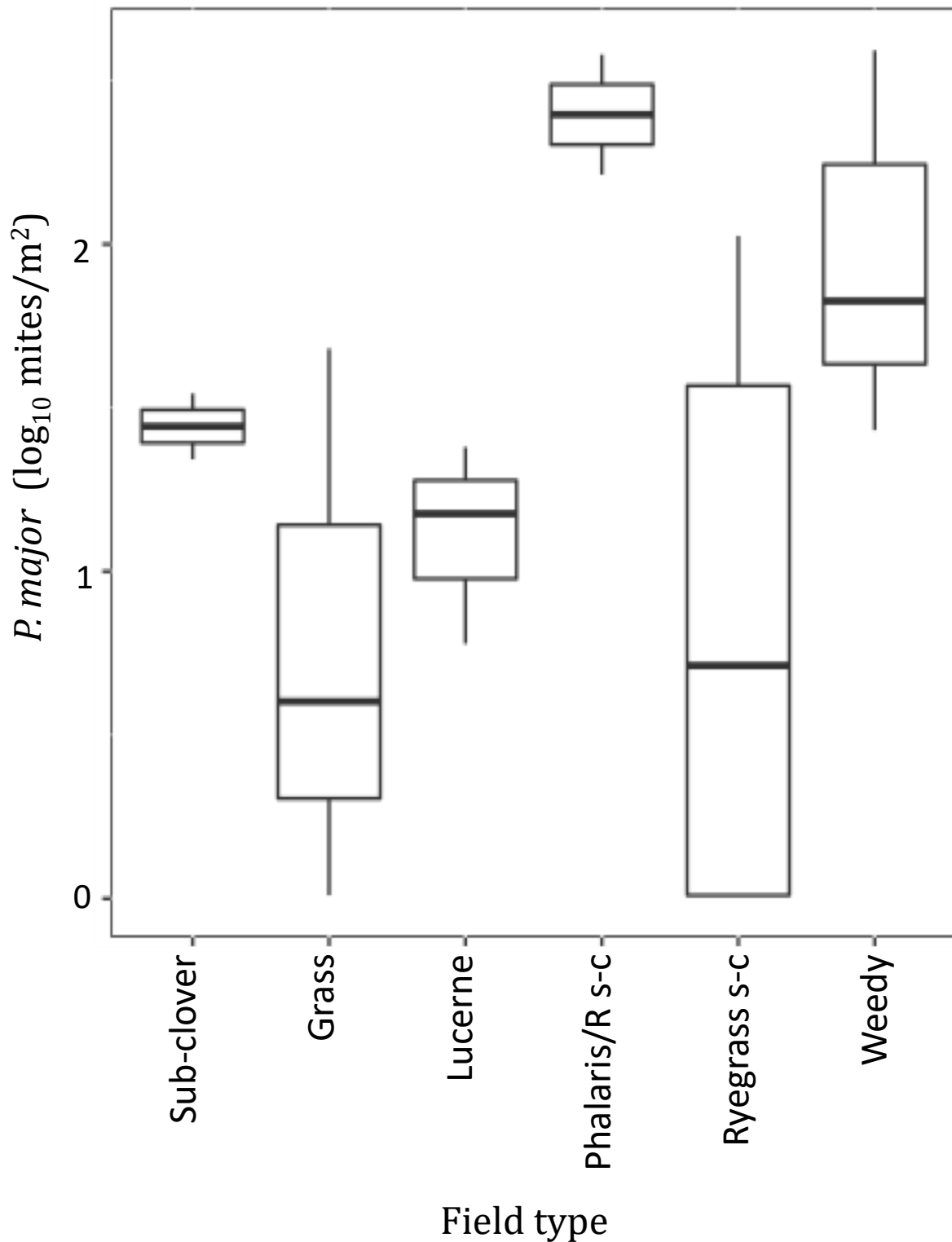


Figure 2.6. Boxplots of *P. major* \log_{10} transformed data (mites per m^2) comparing densities across six field types in the Western District. The horizontal line of the boxplot shows the median value, the edges show interquartile ranges, whiskers show the 95% confidence interval. Phalaris/ryegrass/sub-clover is abbreviated to Phalaris/R s-c and Ryegrass/sub-clover to Ryegrass/s-c.

2.4.4 Relative abundance of *H. destructor* and *P. major* in the Riverina

H. destructor densities showed far more variation between fields than *P. major*. For *H. destructor*, eight fields (19%) had lower than 10 mites per m², but 18 fields (43%) had densities greater than 1000 per m². For *P. major*, one field (2%) had less than 10 per m², but only three fields had densities greater than 1000 per m². The overall composition of mites ($H. destructor / (H. destructor + P. major)$) varied with field type (Table 2.4). Within the three categories of field-type where *H. destructor* flourished (phalaris/ryegrass/sub-clover, ryegrass/sub-clover and weedy), the average proportion of *H. destructor* was around 70-75%. *Pentthaleus major* predominated to the same extent in lucerne and grassy fields. Mite densities of both species were equally low in sub-clover fields (Figure 2.7).

Table 2.4. The significance of selected variables explaining the relative abundance of *H. destructor* versus *P. major* ($H. destructor / (H. destructor + P. major)$) in fields sampled in the Riverina as assessed through linear models. The *p*-value less than 0.05 is bolded. WHC stands for water holding capacity of the soil.

Variable	df 1	df 2	<i>F</i>	<i>p</i>
Year	1	32	0.97	0.33
Field type	5	32	3.61	0.01
WHC	1	32	0.04	0.85
Rainfall	1	32	0.29	0.66

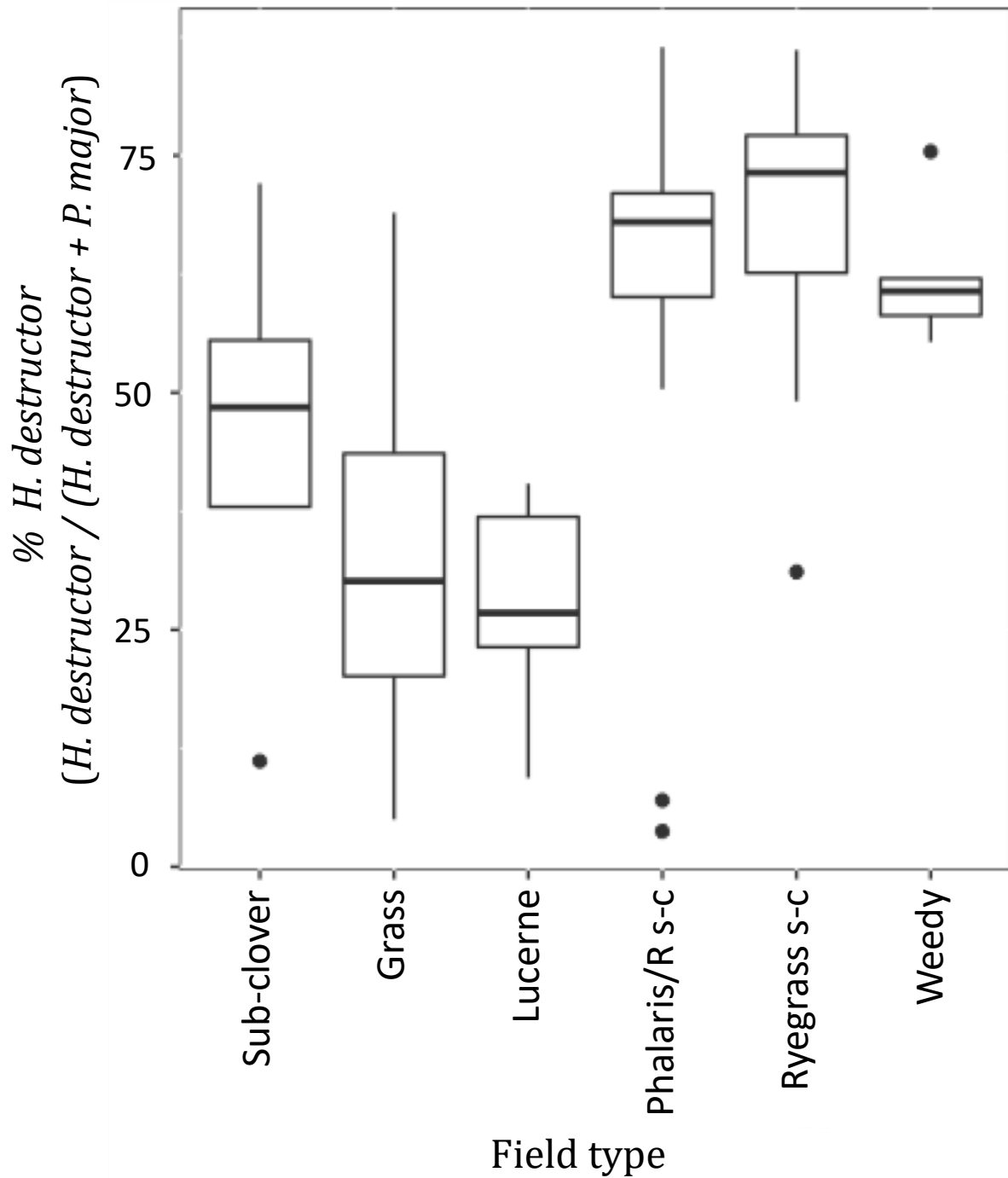


Figure 2.7. Boxplots showing the proportion of *H. destructor* versus *P. major* ($H. destructor / (H. destructor + P. major)$) across six field types in the Riverina. The line and edges of the boxplots show median and interquartile ranges with whiskers showing the 95% confidence intervals. Phalaris/ryegrass/sub-clover is abbreviated to Phalaris/R s-c and Ryegrass/sub-clover to Ryegrass/s-c.

2.5 Discussion

This study assessed whether agronomic and/or environmental variables could be used to predict the population size of the first generation of the key mite pests, *H. destructor* and *P. major*, in pasture fields. For *H. destructor*, the results are encouraging. Estimates of whether high densities of *H. destructor* would be present in fields could be made across two diverse farming regions using only the factor, field-type. For *P. major*, there was a strong regional effect between two farming districts, suggesting predictors will vary between regions. While a field-type effect was evident, *P. major* densities showed smaller fluctuations between field types, with only sub-clover-dominated fields harbouring consistently low mite densities.

For *H. destructor*, lucerne, grassy and sub-clover fields (fields that primarily had grasses or lucerne with minimal amounts of broadleaf weeds or sub-clover, or fields with solely sub-clover) had low to medium densities (0-333 mites per m²). Given *H. destructor* has been shown to quickly build up to the carrying capacity of a pasture (Ridsdill-Smith and Annells 1997, Gower et al. 2008, Ridsdill-Smith et al. 2013), this suggests these fields have a limited carrying capacity for *H. destructor*. Fields with a mix of grasses and sub-clover, and fields with a large broadleaf weed component had much higher densities (greater than 1000 mites per m²) of *H. destructor*, although low numbers were found in the occasional field.

Understanding the in-field factors leading to differences in mite densities between fields is complicated. Of the individual plants, only high levels of broadleaf weeds (defined as >15%) were related to higher mite densities. No association was found between *H. destructor* densities and sub-clover even though this plant is a known source of food and preferred oviposition site (Ridsdill-Smith and Annells 1997), factors thought to limit *H. destructor* population densities (Ridsdill-Smith and Annells 1997, Ridsdill-Smith et al. 2013). Ridsdill-Smith and Annells (1997) were also unable to find an association between mite density with sub-clover in a temporal study across four pastures. Furthermore, sub-clover fields only had low numbers of *H. destructor*, suggesting a mix of grasses and sub-clover may be needed for high *H. destructor* densities. A mixture of plants may provide benefits such as a humid micro-environment (Ridsdill-Smith and Pavri 2000), or by increasing the survival of larvae by promote the growth of microflora (MacIennan et al. 1998).

Several factors considered important for *H. destructor* were not found to be significantly associated with densities in our study. This appears to be due to the overwhelming influence

of field-type. Rainfall during late winter and early spring was not associated with densities even though it is known to promote plant growth and create a humid micro-environment, essential for the survival of juvenile mites as temperatures increase (Solomon 1937b). The water holding content of the soil was weakly significant but appeared to promote increased mite densities in some field-types but not others, indicating there may be an interaction between plant type and soil/rainfall variables. No relationship was found between the amount of food on offer during spring and mite densities across field-types, despite the fact mite densities have been shown to multiply with plant resources (or food on offer) during spring (Grimm et al. 1995) in ryegrass/sub-clover fields (Supplementary material 2). Furthermore, there are a number of variables that were not measured in this study which we know may impact mite abundance. Most importantly, competition between species (Weeks and Hoffmann 2000, Umina and Hoffmann 2005), the role of predatory organisms (Michael et al. 1991, Halliday 2005) and the use of pesticides (Umina and Hoffmann 1999, Robinson and Hoffmann 2000, Umina et al. 2010). In this study, insecticides had been used in a limited number of fields, with applications occurring in the winter prior to our sampling. As insecticide efficacy is highly dependent on the timing of the spray application (Umina and Hoffmann 2003, Ridsdill-Smith et al. 2005) we removed this variable from our analysis after initial consideration (Supplementary material 2).

For *P. major*, the story is different with the strongest effect being a regional difference between fields sampled, with higher numbers found in the Riverina compared with the Western District. Regional effects complicate the ability to predict mite densities. There are many possible reasons for this, including regional fitness differences between genetic clonal types (see Robinson et al. 2002). Field-type showed a weaker association with *P. major* densities than for *H. destructor*, with only sub-clover fields having consistently low densities across fields irrespective of region. Across all fields in the Riverina, *P. major* was found in numbers less than 10 mites per m² in only one field. Both thin-blade and thick-blade grasses were positively related to *P. major* densities (Supplementary material 2). The presence grasses in fields is purported to allow *P. major* populations to survive in drier areas of inland New South Wales (Robinson and Hoffmann 2001) and may be sufficient for *P. major* densities to persist in pasture fields.

Field type determined the species composition of mites in almost all fields, although to some extent this was due to extremes in densities found for *H. destructor*, with *P. major* a more

constant presence. Interspecific effects have been shown to shape the densities of these two earth mites in both crop and pasture fields (Weeks and Hoffmann 2000), with different plants showing considerably different suitability (Umina and Hoffmann 2004). *Pentthaleus major* does not typically feed on sub-clover or broadleaf weeds (Umina and Hoffmann 2004) and this may explain why *H. destructor* predominated in field-types with a significant proportion of broadleaf weeds and/or sub-clover. However, the extent of this relation needs to be investigated over several years given seasonal effects have also been shown to effect intraspecific and interspecific effects on mite abundance (Weeks and Hoffmann 2000).

Predicting the densities of pest mites in pasture fields is complex and requires further research on which variables are important at different time periods throughout the year. The ability to accurately forecast *H. destructor* densities would allow farmers to better focus monitoring practices to those fields with the highest level of risk. We believe the information generated here could be expanded upon, and ultimately be used to create a framework for managing *H. destructor*. This would be particularly powerful if used in collaboration with a recently constructed model predicting the emergence of the first generation of mites (McDonald et al. 2015) and in combination with best sampling techniques (Arthur et al. 2004, Nansen et al. 2015). A predictive framework will also help farmers to assess the mite species likely to be most abundant in a given field. This information is essential in order to assess the appropriate pest management practices given *H. destructor* and *P. major* differ greatly in their sensitivity to chemicals (Umina and Hoffmann 1999, Robinson and Hoffmann 2000, Umina et al. 2010). This information should therefore help farmers guide their pest management practices and reduce their reliance on insecticides. With emerging insecticide resistance issues in *H. destructor* (Umina 2007, Umina et al. 2012, 2017, Maino 2018a, b), as well as several other major Australian pest species, this information is important.

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2.7 Supplementary material 1- Peak larval emergence sampling

Peak larval emergence of first generation of *H. destructor* and *P. major* in fields were confirmed by monitoring mite life-stages in vacuum samples of vegetation in the period leading up to the expected hatch date (as guided by an emergence model for *H. destructor* (McDonald et al. 2015)). A uniform patch of vegetation (either in a field or at a field-edge) was sampled on a roughly weekly basis (3-17days). Vacuum sampling was conducted by pressing the collection sieve onto the soil surface for a period of 5 seconds. During 2014, 200 suction points were made over 200metres. In 2015 this was reduced to 60 suction points over 100metres. The life-stages of mites collected in these vacuum samples were counted under a binocular microscope. Larvae were distinguished as they have six rather than eight legs. Other life-stages were distinguished by size (Baker 1995). Sampling continued until peak larval emergence was determined.

Penthaleus major is known to emerge at a similar time as *H. destructor* (Umina et al 2003) but may emerge slightly earlier (James and O'Malley 1993). During 2014 and 2015, sampling at sites in Victoria and southern New South Wales suggests any difference between the timing of larval emergence in these species was less than a week. Here we present three sites for each year. These sites are presented as sites where both species were similarly represented.

Results and discussion

The timing of peak larvae emergence was similar for *H. destructor* and *P. major* during both 2014 (Figures 2.8 a-c) and 2015 (Figures 2.9 a-c). During 2014, we found maximal numbers of larvae of both species at all sites on the 15th of April (best shown in Figure 2.8 a,b). During 2015, our sampling appeared to miss the peak larval hatch with higher numbers of early nymphs found on the 1st and 6th of May than larvae found at the previous sampling. The proportion of the sizes of nymphs was similar for both species at all three sites (Figures 2.9 a-c). At no site were distinctly different life stages found between species. Based on the life-cycle of individual mites, the timing of peak emergence between *H. destructor* and *P. major* in 2014 and 2015 was likely only a matter of days in the areas sampled.

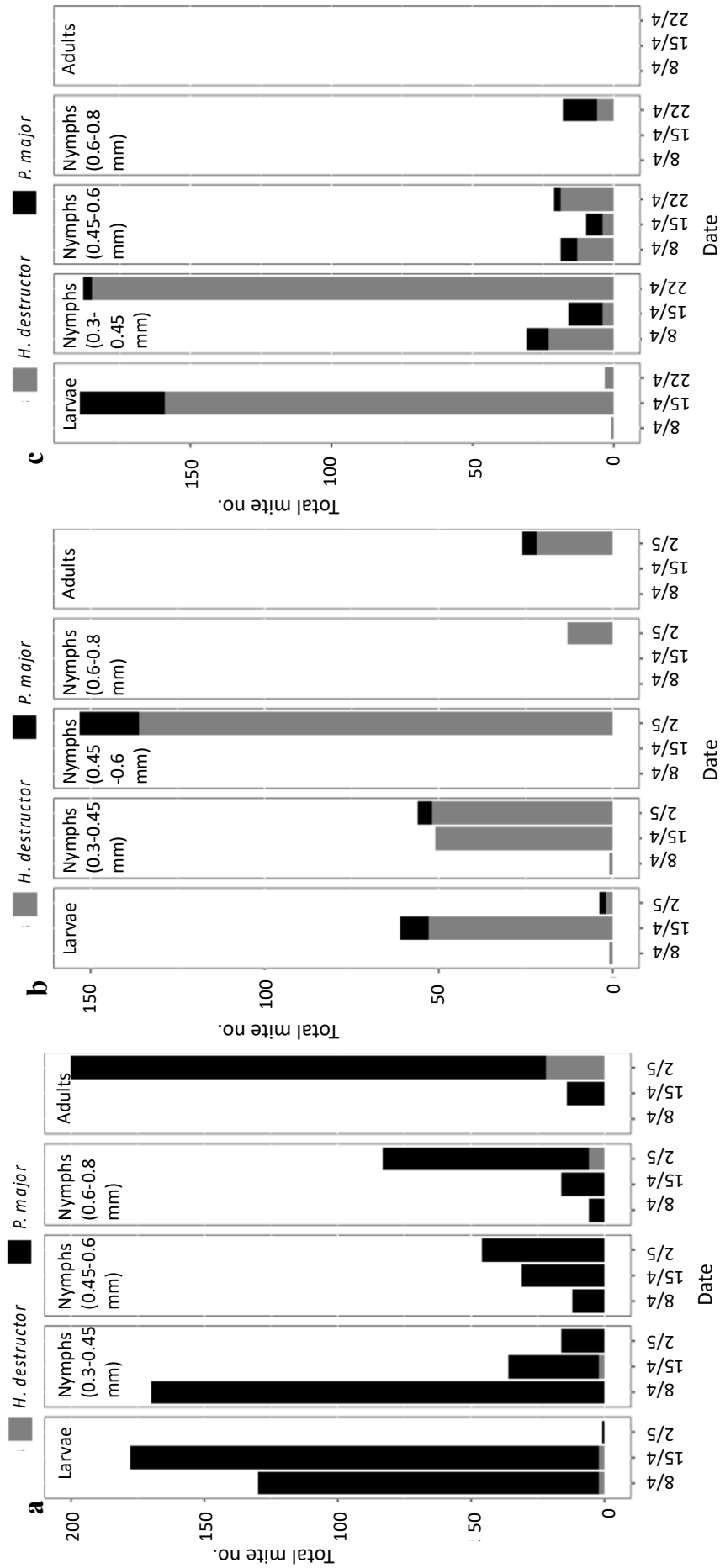


Figure 2.8 Numbers of different life-stages of *H. destructor* and *P. major* collected in 2014 from a) a pasture near Albury, NSW (36.05, 146.84), b) outside a pasture near Seymour, Vic (36.99, 145.14), c) a pasture near Bylands, Vic (37.35, 144.92)

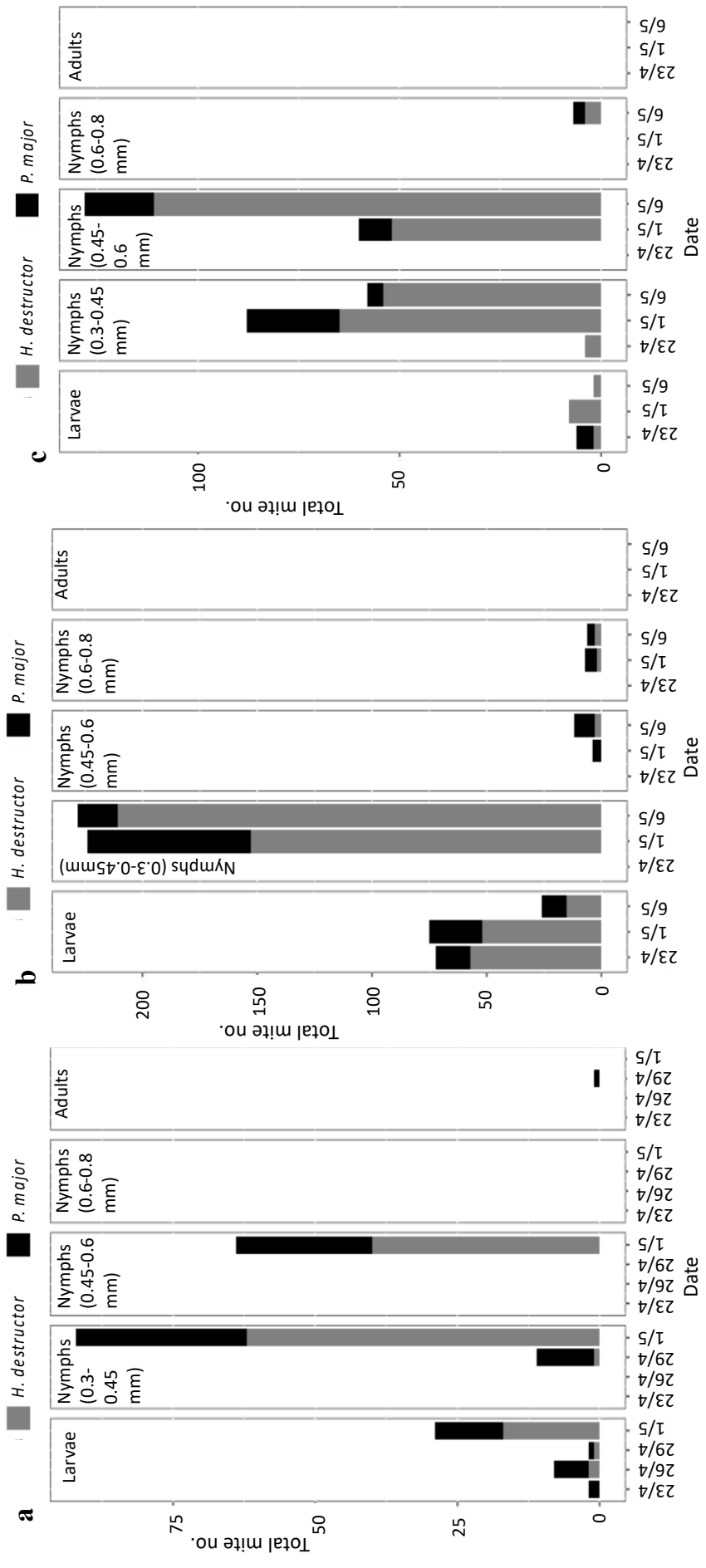


Figure 2.9. Numbers of different life-stages of *H. destructor* and *P. major* collected in 2015 from pasture fields near a) Albury, NSW (36.05, 146.84) b) Moorwatha, NSW (35.92, 146.72) c) Rutherglen, Vic (36.10, 146.50)

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2.8 Supplementary material 2 - Preliminary analysis with vegetative variables

We analysed mite density data against several vegetative variables: plant type (as described by the percentage of plant groups), pasture thickness (as described by the proportion of bare earth in a field) and resource limitation in spring (as described by the amount of food on offer in spring).

Five variables reflecting plant-type were constructed from the five main plant groups (thin-blade grasses, thick-blade grasses, sub-clover, lucerne and broadleaf weeds) (Table 2.5). Thin-blade grasses, thick-blade grasses, lucerne and broadleaf weeds were divided in to two categories, sub-clover was divided in to three based on between-field variation in plant composition (Table 2.5). All plants could be grouped into these categories with the exception of *Erodium* spp. and *Solanum* spp. that were present as weeds in three fields (>15% composition). These plants were removed from analysis as they are not considered to be biologically important for these mites.

The proportion of bare earth in fields during peak production in the previous year was described in two categories. Food on offer in spring was placed in three categories (Table 2.5). When this information was unable to be quantified by the farmer, it was estimated by discussing fluctuations in pasture growth during the past year in comparison to the state of the pasture at the time of sampling, using direct measures (as described in the main document). To estimate food on offer we referred to the Australian Wool Innovation Limited guide (Australian Wool Innovation Limited 2012).

Table 2.5. Agronomic and environmental variables used in the preliminary analysis of *H. destructor* and *P. major* densities.

Variable	Composition of variable and rationale of categories	Field categories
Thin-blade grasses	Present in all fields, primarily <i>L. rigidum</i> , also <i>H. spontaneum</i> , <i>D. glomerate</i> and <i>A. vineale</i> . Composition in fields was highly dependent on the relative abundance of other plants.	Composition of pasture 1) < 50%, 2) > 50%
Thick-blade grasses	<i>P. aquatica</i> present in 22 fields and <i>P. clandestinum</i> in one field. Composition in fields varied from 20-40%.	Composition of pasture 1) < 20%, 2) > 20%
Lucerne	<i>M. sativa</i> present in 16 fields. In 13 fields varied from 30-100% composition of pasture, while in 3 fields < 20%.	Composition of pasture 1) < 20%, 2) > 20%
Sub-clover	<i>T. subterraneum</i> present in almost all fields. Composition varying from minimal to the primary plant.	Composition of pasture 1) < 15%, 2) 15-30%, 3) > 30%
Broadleaf weeds	Present in most fields, primarily <i>A. calendula</i> , also <i>Malva</i> spp., <i>Taxacum</i> spp., <i>E. plantagineum</i> and <i>Plantago</i> spp. Composition was highly variable between fields and through the year. In 12 fields, the composition was >15% through previous year.	Composition of pasture through previous year. 1) < 15%, 2) > 15%
Food on offer during spring	The amount of plant matter for grazing (food on offer) in spring was highly variable depending on plant composition, environmental conditions and agronomic practices.	Maximum food on offer the previous spring 1) < 1.4t/ha, 2) 1.4-4t/ha, 3) > 4t/ha
Bare earth	Proportion of bare earth was highly variable between fields and through the year. This variable assessed proportion during peak production in the previous year.	Proportion of pasture during peak production the previous year 1) < 15%, 2) > 15%
Soil water holding capacity	Varies with a range of soil properties. Data accessed from Australian Soil Resource Information System database (ASRIS 2011).	1) 50-80mm 2) 80-131mm

Rainfall	Rainfall from 16 th of August until 15 th of October. Data accessed from Australian Water Availability Project (AWAP) (Raupach et al. 2012).	Quantitative rainfall data
Region	Sites were grouped into large geographical regions encompassing approximately 100 km.	1) Riverina, 2) Western District
Year sampled	Sampling was conducted during May or June in 2014 or 2015	1) 2014, 2) 2015
Insecticide use	Not used in any field in the year of sampling. Used in 12 fields in the previous year, however 3 fields were sprayed out of the typical season for mites (prior to April and after October 15 th). In the remaining 9 fields, all but one application occurred in winter.	1) sprayed 2) not sprayed (NB: removed after initial analysis).

Data analysis

We analysed the data by considering the density of each mite species (log₁₀ transformed mites per m²) and tested all predictive variables. This included the individual plants (clover, broadleaf weeds, lucerne, phalaris), the pasture production variables (bare earth and food on offer), the soil variables (clay content and water holding capacity), environmental variables (region and rainfall) as well as the year (Table 2.5). All variables were treated as categorical except for rainfall which was continuous. We conducted an initial analysis of the effect of mites per m² using individual variables in general linear models (categorical variables logit link) or regressions (continuous variables).

Variables were considered significant at $p < 0.05$. Boxplots and scatterplots were used to visualise the relationship between densities and predictive variables (R studio, *ggplot2* package). For *P. major*, we found a regional effect, thus the pasture variables were assessed using regional datasets.

Results

H. destructor

For *H. destructor*, the abundance of broadleaf weeds was the only vegetative variable significantly associated with mite density across fields ($p < 0.05$) (Figure 2.10). Of the other plant-types, lucerne had a weakly non-significant negative association ($p = 0.07$), while other plant types (thin-blade grasses, thick-blade grasses and sub-clover) showed no evidence of an association. The proportion of bare earth in a field or the amount of food on offer was not significantly associated (Table 2.6).

As found in the analysis using field-type (main document, Table 2.3), year, region and rainfall showed no association, while soil water-holding capacity was weakly associated ($p < 0.05$) (Figure 2.11).

P. major

For *P. major*, densities varied with region ($p < 0.001$), with higher median densities in the Riverina than Western Districts (Supplementary material 2 Figure 2.12). In fields in the Riverina, *P. major* densities were significantly associated with thin-blade ($p < 0.001$) (Figure 2.13) and thick-blade ($p < 0.01$) grasses (Figure 2.14). In fields in the Western District, broadleaf weeds were associated with *P. major* densities.

Other plant-types (thin-blade grasses, thick-blade grasses, lucerne and sub-clover) and plant production variables (bare earth and food on offer) were not significantly associated with *P. major* densities in either region (Table 2.6). Year and rainfall were non-significantly associated. Soil water-holding capacity was weakly associated with density in the Riverina ($p < 0.05$) but not in the Western District.

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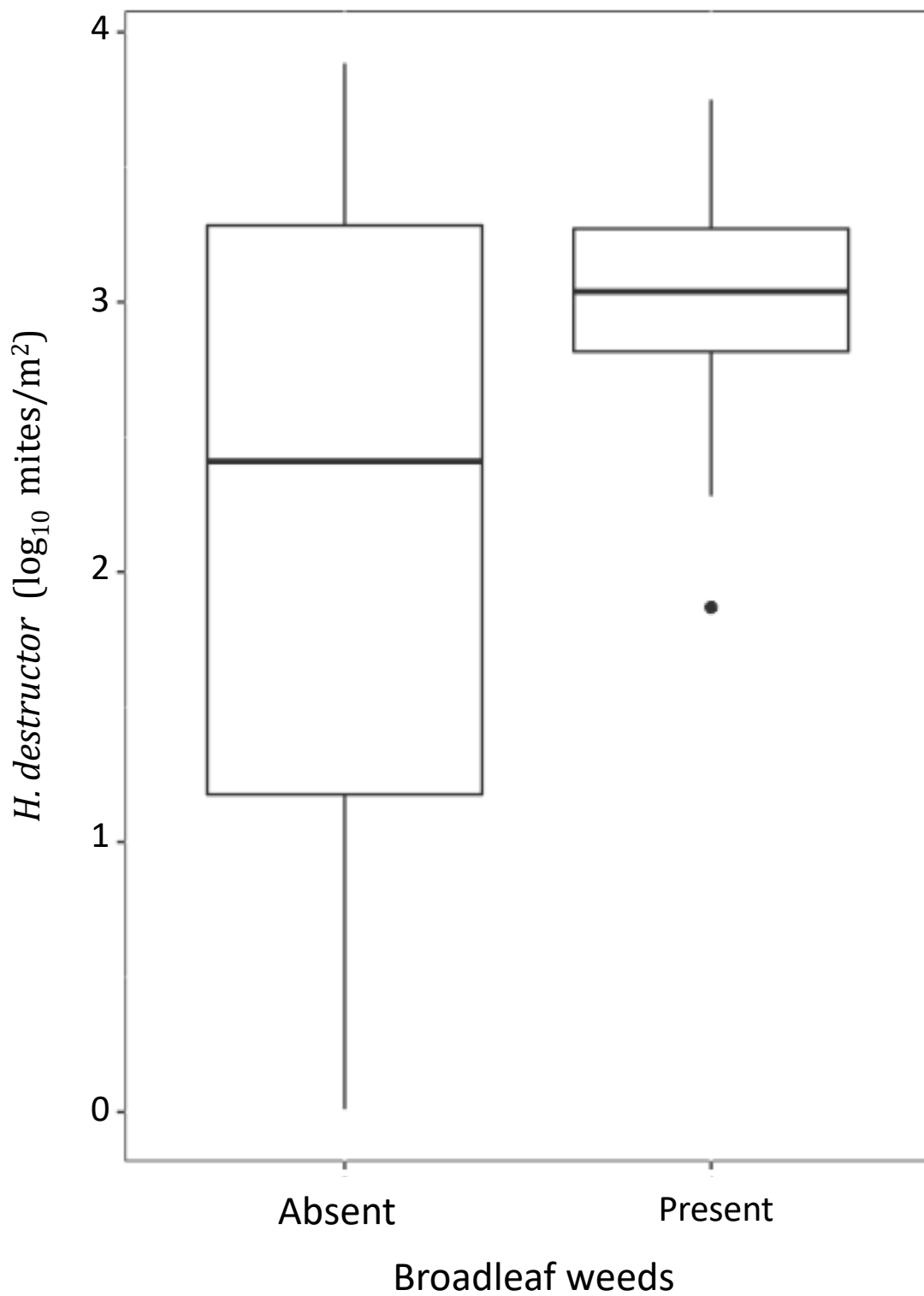


Figure 2.10. Boxplot of *H. destructor* log₁₀ transformed data (mites per m²) comparing densities against fields with a significant proportion of broadleaf weeds. The horizontal line of the boxplot shows the median value, the edges show interquartile ranges, whiskers show the 95% confidence intervals. Closed circles show outlier values outside of this interval.

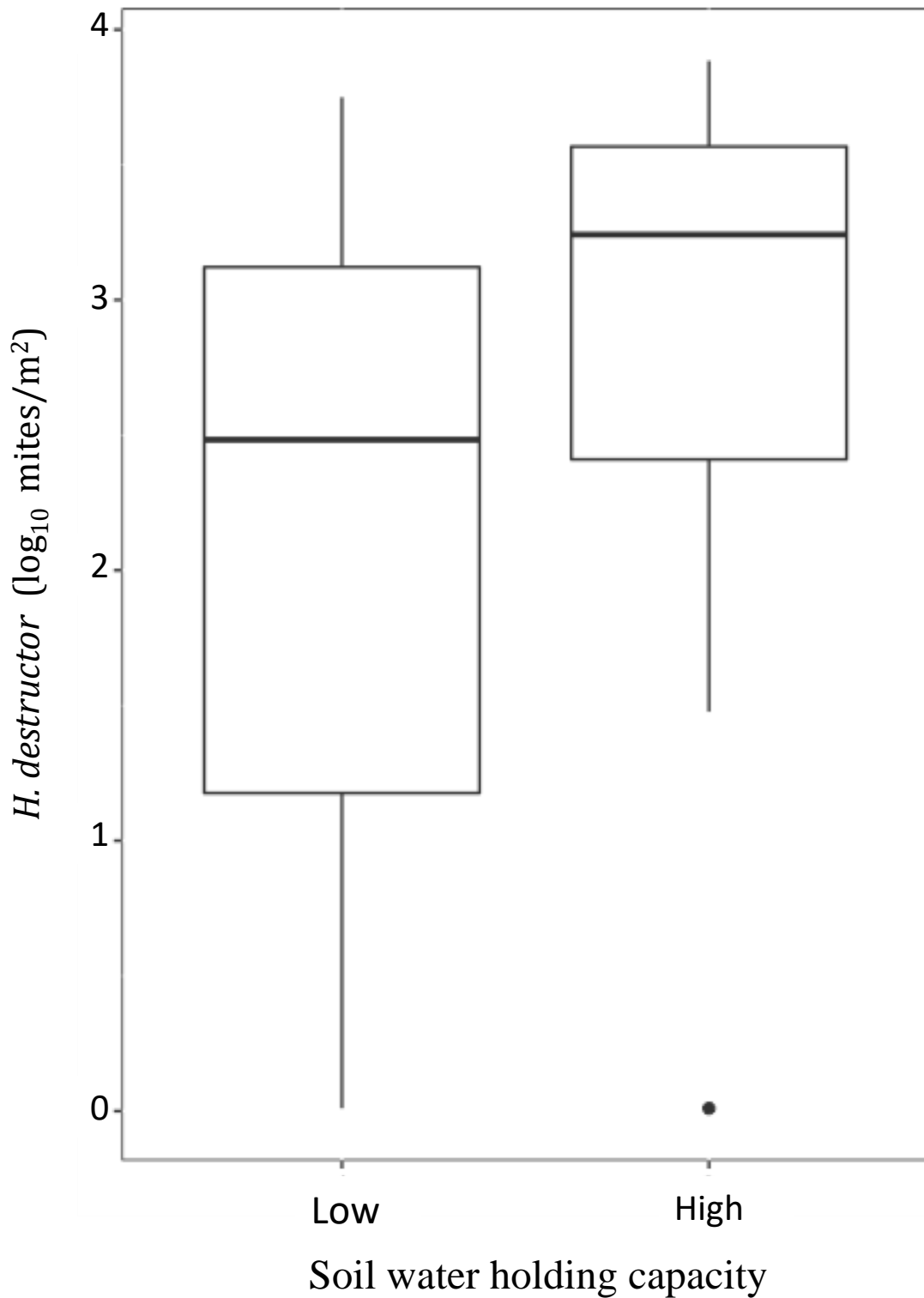


Figure 2.11. Boxplot of *H. destructor* \log_{10} transformed data (mites per m^2) comparing densities of mites in fields with soils of low (50-80mm) and high (80-130mm) water holding capacity. The horizontal line of the boxplot shows the median value, the edges show interquartile ranges, whiskers show the 95% confidence intervals. Closed circles show outlier values outside of this interval.

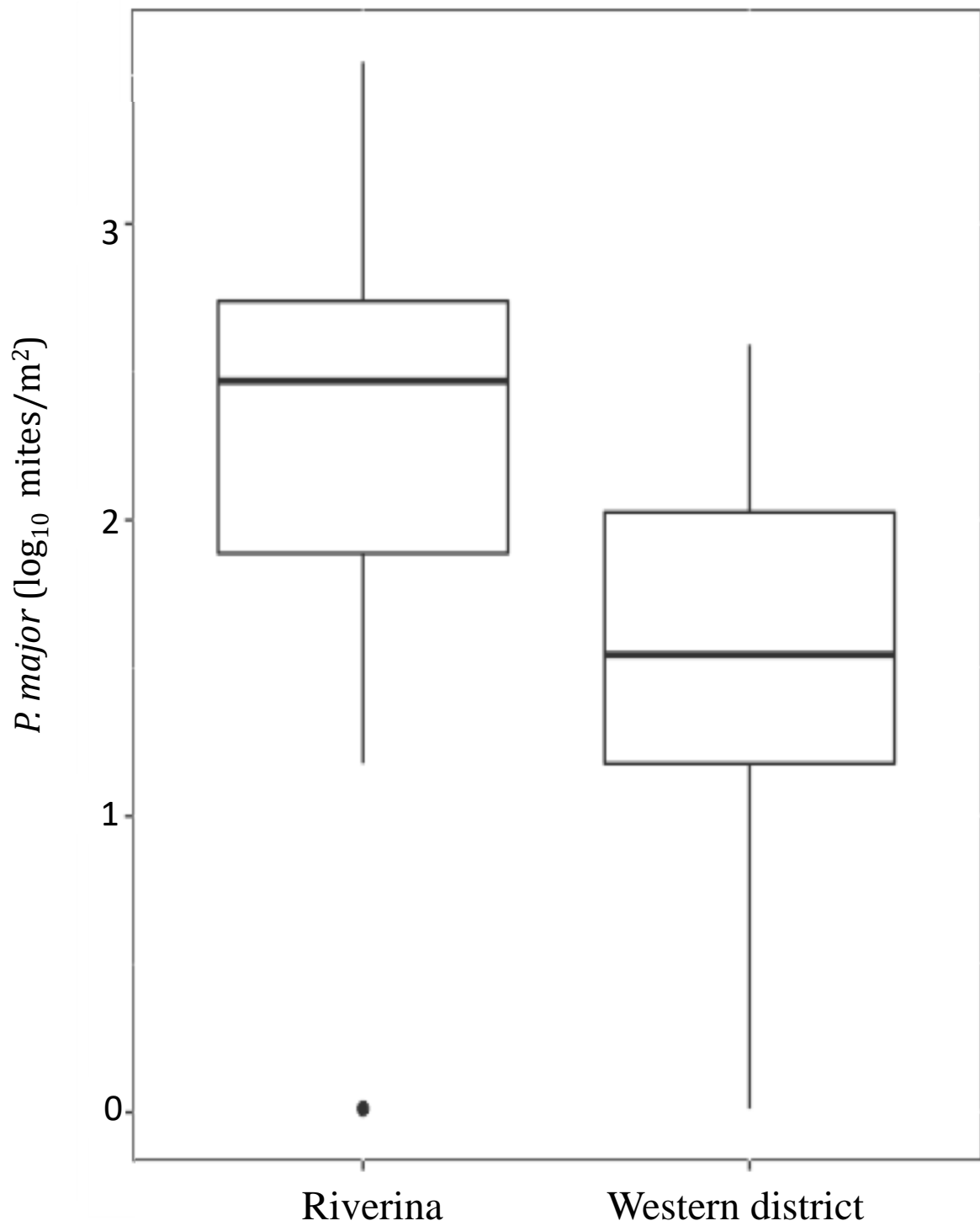


Figure 2.12. Boxplot of *P. major* \log_{10} transformed data (mites per m^2) comparing densities in fields in the Riverina and the Western District. The horizontal line of the boxplot shows the median value, the edges show interquartile ranges, whiskers show the 95% confidence intervals. Closed circles show outlier values outside of this interval.

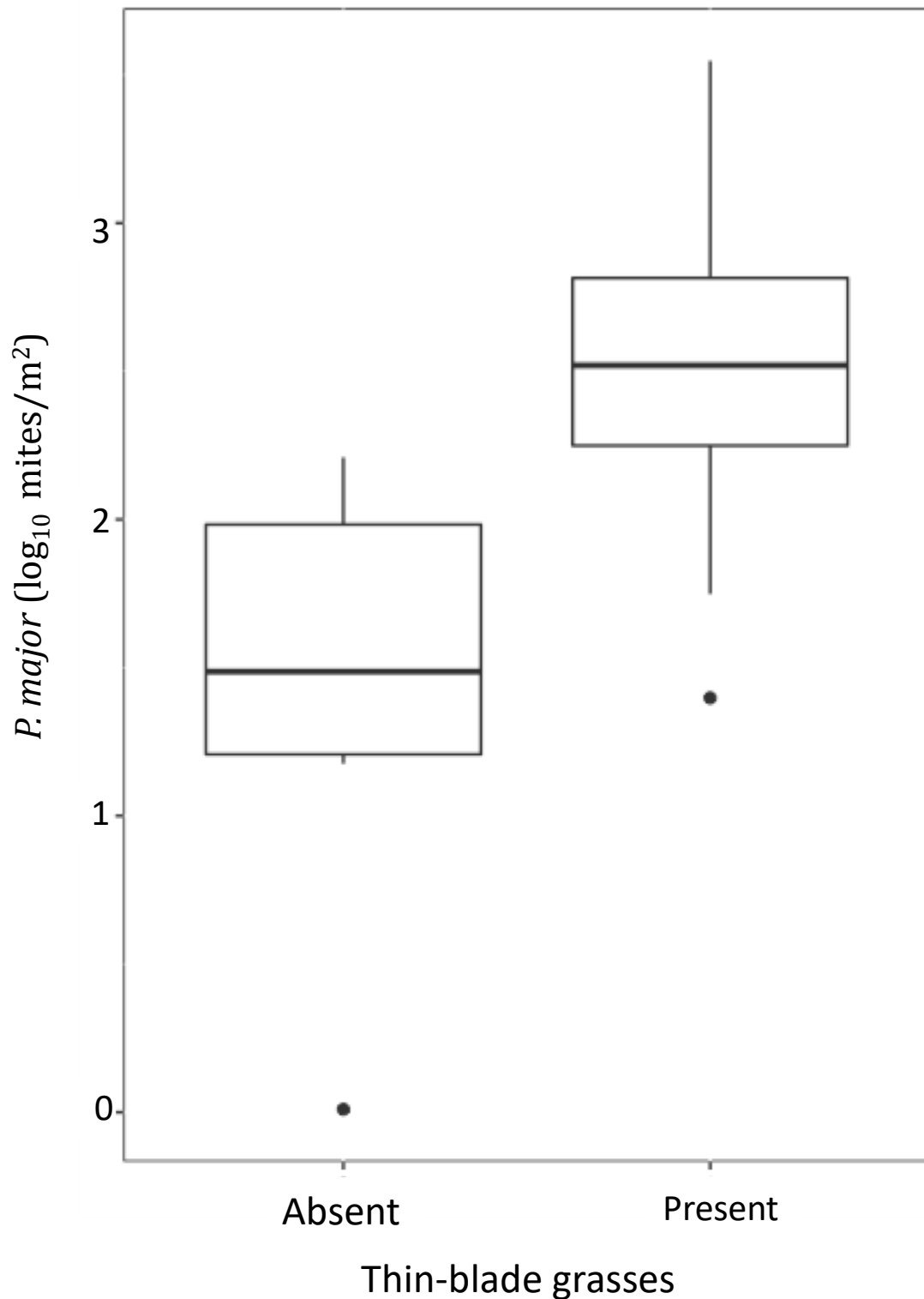


Figure 2.13. Boxplot of *P. major* log₁₀ transformed data (mites per m²) comparing densities in fields where the composition of thin-blade grasses was greater than 50%. Data includes only fields sampled in the Riverina. The horizontal line of the boxplot shows the median value, the edges show interquartile ranges, whiskers show the 95% confidence intervals. Closed circles show outlier values outside of this interval.

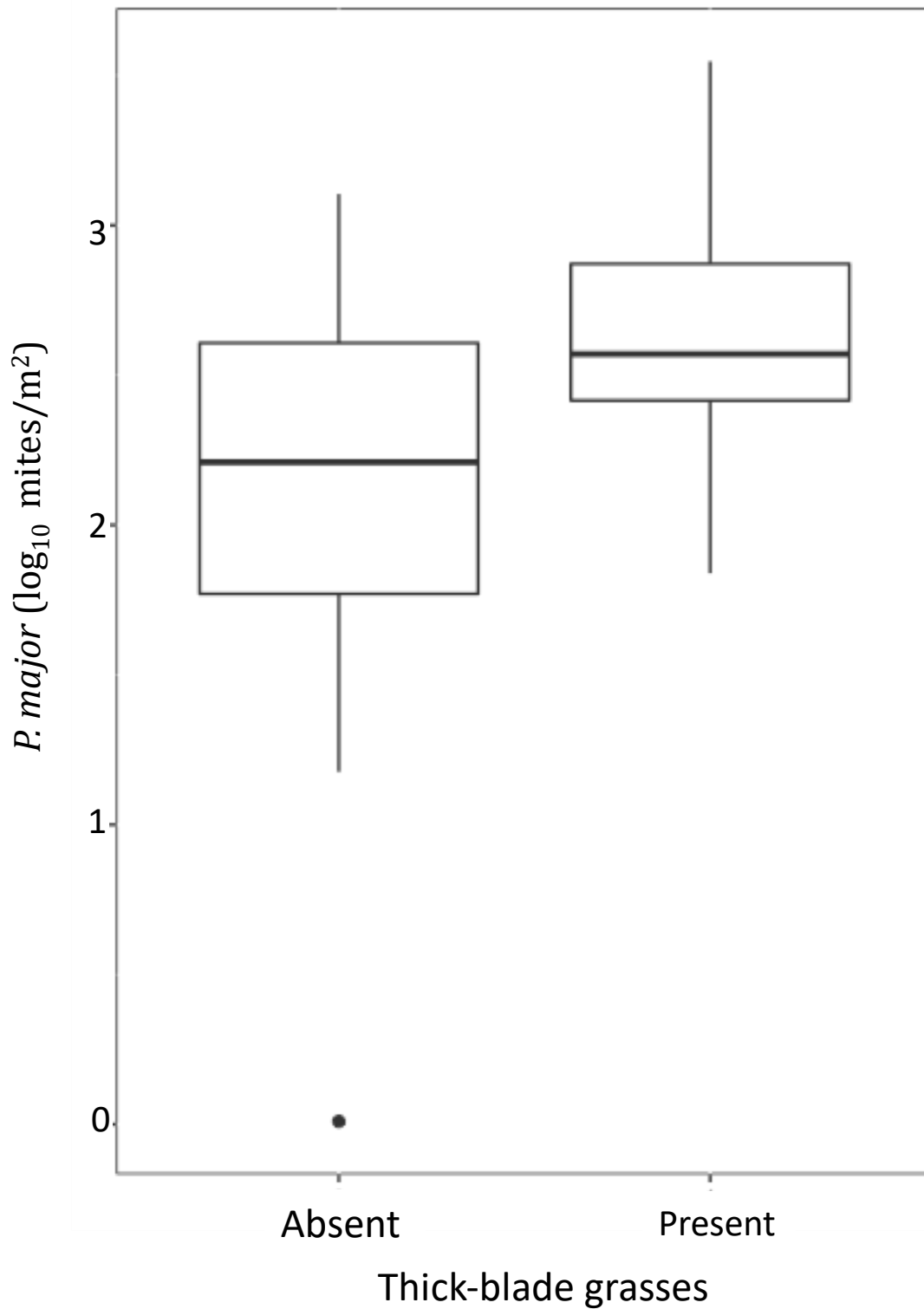


Figure 2.14. Boxplot of *P. major* log₁₀ transformed data (mites per m²) comparing densities in fields where the composition of thick-blade grasses was greater than 20%. Data includes only fields sampled in the Riverina. The horizontal line of the boxplot shows the median value, the edges show interquartile ranges, whiskers show the 95% confidence intervals. Closed circles show outlier values outside of this interval.

Table 2.6. The significance of all variables explaining the density of *H. destructor* and *P. major* in sampled pasture fields as assessed through linear models. The *p*-values < 0.05 are bolded. The significance of variables for the total dataset is shown for both species. For *P. major*, the significance of variables is also displayed using the regional-specific data. WHC = water holding capacity of the soil.

	<i>H. destructor</i>	<i>P. major</i>	<i>P. major</i> in the Riverina	<i>P. major</i> in the Western District
Year	F _{1,60} = 0.03, \ p = 0.86	F _{1,60} = 0.06, p = 0.43	F _{1,39} = 0.32, p = 0.57	F _{1,19} = 2.31, p = 0.15
Region	F _{1,60} = 0.40, p = 0.53	F _{1,60} = 21.72, p < 0.001	NA	NA
Clover	F _{2,59} = 1.30, p = 0.28	F _{2,46} = 1.49, p = 0.23	F _{2,38} = 2.82, p = 0.07	F _{2,18} = 0.19, p = 0.83
Broadleaf weeds	F_{1,60} = 5.39, p < 0.05	F _{1,60} = 0.07, p = 0.93	F _{1,39} = 0.03, p = 0.86	F _{1,19} = 4.40, p < 0.05
Thick-blade grasses	F _{1,60} = 0.13, p = 0.72	F _{1,60} = 6.87, p < 0.05	F_{1,39} = 8.21, p < 0.01	F _{1,19} = 0.01, p = 0.93
Thin-blade grasses	F _{1,60} = 1.94, p = 0.17	F_{1,60} = 4.69, p < 0.05	F_{1,39} = 21.04, p < 0.001	F _{1,19} = 3.43, p = 0.08
Lucerne	F _{1,60} = 3.39, p = 0.07	F _{1,60} = 0.02, p = 0.89	F _{1,39} = 1.38, p = 0.25	F _{1,19} = 3.40, p = 0.10
Food on offer	F _{2,59} = 0.64, p = 0.53	F_{1,60} = 8.03, p < 0.001	F _{1,39} = 1.34, p = 0.25	F _{1,19} = 0.10, p = 0.76
Bare earth	F _{1,60} = 0.003, p = 0.96	F _{1,60} = 2.03, p = 0.16	F _{1,39} = 1.13, p = 0.29	F _{1,19} = 1.12, p = 0.30
WHC	F_{1,60} = 4.09, p < 0.05	F_{1,60} = 6.79, p < 0.05	F_{1,39} = 5.37, p < 0.05	F _{1,19} = 1.67, p = 0.21
Rainfall in spring	F _{1,60} = 3.59, p = 0.06	F _{1,60} = 15.15, p < 0.001	F _{1,39} = 1.66, p = 0.21	F _{1,19} = 0.17, p = 0.68

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Canberra

2.9 Appendix- Earth mite densities in crop fields

Methods

Forty-one fields were sampled (21 in 2014 and 20 in 2015). A maximum of four crop fields were sampled from a single farm. As crop fields are known to support limited densities of earth mites (Umina and Hoffmann 2004, Macfadyen 2014), I targeted fields that were most likely to have high densities. These were fields coming out of susceptible crop-types, such as canola, bean, lupin and field-pea and with a low pesticide history.

Field sampling was conducted similarly to pasture fields with sampling conducted at three replicates at ten points in the field thirty metres apart. Of the three replicates taken, two replicates were taken from neighbouring rows and one replicate was taken from the inter-row. The three samples were pooled in the field.

In most cases, sampling was done in the middle of the field, however in some huge or hard to access fields, the sampling location was modified with a location 300 metre in to the field. When sampling crop fields prior to the emergence of seedlings, sampling was conducted on the available vegetation, with replicates taken at the nearest available vegetation (with a focus on collecting from plant types known to be attacked by *H. destructor*). In cases where vacuum sampling was determined not to be successful, a visual assessment was made. In some cases, in crop fields the use of a blower-vacuum was not possible as the sieve immediately became coated in dust restricting any further suction. In these instances only a visual assessment was made.

Results

Both earth mite species were found in low densities in cropping fields. *P. major* was found in more fields, with 5 fields (12%) found with densities greater than 10/m². For *H. destructor* high densities were found in two fields. This appeared to be due to the high proportion of broad-leaf weeds that had been allowed to grow throughout the previous year. Given the low numbers of mites found in these fields, further analysis was unable to be conducted.

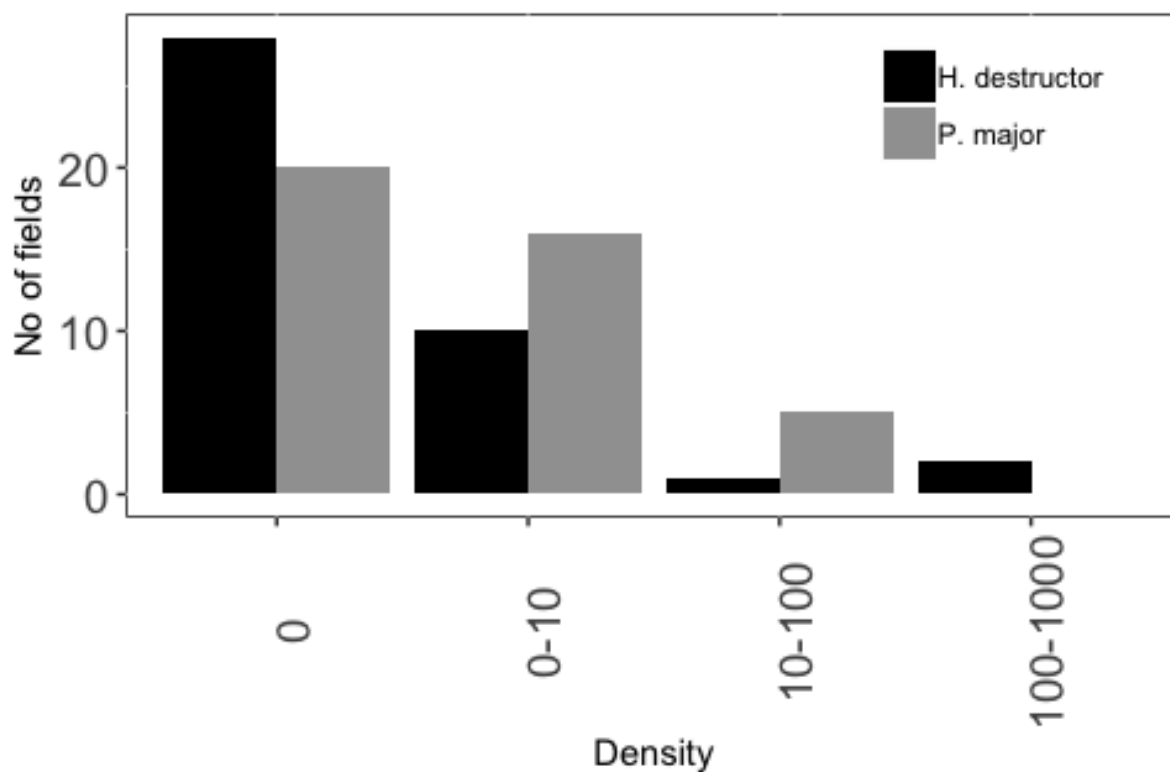


Figure 2.15. The presence and density (mites per m²) of *H. destructor* and *P. major* in fields sampled from cropping environments. Densities have been divided into four categories (absent, 0-10, 10-100, <100).

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CHAPTER 3: Crop seedling susceptibility to *Armadillidium vulgare* (Isopoda: Armadillidiidae) and *Ommatoiulus moreleti* (Diplopoda: Julidae)

3.1 Abstract

The isopod, *Armadillidium vulgare* (Latreille) (Isopoda: Armadillidiidae), and the millipede, *Ommatoiulus moreleti* (Lucas) (Diplopoda: Julidae), are increasingly being reported as pests of emerging broadacre crop seedlings in southern Australia. This is thought to be due to the increased adoption of stubble retention practices, leading to increased abundance of these soil-dwelling organisms. Here, we evaluate the propensity of *A. vulgare* and *O. moreleti* to damage a range of crop seedlings. Through the combined analysis of a controlled feeding trial and field reports, we show *A. vulgare* is able to feed on and damage a range of pulses, legumes, cereals, and oilseeds, as emerging seedlings. *O. moreleti* had a more restricted range of feeding, being limited to lupin, lucerne, and canola in the feeding trial. These results are discussed in the context of developing pest management guidelines for these species.

3.2 Introduction

Millipedes and terrestrial isopods generally feed on decomposing plant matter and play a beneficial role in recycling nutrition back in to the soil (Vos et al. 2011). They also occasionally feed on living plant tissue (Ebregt et al. 2007, Saska 2008, Farmer and Dubugnon 2009, Koprdoová et al. 2010, 2012) and are considered to be a pest in some agricultural systems (Baker 1974, Ebregt et al. 2005, Paoletti et al. 2008, Martínez et al. 2014). The isopod, *Armadillidium vulgare* (Latreille) (Isopoda:Armadillidae), and the millipede, *Ommatoiulus moreleti* (Lucas) (Diplopoda:Julidae), are commonly found in the soil and litter layer of gardens and bushland across southern Australia, as well as other Mediterranean-type climates around the world (Paris 1963, Baker 1984, Paoletti and Hassall 1999, Paoletti et al. 2007). The broadacre farming environment does not generally provide a soil environment conducive to the build-up of these organisms. Fields that use ‘conservation’ tillage practices, however, provide a more favorable environment for these organisms, in relation to fields that are annually tilled (Holland 2004).

The recent trend of farmers in Australia and elsewhere to adopt conservation tillage practices is thought to have led to the build-up of millipedes and isopods in broadacre farming systems. Conservation tillage practices aim to improve the soil health by maintaining top-soil and moisture, as well as recycling the remaining plant nutrition back in to the soil after the crop has been harvested (Stinner and House 1990). These practices improve the field environment for millipedes and isopods by providing them with shelter, an increased source of moisture, and decaying crop residue as a food source (Holland 2004). Other factors, such as the simplification of the landscape through the removal or absence of shelterbelts, leading to the loss of predators, may also be important in the build-up of these organisms (Paoletti et al. 2008).

Australian farmers commonly report that these organisms can be present in fields without causing damage (Perry 2012). Why they turn to feeding on living rather than decaying plant matter is unknown but may be linked to changes in the density and quality of decaying plant matter, environmental factors, and/or the susceptibility of living plants. *A. vulgare* is now reported to be one of the most significant pests of oilseed crops in Argentina (Faber et al. 2011) and soybean in parts of the United States (Johnson et al. 2012). For millipedes, this is one of the first instances of reported damage to crop seedlings (Baker 1974).

Given these species generally feed on decaying plant matter, where hard to digest plant compounds have been reduced through microbial breakdown, it is likely they have a limited ability to feed on living plants. Understanding which crops an organism is likely to damage is a vital first step in developing integrated pest management programs (Umina and Hoffmann 2004, Horne et al. 2008). This knowledge allows farmers to limit crop damage by planting less susceptible crops and to limit control practices to susceptible crops.

In this study, we examine the susceptibility of a range commonly grown crop seedlings to be damaged at three early seedling growth stages by *O. moreleti* and *A. vulgare*. The findings from these feeding trials were then correlated with records of damage on commercial fields in Australia. Together these results are used to assess the potential of these species to cause crop damage under variable field environments.

3.3 Methods

3.3.1 Shadehouse trial

Microcosm setup

The ability of *O. moreleti* and *A. vulgare* to damage crop plants was tested using microcosms in a shadehouse. This method has been used previously to determine numerous aspects of invertebrate biology, including plant host associations (Umina and Hoffmann 2004). Each microcosm consisted of a 3L plastic container (15cm long × 10cm wide × 21cm deep) containing 1:1 sterilized sandy loam:potting mix. To create a favorable environment for the survival of organisms as well as plant growth, microcosm tubs were watered every 2–3 d as required throughout the trial. Because both species are prone to desiccation, a shelter, created by a piece of moist cloth, was added across a quarter of each microcosm.

Crops and varieties assessed were canola [*Brassica napus* L. (Brassicales: Brassicaceae) cultivar ‘Gem’], wheat [*Triticum aestivum* (Poales: Poaceae) cv. ‘Lancer’], oat [*Avena sativa* (Poales: Poaceae) cv. ‘Mammoth’], lucerne [*Medicago sativa* (Fabales: Fabaceae) cv. ‘Sardi 7’], red lentil [*Lens culinaris* (Fabales: Fabaceae) cv. ‘Ace’], chickpea [*Cicer arietinum* (Fabales: Fabaceae) cv. ‘Seamer’], faba beans [*Vicia faba* L. (Fabales: Fabaceae) cv. ‘Warda’] and lupin [*Lupinus angustifolius* (Fabales: Fabaceae) cv. ‘Barlock’]. These

were chosen because they are widely grown and among the most economically important broadacre crops in Australia.

For each crop, *O. moreleti* and *A. vulgare* were introduced at three different stages of seedling development (Table 3.1). To synchronise the introduction of *O. moreleti* and *A. vulgare* with these growth stages, germinated seeds were sown into microcosms at a planting interval of 4–12 d. Eight seeds were sown in canola, lucerne, wheat, and oat microcosms; six for lentil, lupin, and chickpea microcosms; and four seeds were sown in faba bean microcosms. Reductions in sowing density reflected the larger size of these seedlings. Following sowing, each microcosm was watered, enclosed with a clear plastic lid that had a large gauze window for ventilation, and placed in a shadehouse. For each treatment, five replicate tubs were established in this manner. In four microcosms, organisms were introduced at the appropriate growth stage, while a single replicate microcosm had no organisms introduced; this acted as a control to ensure there was no damage to seedlings due to other factors.

Table 3.1. Crop type and growth stages at the time of *A. vulgare* and *O. moreleti* introductions into the shadehouse feeding trial.

Crop	Growth stage		
	First	Second	Third
Lucerne	D9- unfolding cotyledons	D11- one true leaf	D12/13- two or three leaves
Canola	D9- unfolding cotyledons	D11- one true leaf	D12/13- two or three leaves
Lupin	D9- unfolding cotyledons	D12- two trifoliolate leaves	D14- four trifoliolate leaves
Lentil	D9- shoot through the soil surface	D13- three multifoliolate leaves	D17- seven multifoliolate leaves
Chickpea	D9- shoot through the soil surface	D13- three multifoliolate leaves	D17- seven multifoliolate leaves
Faba bean	D9- shoot through the soil surface	D12- two leaves	D14- four leaves
Wheat	G10- first leaf through coleoptile	G12- two leaves	G13- three leaves

Oat	G10- first leaf through coleoptile	G12- two leaves	G13- three leaves
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Plant growth stages are defined according to the BBCH scale (Hess et al. 1997, Lancashire et al. 1991).

3.3.2 Specimen collections and feeding trial

Large adults of *A. vulgare* (>80 mg) and *O. moreleti* (>120 mg) were collected in 20 liter polystyrene cold boxes lined with moist paper towel. *A. vulgare* were collected from a domestic garden and under oat straw in a mixed orchard (36° 3'9"S, 146° 50'24"E), while *O. moreleti* were collected under leaf litter near a wetland (36° 4'7"S, 146° 51'16"E). *A. vulgare* were starved for 3–4 d and *O. moreleti* starved for 12–24 h prior to introduction so that specimens were likely to feed once placed into microcosms. Starvation periods were determined from preliminary studies as the minimum time to promote active feeding, but not too long that the starvation period would reduce the microbial content of the gut (Zimmer 2002) and thus the ability of each species to feed on plants. For each species, 12 randomly selected specimens were introduced into microcosms, which were placed in a random arrangement in a shadehouse.

Assessments of feeding damage to seedlings were made five times over 15 d (on days 2, 4, 7, 10, and 15). Any dead individuals were removed as soon as they were observed to avoid cannibalism (Tuf et al. 2013). Plant damage was assessed by recording the amount of damage to each part of the plant. This damage was converted to a percentage, by assessing the amount of damage relative to the plant tissue remaining in each microcosm tub. The total number of living crop seedlings within each tub was also recorded on each sampling day. Seedlings were determined to be killed when they had been defoliated or removed.

At the conclusion of the trials, the total number of live specimens was assessed in all microcosm tubs through a destructive sampling effort. For *O. moreleti*, two microcosms (one from the lucerne treatment and one with chickpea) were excluded from the analysis because some individuals escaped during the trial, while a single microcosm of canola was excluded due to an aphid infestation affecting plant damage scores. For *A. vulgare*, a single microcosm with oats was excluded as it was tipped over during the trial. All microcosms in the second

and third growth stages of lupin, including the controls, wilted at day 10, so no further assessments were undertaken beyond this day.

3.3.3 Data analysis

Percentage data for seedlings damaged and seedlings killed in each microcosm were modeled using generalized linear models (GLMs). Separate models were constructed for four response variables: *A. vulgare* seedling damage and seedlings killed and *O. moreleti* seedlings damaged and seedlings killed. The explanatory variables of crop type, growth stage and time, and the interactions between crop type and growth stage were included in the model. Binomially distributed errors and a logit link were used to fit the GLM to these variables. Models were constructed using a process of sequential addition of explanatory variables, using time as the blocking factor. The best model was identified by AIC values (Akaike 1974) and validated by assessing the distribution and homogeneity of the residuals.

Data analysis included only the crop types that were damaged. No feeding damage was observed in several treatments. For *O. moreleti*, the crop types, wheat, oat, chickpea, lentil, and faba bean were, therefore, excluded, while for *A. vulgare* faba bean was excluded. Data analysis for *A. vulgare* was reduced to three time points (2, 4, and 7 d) as all seedlings in several treatments had been completely killed by day 7.

To assess whether feeding on plants affected mortality in *A. vulgare* and *O. moreleti*, the percentage mortality, at the conclusion of the trial, was analyzed against crop and growth stage using GLMs. Binomially distributed errors and a logit link were used to fit the GLM to this variable. All analyses were conducted with R Studio v 0.99.893 (R Studio Team 2015).

3.3.4 Reports of damage from commercial fields

In southern Australia, reports of pest outbreaks in broadacre fields are generated by an extensive network of farmers, agronomists, and extension workers, relayed to researchers to inform the farming community of pest issues through industry newsletters. These reports collect information on 1) the species causing crop damage, 2) the crop that has been damaged, and 3) the amount of damage that has occurred. We compiled and then investigated

reports of millipedes and isopods causing damage to crop and pasture seedlings in Victoria, New South Wales, South Australia, and Western Australia. The Western Australian information has been collated by the Department of Agriculture and Food Western Australia from 1996 to 2015 (DAFWA 2016). Information from New South Wales and Victoria has been collected by Cesar and the University of Melbourne from 2006 to 2015 (Cesar 2016), while South Australian records have been compiled by the South Australian Research Development Institute from 2006 to 2015 (SARDI 2016).

In the case of isopods, 12 reports were identified as *Australiodillo bifrons* and these were excluded from analysis. This species is restricted to the north coast of NSW and readily identified in the field by its morphology and unique behavior of swarming (Paoletti et al. 2008). All other isopod reports were either identified or assumed to be *A. vulgare* based on appearance and/or behavior. All millipede reports were identified as *O. moreleti*. In almost all cases, the damage was reported as being patchy across affected paddocks; however, the severity of feeding damage differed considerably. Records of feeding damage to crop plants were split into three categories: severe (greater than 10% of the crop loss), moderate (only small sections of the paddock with severe damage), and minimal/unknown (little to no damage recorded or this information was unknown). In the case of multiple fields being damaged, we recorded these reports as two fields.

3.4 Results

3.4.1 *Armadillidium vulgare*

A. vulgare fed on and killed seedlings of all crops except for faba bean. The greatest degree of seedling damage occurred to lucerne and then canola, followed by oat and lupin (Fig 3.1; Table 3.3). At the completion of these trials, all lucerne and all (but one) canola seedlings were consumed. Lupin and oat seedlings were also substantially damaged, while wheat, chickpea, and lentil seedlings received comparatively little damage (Table 3.3). Wheat, chickpea, and lentil seedlings were only killed in the first two growth stages. For chickpea and lentil, no feeding damage was recorded in most microcosms. Wheat seedlings were chewed throughout the study in all replicate microcosms; however, this feeding did not greatly affect the seedling.

For those seedlings that were impacted (all crops except faba bean), crop type and growth stage were significant predictors for the percentage of seedlings damaged and the percentage of seedlings killed in the GLMs (Table 3.2). Time was not significant for either seedlings damaged or seedlings killed (Table 3.2), and there was no interaction between crop type and growth stage. Seedling mortality was greatest for all crops when *A. vulgare* was introduced on 1 d old seedlings (Table 3.3; Fig 3.1). More than any other crop, oats showed considerable variation in seedling mortality with growth stage; approximately 70% of the seedlings were killed at the first growth stage, compared with 9% and 4% at the second and third growth stages, respectively (Fig 3.1d). While seedling mortality in the 2nd and 3rd growth stages of oat seedlings was relatively low, percentage feeding damage was far higher (Table 3.3).

Less than 10% of *A. vulgare* died during these trials. There was no effect of treatment on mortality (crop type: $\chi^2(6) = 11.10, P = 0.95$; growth stage: $\chi^2(2) = 10.63, P = 0.71$), suggesting that neither plant toxins nor starvation affected the survival of individuals over the course of the trial.

3.4.2 *Ommatoiulus moreleti*

O. moreleti did not damage the majority of crop seedlings in these trials. No feeding was observed on chickpea, lentil, faba bean, wheat, or oat at any growth stage. Lupin and lucerne seedlings were substantially damaged at all growth stages, while canola suffered damage at each growth stage (most notably at the first stage) (Table 3.4). The GLM fitted to data from the crops that were damaged (canola, lucerne, and lupin) showed crop type and time, but not growth stage, had a significant effect on seedlings damaged (Table 3.2). Time, crop type, and growth stage were significant predictors for the percentage of seedlings killed (Table 3.2).

Only a single seedling of canola (in the first growth stage) was killed across all tubs, while seedlings of lupin and lucerne were killed in all tubs, at all growth stages. Higher seedling mortality was observed in the earlier growth stages for both lupins and lucerne (Fig 3.2). Seedling mortality also increased over the course of the trial.

Mortality of *O. moreleti* was low across all tubs in this study, with 4% deceased at the end of the trial. There was no effect of treatment on mortality (for crop type: $\chi^2(7) = 8.95, P = 0.98$;

for growth stage: $\chi^2(2) = 8.27, P = 0.71$), suggesting that neither plant toxins nor starvation affected the survival of individuals over the course of the trial.

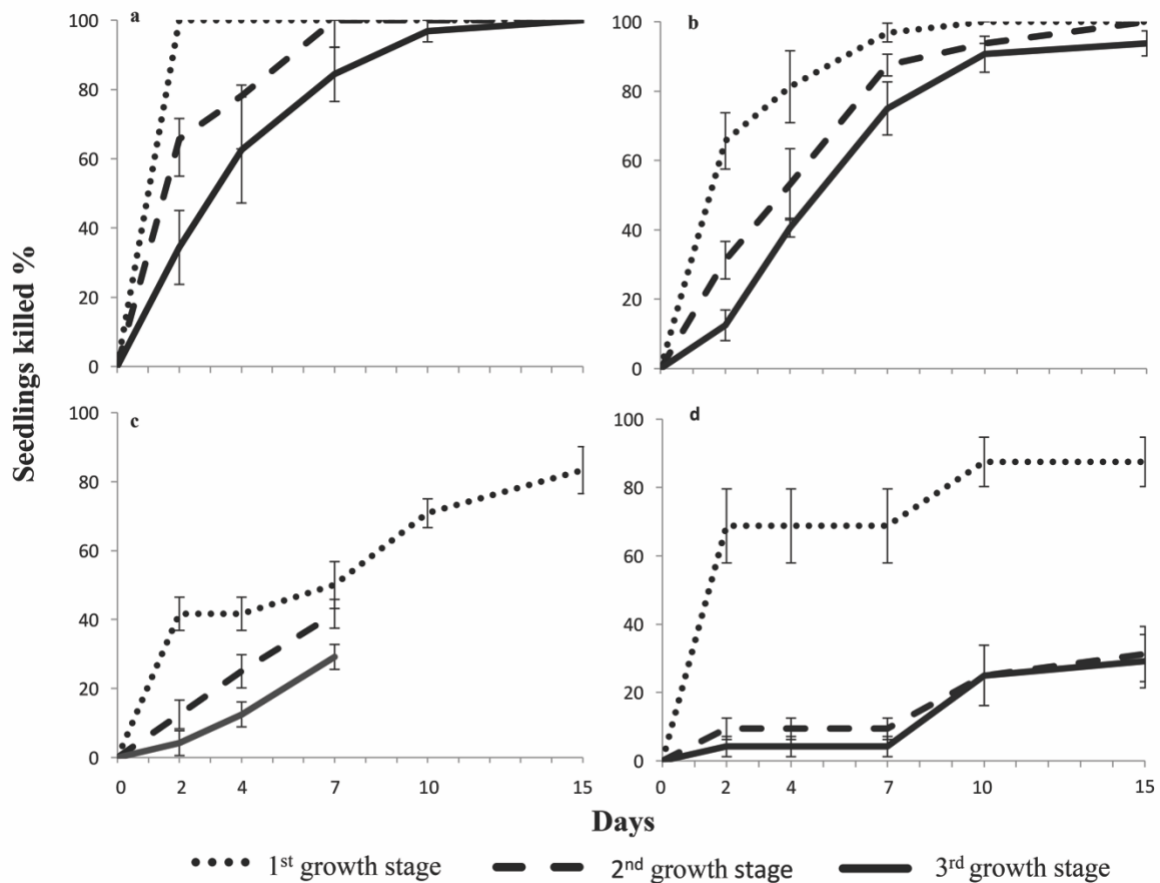


Figure 3.1. Percentage seedlings killed by *A. vulgare* feeding on (a) lucerne, (b) canola, (c) lupin, and (d) oat at three growth stages (refer to Table 3.1 for growth stages). Error bars represent standard error of the mean.

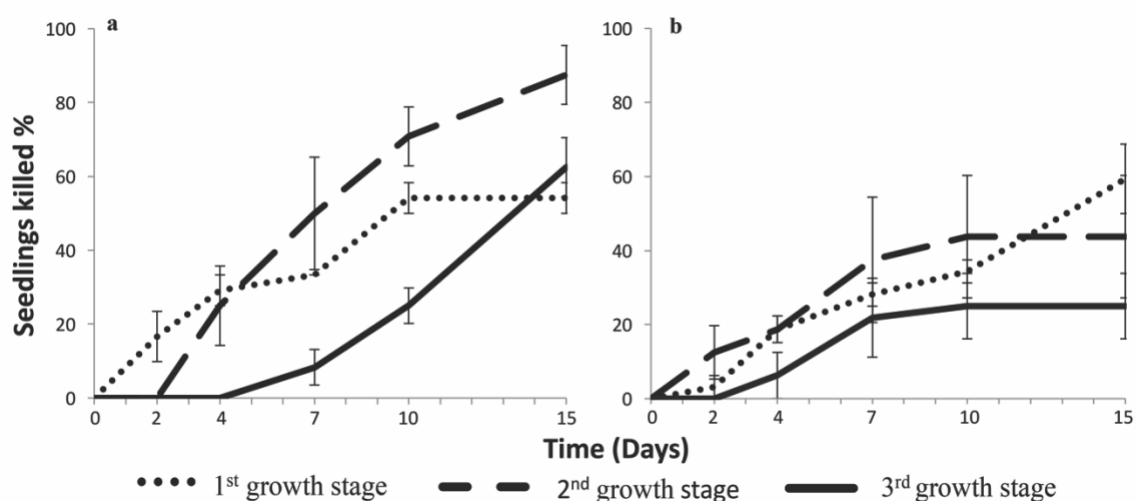


Figure 3.2. Percentage seedlings killed by *O. moreleti* feeding on (a) lupin and (b) lucerne at three growth stages (refer to Table 3.1 for growth stages). Error bars represent standard error of the mean.

Table 3.2. The significance of variables explaining seedlings damaged and seedlings killed by *A. vulgare* and *O. moreleti* in generalized linear models assessed through a chi-squared goodness of fit test (df in brackets)

Response variable	Time	Crop type	Growth stage	Crop type - growth stage interaction
<i>A. vulgare</i> seedlings damaged	$\chi^2 (2) = 4.13,$ p=0.13	$\chi^2 (6) = 125.40,$ p<0.001	$\chi^2 (2) = 16.19,$ p<0.001	$\chi^2 (2) = 8.81,$ p=0.77
<i>A. vulgare</i> seedlings killed	$\chi^2 (2) = 4.14,$ p=0.13	$\chi^2 (6) = 118.16,$ p<0.001	$\chi^2 (2) = 26.70,$ p<0.001	$\chi^2 (2) = 6.58,$ p=0.88
<i>O. moreleti</i> seedlings damage	$\chi^2 (4) = 13.74,$ p=0.01	$\chi^2 (2) = 41.71,$ p<0.001	$\chi^2 (2) = 1.17,$ p=0.56	$\chi^2(4) = 3.97,$ p=0.41
<i>O. moreleti</i> seedlings killed	$\chi^2 (4) = 17.97,$ p=0.001	$\chi^2 (2) = 31.7,$ p<0.001	$\chi^2(2) = 7.14,$ p=0.03	$\chi^2(4) = 0.83,$ p=0.93

Table 3.3. Percentage damage to eight crop seedlings (\pm se) caused by *A. vulgare* after 15 days when introduced at three growth stages

Crop type	Growth stage		
	First	Second	Third
Lucerne	100 (D9)	100 (D11)	100 (D12/13)
Canola	100 (D9)	100 (D11)	96 \pm 4 (D12/13)
Lupin	63 \pm 7* (D9)	56 \pm 6* (D12)	56 \pm 5* (D14)
Lentil	4 \pm 3 (D9)	0 (D13)	0 (D17)
Chickpea	4 \pm 3 (D9)	4 \pm 3 (D13)	0 (D17)
Faba bean	0 (D9)	0 (D12)	0 (D14)
Wheat	9 \pm 3 (G10)	6 \pm 1 (G12)	5 \pm 0 (G13)
Oat	88 \pm 7 (G10)	75 \pm 3 (G12)	40 \pm 9 (G13)

Seedling growth is given in brackets (as defined by the BBCH scale (Hess et al. 1997, Lancashire et al. 1991)). * Damage to lupin seedlings is given at 7 days due to plants wilting at the second and third growth stage prior to day 10.

Table 3.4. Percentage damage to eight crop seedlings (\pm se) caused by *O. moreleti* after 15 days when introduced at three growth stages

Crop type	Growth stage		
	First	Second	Third
Lucerne	65 \pm 5 (D9)	60 \pm 13 (D11)	32 \pm 9 (D12/13)
Canola	25 \pm 3 (D9)	5 \pm 2 (D11)	1 \pm 1 (D12/13)
Lupin	66 \pm 3 (D9)	88 \pm 7 (D12)	85 \pm 4 (D14)
Lentil	0 (D9)	0 (D13)	0 (D17)
Chickpea	0 (D9)	0 (D13)	0 (D17)
Faba bean	0 (D9)	0 (D12)	0 (D14)
Wheat	0 (G10)	0 (G12)	0 (G13)
Oat	0 (G10)	0 (G12)	0 (G13)

Seedling growth is given in brackets (as defined by the BBCH scale (Hess et al. 1997, Lancashire et al. 1991)).

3.4.3 Pest reports from the field

A. vulgare has been reported damaging a variety of broadacre crops in Australia, including New South Wales, Victoria, South Australia, and Western Australia. In total, 67 reports were received during the period 2006–2015, with two reports recorded from Western Australia during 1996–2005 (Table 3.5). *A. vulgare* was reported to have damaged a range of cereal, pulse, and oilseed crops, but the greatest number of reports involved canola in all states (~70% of all reports). More than 50% of reports from canola were of *A. vulgare* causing severe feeding damage (Table 3.5), resulting in greater than 10% crop loss. Several reports stated fields needed to be completely resown following attack by *A. vulgare*. Severe damage was also reported in fields sown to lentil, lucerne, chickpea, and field peas. For cereals, nine reports were received of *A. vulgare* damaging crops, but none specified severe or patchy damage (Table 3.5).

O. moreleti was reported damaging crops from South Australia, Victoria, and New South Wales during the period 2006–2015. No reports of crop damage by millipedes were reported in Western Australia from 1996–2015. The greatest number of reports involved canola damage (>90% of all reports; Table 3.6). Of the 34 cases of crop damage to canola, 9 (~27%) reported losses of greater than 10%. Several reports stated fields needed to be completely resown following attack by *O. moreleti*. Damage to lupin was reported in two cases; both involved severe feeding damage. There was a single report of *O. moreleti* feeding on a cereal crop, but minimal/no damage was found (Table 3.6).

Table 3.5. Total number of broadacre crop fields reported to have been damaged by *A. vulgare* as emerging seedlings in South Australia, New South Wales and Victoria from 2006–2015, and in Western Australia from 1996–2015

Crop type	Severity of damage (no of fields)			Total (no of fields)
	Severe	Moderate	Minimal/ Unknown	
Canola	26	6	17	49
Lucerne	1	1	0	2
Lentil	1	5	0	6

Chickpea	1	0	0	1
Field peas	1	0	0	1
Wheat	0	0	2	2
Oat	0	0	1	1
Barley	0	0	1	1
Cereal*	0	0	5	5
Rye grass	0	0	1	1
Total	30	12	27	69

Severe (greater than 10% of the crop loss), moderate (only small sections of the paddock with severe damage), and minimal/unknown (little to no damage recorded, or this information was unknown).

*cereal species not specified

Table 3.6. The number of broadacre crop fields reported to have been damaged by *O. moreleti* as emerging seedlings in South Australia, New South Wales and Victoria from 2006-2015

Crop type	Severity of damage (no of fields)			Total (no of fields)
	Severe	Moderate	Minimal/ Unknown	
Canola	9	6	19	34
Lupin	2	0	0	2
Cereal*	0	0	1	1
Total	11	6	20	37

Severe (greater than 10% of the crop loss), moderate (only small sections of the paddock with severe damage), and minimal/unknown (little to no damage recorded, or this information was unknown).

* cereal species not specified

3.5 Discussion

The millipede, *O. moreleti*, and the isopod, *A. vulgare*, represent a new and increasing problem for Australian farmers. Understanding which crops these species are likely to damage is a vital first step in developing an integrated pest management program (Umina and Hoffmann 2004, Horne et al. 2008). Of the eight crop species assessed here, canola, lucerne,

and lupin were damaged by both species at all growth stages. *O. moreleti* did not damage any other crops. *A. vulgare* damaged chickpea, lentil, wheat, and oat seedlings. Neither species caused damage to faba bean seedlings.

For *A. vulgare*, crop type and the growth stage of seedlings were important factors influencing the amount of feeding damage caused. Seedlings were most vulnerable to attack when *A. vulgare* were introduced at the first growth stage; they were typically severed at the base by chewing through the plant hypocotyl. This type of feeding is consistent with damage *A. vulgare* causes to soybean and sunflower crops overseas (Johnson et al. 2012, Faberi et al. 2014). Lucerne was the most susceptible crop to *A. vulgare*, with all seedlings in the first growth stage killed within 2 d of introduction. A similar pattern was observed for canola. In the case of chickpea, lentil, and wheat seedlings, a small amount of damage was caused to plants at the earliest growth stages. For *O. moreleti*, the crop type but not the growth stage of plants was the most important factor influencing the amount of feeding damage. *O. moreleti* individuals were commonly found on lentil, chickpea, wheat, oat, and faba bean, but no feeding damage was observed (except for a tiny nibble on an emerging oat seedling in one microcosm tub). Lupin was most susceptible to *O. moreleti* feeding, with leaf damage accumulating over the course of the study, regardless of growth stage.

Although not examined directly in this study, variation in the physical architecture and chemical defences of plant seedlings likely influenced their susceptibility to attack. Lucerne for instance may be vulnerable to chewing damage due to the slender nature of the stems of establishing seedlings and faba bean may be resilient due to the thickness of the plant tissue. The tough outer cuticle and lignified cell walls of cereal grasses (Bernays 1994) may prevent feeding by *O. moreleti* but not *A. vulgare*, given isopods are known to be able to feed on some tough sources of organic material, such as hard seeds (Saska 2008), while millipedes have been found to be limited in their ability to feed on tougher detritus (Bailey and Mendonça 1990, Ashwini and Sridhar 2005). In the case of canola and lupin, commercial breeding programs have selectively removed deterrent chemicals such as glucosinolates, erucic acid, and alkaloids due to taste preference of humans (de Cortes Sánchez et al. 2005, Elahi et al. 2016). These crop types have likely become more palatable to a wide range of arthropod species. This has potentially facilitated damage from species such as *A. vulgare* and *O. moreleti*, which primarily feed on decaying plant matter (Hassall and Rushton 1984, Bailey and Mendonça 1990), but have the potential to become facultative herbivores (Cole

1997, Adhikari et al. 2012). *Arabidopsis thaliana* seedlings have been found to be more vulnerable to damage by *A. vulgare* when the jasmonate defence pathway is silenced (Farmer and Dubugnon 2009).

In the field, the impact a species has on a crop is dependent on a wide range of agronomic and environmental factors that can influence both the feeding behavior of the organism (Bailey and Kovaliski 1993, Agrawal et al. 1999, Mensink and Henry 2011) and the susceptibility of the plant (Hanson et al. 1981, Niemeyer et al. 1989, Arthur 2013, Philippi et al. 2016). In some instances, we found crop types showing low susceptibility in the feeding trial reportedly damaged in the field. For example, there have been numerous field reports of *O. moreleti* causing severe damage to canola and multiple reports of *A. vulgare* causing moderate damage to lentils; however, in our feeding trial, these crops sustained little damage. Nevertheless, we found a reasonable agreement between the results from the feeding trial and field reports. *O. moreleti* has only been reported damaging canola and lupin in the field, strongly supporting the hypothesis that *O. moreleti* is limited in its ability to feed on a range of crop plants. Field reports demonstrate *A. vulgare* can be a pest of a wide range of crops, with canola, lucerne, lentil, chickpea, and field peas all reported to have been severely damaged. The lack of field reports of severe damage to wheat, the most commonly planted crop in southern Australia, provides confidence that varieties of this plant show a high degree of tolerance to *A. vulgare* feeding in a range of environments and presumably under high pest densities.

From this research, we can have confidence, particularly in the case of *O. moreleti*, that crop damage can be limited, by sowing crops with low susceptibility such as wheat or faba bean, instead of a susceptible crop like canola, lucerne, or lupin. In the case of *A. vulgare*, this species is likely to cause damage to a variety of crops, but in the event of damage farmers may consider sowing wheat, which is less susceptible to this pest. This study also demonstrates the variability in susceptibility to feeding damage with plant growth stage. This information can be used to inform farmers of the likely risk, and therefore potential need for chemical intervention, when these species are found within broadacre crops. Additionally, our work provides a framework for future studies on the factors affecting the feeding behavior of *A. vulgare* and *O. moreleti* in different farming environments.

Research is needed to determine how the quantity and quality of crop residues (crop residue type, extent of crop residue breakdown, and moisture levels) affects feeding on different

crops under different environmental conditions. Further research should focus on helping farmers identify which fields are at risk and to develop control strategies for susceptible crops, including the need for chemicals to be registered. In parts of the Americas, where *A. vulgare* is now described as a perennial pest, economic thresholds have been developed in sunflower (Faber et al. 2014) and soybean crops (Saluso 2004) and integrated pest management strategies, such as the burning of stubble, have been investigated (Alfaress 2012, Johnson et al. 2012).

3.6 References

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CHAPTER 4- Factors influencing damage by the Portuguese millipede, *Ommatoiulus moreleti* (Julida: Julidae), to crop seedlings

4.1 Abstract

In different parts of the world, the increasing agricultural practice of retaining crop stubble in fields across seasons has led to population increases of soil-dwelling arthropods, primarily detritivorous species. These species typically play a beneficial role in the ecosystem, but some, including the Portuguese millipede (*Ommatoiulus moreleti*) can be sporadic pests. To assist in better understanding pest risk, this study examines why *O. moreleti* feeds on crop seedlings. For lupin, seedling susceptibility appears to be related to plant properties, with greatly different levels of damage caused to the two cultivated species (*Lupinus angustinus* and *Lupinus albus*) and particularly between cultivated and wild-type *L. angustinus* seedlings. Millipedes feeding on lupin (cultivated *L. angustinus*), but not lucerne (*Medicago sativa*), gained a similar amount of weight to those feeding on other foods known to be readily consumed. The life-stage of *O. moreleti* was found to be related to seedling damage. The presence of crop stubbles (as alternate food sources) did not limit the damage *O. moreleti* caused to lupin, suggesting that the presence of stubble in a field situation may not preclude feeding on crop seedlings. We discuss how results from these controlled environment trials can build a basis for understanding variable crop damage by *O. moreleti* in the field.

4.2 Introduction

Arthropod species with a broad feeding range can behave as both a beneficial or pest species in agricultural environments (Coll and Guershon 2002). Some species switch from feeding on arthropods to feeding on crop plants depending on relative food quality and availability (Agrawal et al. 1999, Agrawal and Klein 2000, Eubanks and Denno 2000). Some species switch from being predatory as juveniles to primarily plant-feeders as adults (Limburg and Rosenheim 2001, Jonsson et al. 2009). Similarly, species that primarily feed on decaying plant matter, and help recycle plant nutrition back in to the soil, can also be pests by attacking living plants (Faberi et al. 2011, Johnson et al. 2012, Douglas et al. 2017). The factors involved in these switches in feeding behaviour are poorly understood.

In comparison to herbivores, few studies have considered the feeding behaviour of detritivores on living plants. Some detritivorous species may be best described as scavengers, as while they primarily feed on decaying plant matter, they also feed on a wide range of food sources (David and Célérier 1997, Zimmer 2002, Martinson et al. 2008, Saska 2008, Koprdoová et al. 2010), including living plant tissue such as seeds (Saska 2008, Koprdoová et al. 2010), fruit and vegetables. Thus some species of terrestrial isopods and millipedes are considered pests in gardens and horticultural crops (Sutton 1980, Blower 1985, Warburg 1993, Sierwald and Bond 2007). While they are known to occasionally feed on seedlings (Paris 1963, Francisco and Fontanetti 2015), this is thought to be limited by their ability to digest cellulose or detoxify deterrent plant compounds. However, their capacity to feed on seedlings may vary with plant defences or due to adaptations in their gut physiology which allows them to process different food sources in different environments (Taylor 1982b, Sutton 1984, Kukor and Martin 1986). For primarily detritivorous feeding terrestrial gastropods, the proportion of living leaf matter plant consumed can be higher when deterrent plant chemicals are lower (Speiser and Rowell-Rahier 1991) or when dead plant matter is dry (Mensink and Henry 2011).

In different parts of the world, the increasing agricultural practice of farmers retaining their crop stubble in fields across seasons has led to population increases of soil-dwelling, primarily detritivorous arthropods (Holland 2004, Faberi et al. 2011, Johnson et al. 2012). While agricultural crop fields are generally unsuitable, stubble retention practices improve the field environment for these organisms. Crop stubble provides these organisms with shelter, a suitable habitat and an abundant food source (Holland 2004). Feeding on the

stubble helps break down this plant matter, releasing nutrients into the soil (Stinner and House 1990, Holland 2004). However, some detritivorous species can also be widespread pests of crop seedlings (Faberi et al. 2011, Johnson et al. 2012, 2013, Douglas et al. 2017).

The Portuguese millipede, *Ommatoiulus moreleti* (Lucas) (Julida: Julidae), was first found in Australia in 1953 and has since spread across southern Australia (Baker 1985, Baker et al. 2013). Due to its attraction to lights, it is a well-known urban nuisance, invading homes in large numbers in autumn when it is most active (Baker 1979, McKillup 1988). *Ommatoiulus moreleti* is also known to be a nuisance in domestic gardens and horticultural fields, damaging fruit and vegetables (Baker 1984, Baker et al. 2013). Reports of *O. moreleti* causing widespread damage to broad-acre crop seedlings were initially met with some skepticism by the grains industry (Henry, K. pers. comm.), given millipedes have rarely been reported to be pests of crop seedlings elsewhere in the world (Francisco and Fontanetti 2015). However, reports of *O. moreleti* damaging crop seedlings have been increasing over the past decade (cesar 2016, SARDI 2016) and it is now accepted that *O. moreleti* can cause crop damage. More recently, Douglas et al. (2017), found *O. moreleti* will feed on a limited range of broad-acre crop seedlings (canola, lupin and lucerne). However, large numbers of this species can be present in fields with susceptible crop seedlings without causing damage (Perry 2012).

This study aims to build a framework for future research on the feeding behaviour of detritivores by using controlled environment studies to improve our understanding of why *O. moreleti* feeds on crop seedlings. We address two questions. Firstly, what are some of the factors that influence feeding on susceptible crop seedlings, and secondly, does *O. moreleti* obtain a fitness benefit from feeding on crop seedlings? We investigate how the level of damage *O. moreleti* varies with 1) the size and lifestage of *O. moreleti*, 2) the presence of alternate food sources and 3) with reduced chemical defences in commercial lupin seedlings. To investigate the fitness benefits of feeding on seedlings in our final trial, we compare weight gain obtained from feeding on seedlings in comparison to food sources on which they are known to regularly feed.

4.3 Materials and Methods

4.3.1 Study outline

The trials involved feeding chambers with seedlings (and in some cases alternative sources of food) and introduced field-collected millipedes. The feeding chambers were set up with different combinations of *O. moreleti*, crop-seedlings and alternate food sources to progressively assess how specific factors affected the level of feeding damage (Table 1). Environmental effects were limited by conducting trials under conditions we considered optimal for *O. moreleti*. Trials one and two were conducted in a shade-house that reflected ambient conditions in early autumn of 2017 and 2018. Average daily temp (min. - max.) was 3°C - 19.7°C for trial one and 5°C - 17.2°C for trial two. Trials three and four were conducted in a controlled-temperature room at an optimal temperature of 20° C for *O. moreleti* (Baker 1979, 1980, Bailey and Kovaliski 1993) in order to monitor the effect of time under stable conditions. Trials ran between 14 and 22 days with plant feeding damage and millipede mortality assessed (and weight gain for trial 4) after *O. moreleti* were introduced.

The feeding chambers were 600 mL plastic cups with a gauze-covered lid and 200 mL soil (1:1 sand: potting mix (Debco Potmate, Debco Pty Ltd, AUS)). Cotton wool was added to the soil surface to provide a moist shelter for *O. moreleti*. Cups were watered as required for healthy plant growth and to ensure the soil microenvironment and the shelter remained moist, but not wet for *O. moreleti* (Baker 1980). Alternate food sources, when present (Table 1), were kept moist and soft. Moisture was monitored visually with humidity inside the cups recorded using a Hygrochron iButton (DS1923, Thermodata Pty Ltd, AUS). We introduced millipedes on to seedlings grown in the feeding chambers shortly after emergence, as feeding by *O. moreleti* is limited to the emerging seedling growth stages, especially for canola and lucerne (Douglas et al. 2017). Lucerne (*Medicago sativa*) and canola (*Brassica napus L.*) had unfurled cotyledons. The two species of lupin (*Lupinus angustinus* and *Lupinus albus*) had two unfurled multi-foliolate leaves. Throughout all trials, we monitored *O. moreleti* activity and survival. Mortality, recorded by the lack of antennal reflex (O'Neill 1969), varied from 0-8.5% between trials. All other millipedes appeared healthy and active.

O. moreleti were collected from the field by hand. For trial one, the collection was from the garden and orchard of a farm near Albury NSW (36° 3'9"S, 146° 50'24"E). For collections for trials 2, 3, 4 we located a crop field (37° 49'46"S, 142° 22'52"E) with ongoing pest issues

with *O. moreleti*. Individuals were taken back to the laboratory in polystyrofoam containers lined with a moist paper towel and some soil where they remained for up to 4 days before being introduced in to feeding chambers. This was done to ensure they were hungry but not water stressed at the commencement of the trial (see Douglas et al. 2017).

4.3.2 Trial methods

For trial one, we introduced three individuals of different sizes, lifestages and sexes in to feeding chambers with a single crop seedling of canola, lupin and lucerne (Table 4.1). Based on the results from trial one, trials two to four focused primarily on lupin, (as it is the most susceptible crop (Trial 1, Douglas et al (2017)) and used only large females (as they cause most feeding damage (Trial 1)). For trials one to three we introduced three individuals per cup. For trial four we only introduced a single individual as were interested in individual effects. Details, replications and set-up given in Table 4.1.

For trial 1 field collected *O. moreleti* were initially grouped as adults or juveniles by size and colour. Adults were divided by sex by examining the seventh pairs of legs that appear absent on males (as their gonopods are modified seventh legs) (Baker 1978). Live juveniles of this species cannot be sexed (Baker 1978). For each group (juveniles, males, and females), individuals were then divided by size into large and small categories (large females, small females, large males, small males, juveniles, small juveniles). At the conclusion of the trial, we examined 15 individuals in each category to determine the stadia, by counting the rows of ocelli (Vachon 1947), as well as size (length and weight) by measuring and weighing them (see Table 4.2). The trial ran for 15 days with assessments made on days two, four, seven, 10 and 15. Three out of a total of 540 millipedes died.

Table 4.1. Description of experiments including variables considered, life-stages used, and food sources.

Trial and aim	<i>O. moreleti</i> life-stage, replication	Food sources in treatments
1. Test effect of <i>O. moreleti</i> life-stage/sex and size on feeding damage	Various life-stages, 3 individuals/replicate, 10 replicates	1) <i>L. angustinus</i> cv. ‘Mandelup’, 2) <i>B. napus</i> L. cv. ‘Gem’, 3) <i>M. sativa</i> cv. ‘Sardi 7’
2. Compare effect of crop cultivar and species	Large females, 3 individuals/replicate, 15 replicates	<i>L. angustinus</i> 1) cv. ‘Barlock’, 2) cv. ‘Jurien’, 3) cv. ‘wild-type*’), 4) <i>L. albus</i> (cv. Luxor)
3. Test effect of food choice on damage level	Large females, 3 individuals/replicate, 11 replicates	<i>L. angustinus</i> cv. ‘Mandelup’ (1) no-choice, (2) with canola stubble, (3) with wheat stubble, (4) with mixed stubble
4. Assess impact of seeding feeding on fitness	Large females, 1 individual/replicate, 20-50 replicates **	<i>L. angustinus</i> cv. ‘Barlock’, <i>M. sativa</i> cv. ‘Sardi 7’, various food sources (see Table 4.3)

* Wild-type seeds came from a plant that expressed quinolizidine alkaloids within a cultivated variety ** 50- for plants, 20 for other food sources

Table 4.2. The range (min-max) of stadia and size ((length (mm) and weight (mg \pm standard error)) of *O. moreleti* in the six categories used in the analysis of feeding damage in Trial 1.

	Females		Males		Juveniles	
	Large	Small	Large	Small	Large	Small
Stadia	10 th -12 th	9 th or 10 th	9 th -12 th	8 th or 9 th	7 th	5 th or 6 th
Length (mm)	30-39	21-28	25-35	15-25	11-13	6-8
Weight (mg)	164-324, 244.4 \pm 12.1	50.2-90.7, 76.7 \pm 3.3	73.5-178.6, 115.6 \pm 8.8	41.6-96.8, 67.1 \pm 4.0	23.2-43.3, 32.0 \pm 1.1	9.6-16.1, 12.7 \pm 0.4

Trial 2 investigated how variation in host plant quality influenced the level of feeding damage to of lupin. We hypothesised that the susceptibility of *Lupinus angustinus* (Trial 1, Douglas et al (2017)) could be linked to the commercial breeding practices that have removed the defensive compound, quinolizidine alkaloids, due to the taste it has on the crop (Chen et al. 2007, Adhikari et al. 2012). *L. angustinus* seeds that express quinolizidine alkaloids were obtained from the Australian Grains Genebank. These seeds were collected from a cultivated *L. angustinus* crop as part of a monitoring process to remove plants expressing quinolizidine alkaloids. For this trial, we refer to them as ‘wild-type’ seedlings but they show little variation from the cultivated varieties used in this study, except for the expression of quinolizidine alkaloids.

We investigated the relative feeding damage *O. moreleti* caused to seedlings of two cultivated varieties of *L. angustinus* (cv. ‘Mandelup’ and cv. ‘Jurien’), ‘wild-type’ *L. angustinus* and one cultivated variety of *Lupinus albus* (cv. ‘Luxor’). *L. albus* is the other species of lupin grown in Australia. This species was included in the trial as while it has also been bred to remove quinolizidine alkaloids, these two species differ greatly in their physical architecture, with *L. albus* having significantly thicker leaf tissue. Thus while we can’t directly compare these species, we hypothesised *L. albus* would have lower susceptibility given millipedes are known to be limited in feeding by tissue toughness (Bailey and de Mendonça 1990, Ashwini and Sridhar 2005). The trial ran for 14 days with assessments made on days two, four, seven, 10 and 14. None of the 180 millipedes died.

Trial 3 investigated whether providing *O. moreleti* with alternate food sources would affect the level of feeding damage caused to *L. angustinus* (cv. Mandelup). Millipedes were placed in cups in a no choice situation (with bare soil, as in Trials 1 and 2) or in cups in a choice environment. To construct a choice environment, we chose alternate food sources used as food by *O. moreleti* in the field of the collection. These alternate food sources were canola and wheat crop stubbles. Four treatments were established: (1) *L. angustinus* seedling alone (bare soil), (2) *L. angustinus* seedling plus canola stubble, (3) *L. angustinus* seedling plus wheat stubble, and (4) *L. angustinus* seedling plus a mixture of canola and wheat stubble. Stubble was provided in excess quantity, as determined from preliminary trials (1 g of wheat stubble and 2 g of canola stubble). Potential variability in the habitat of the microcosm between treatments was limited by providing a moist shelter (as described in 4.3.1) in all

cups. Controls without millipedes were added to monitor for treatment effects on plant growth; no variability was observed.

Crop stubbles were hand-collected (in the same field *O. moreleti* were collected the following week). Wheat stubble in a state of advanced decay was collected. Canola stubble was collected as uneaten, free-standing stubble. This disparity in the condition of stubble selection was due to the relative palatability of these stubble types noted in previous field and shadehouse observations. Millipedes are found inside canola stubble, feeding on the soft lumen of canola stubble. For wheat, feeding is limited by the hardness of this tissue (Bailey and de Mendonça 1990, Ashwini and Sridhar 2005). We therefore selected soft, decayed wheat stubble, including stubble where feeding had been observed in the field. In the laboratory, collected stubble was sieved to remove excess soil, wetted, and kept moist under cardboard prior to the trial. Prior to the trial we used surplus millipedes to confirm *O. moreleti* would feed on both stubble samples (Douglas, J. unpublished data).

The trial ran for 18 days with assessments of plant damage and millipede survival made on days three, six, nine, 12, 15 and 18. On days six and 12 we moved individuals into identical feeding chambers. This was done as 1) seedling growth limits feeding (Douglas et al 2017). Placing individuals on fresh seedlings allowed treatment effects on the level of feeding over time to be assessed on a standardised seedling growth stage 2) to limit fungal growth occurring on the alternate food sources, 3) to limit variability in plant growth between treatments. Six out of 132 *O. moreleti* introduced died during the course of the study. Replicates where mortality occurred were removed from the analysis (two replicates from Treatment 2 and 3, and one replicate from Treatment 1 and 4, treatment numbers provided above).

Trial 4 investigated whether *O. moreleti* would obtain a fitness advantage from feeding on seedlings. We examined the weight gain of individual *O. moreleti* in response to feeding on lucerne and lupin seedlings over the course of 22 days. Three other alternative food sources, stubble, seed and potato were chosen to represent a spectrum of foods known to be fed on by *O. moreleti* (Table 4.3). All food sources were provided in excess quantity and prepared to ensure they could be fed on throughout the trial (as described in Table 4.3). For a positive control, individuals were provided both seed and potato. For a negative control, individuals were placed in cups with bare soil only (Table 4.3). The trial ran for 22 days, with individuals placed in in a fresh cup containing the same food source at day 11 individuals (as per trial 3).

To assess treatment changes in the weight of millipedes, we weighed individuals to 0.01 mg at day 0, 11 and 22.

As we anticipated a low proportion of millipedes may feed on seedlings, 50 replicates were used for the treatments containing crop seedlings and 20 replicates were used for the other food sources. Of the 200 millipedes introduced, a total of 17 died during this trial. For lupin and lucerne treatments respectively, five and seven out of 50 millipedes died. For other feeding treatments 0-2 out of 20 introduced millipedes died. Dead individuals were removed from all further analysis.

As a secondary question, trial 4 investigated whether feeding on lucerne and lupin seedlings during the first 11 days changed the chance of feeding in the second 11 days. Feeding on seedlings was categorized as either “no/minimal feeding” or “substantial feeding” if one or more multifoliate lupin leaves were consumed or if one or more lucerne seedlings was consumed.

Table 4.3. Food sources provided to *O. moreleti* and details of each treatment used in Trial 4.

Food source	Feeding value	Preparation and quantity provided
Lupin seedling	Shown to be a potential food source in Trial 1 and Douglas et al. (2017)	One seedling. All seedlings had two true leaves with the 3 rd and 4 th leaves emerging.
Lucerne seedlings	Shown to be a potential food source in Trial 1 and Douglas et al. (2017)	Five seedlings. All seedlings had fully unfurled cotyledons.
Canola stubble	Millipedes readily feed on the lumen of canola stubble in the field (J.D. personal observation).	Canola stubble was collected from the same farm as millipedes and prepared as described in Trial 2. 5 pieces of stubble approximately 5cm long were added to each cup (1.86g mean weight).

Seed	Seeds are known to be readily fed on by millipedes (Koprdoová et al. 2010) including <i>O. moreleti</i> (J.D. personal observation).	<i>L. angustinus</i> (cv. ‘Barlock’) seeds were germinated to break the seed coat before being left to die in the sun. 2 seeds were added to each cup (0.98g mean weight). In 3 out of 80 cups, mould grew on seeds and were replaced.
Potato	Potato slices have previously been used to culture <i>O. moreleti</i> (Baker 1978).	2 potato slices, approximately 5mm thick were added to each cup (1.67g mean weight). In 2 out of 40 cups, potatoes developed a hard crust and were replaced.
Positive control- Potato and seed	As multiple food sources may be needed for nutritional benefits, lupin seeds and potato, determined to be quality food sources were provided as a positive control.	Potato and lupin seeds were prepared and distributed to cups as described above.
Negative control- Bare soil	Only soil was provided in the feeding chamber. Soil is commonly found in the gut (Bailey & Kovalski 1990).	200 ml soil (1:1 sand: Décor potting mix). This potting mix was chosen as it is composed of low organic matter.

4.3.3 Data analysis

As *O. moreleti* mortality was limited to 0-2 individuals per treatment in Trial 1-3, treatment effects were only considered in Trials 4. For Trial 4 treatment effects on millipede mortality were investigated by a Fisher’s exact test using a paired contingency table, with no effect found between any treatment pair ($p > 0.15$).

Feeding damage was recorded differently for the different trials. For trial 1 we recorded the level of feeding damage to the plant as the percentage of the initial leaf matter removed in order to compare feeding between crop species. Trial 2 and 3, which only used lupin

seedlings assessed feeding damage as the cumulative number of leaf foliates removed. In trial 4, we recorded feeding as the number of lupin foliates and lucerne seedlings consumed and then converted this to an estimate of dry plant tissue weight (mg). This estimate was determined by multiplying the amount of leaf matter consumed by an estimate of the mean dry weight of leaf tissue in each seedling. An estimate of the dry weight of lupin and lucerne seedlings was obtained by oven-drying lucerne and lupin seedlings (20 seedlings for each) at 40°C for 48 hours and recording mean weight.

For trial 1 the proportional plant feeding damage data (%) was not normally distributed, therefore the data was transformed into a dichotomous variable (seedling consumed/not consumed). We conducted a generalised linear model (GLM) using seedlings consumed at 15 days as the response variable and crop-type, sex/life-stage and size as predictor variables. Six models were constructed using a process of sequential addition of significant predictor variables and the interaction between crop type and other predictors. The best model was identified by restricted maximum likelihood estimation. Binomially distributed errors and a logit link function were used to fit the GLM to these variables (R Core Team 2018).

For trial 2 and trial 3 treatment effects on the level of feeding damage caused to lupin seedlings over the course of the trials were investigated using a linear mixed effects models (Pinheiro et al. 2018). For trial 2 the model was constructed with variety and time as fixed effects and replicate as a random effect. For trial 3 the model was constructed with alternate food sources and time as fixed effects and replicate as a random effect. Models were fitted by restricted maximum likelihood estimation. Significant effects were investigated by pairwise comparison of the slope using Tukey's HSD post-hoc tests (Lenth 2018).

For trial 4 equality of variances in weight gain (mg per individual millipede) were determined using Levene's test. A repeated measures ANOVA was performed on weight gain to investigate treatment differences in weight gain when averaged over the two time periods and test for an interaction between weight gain and time. A Tukey's HSD test was performed for *posteriori* comparisons.

Differences in the amount of lucerne and lupin leaf tissue consumed (mg) over the 22 days of the study were tested using an analysis of variance (ANOVA). Linear models were then used to test the relationship between weight change in millipedes and plant tissue consumed. As

little lucerne was eaten compared with lupin (see Results) these were run as individual models for each crop type.

To understand whether feeding on seedlings during the first 11 days changed the chance of feeding in the second 11 days, the amount of feeding on lucerne and lupin was analysed through McNemar's test with continuity correction using a two by two matrix. All analyses were conducted in R Studio (RStudio Team 2015).

4.4 Results

Trial 1 investigated the level of feeding damage (proportion of seedling removed) different life-stages/sexes (juveniles, males, and females) of *O. moreleti* caused to canola, lucerne and lupin seedlings. The influence of size was investigated by dividing each life-stage/sex category into large and small individuals. Large individuals weighed roughly 2-3 times more than small individuals within each category (Table 4.2). Over the course of 15 days, some feeding damage was observed by all sizes and life-stages (Figure 4.1). All factors (crop-type, life-stage/sex and size) were related to feeding damage; for crop ($\chi^2_{(2)} = 42.2$, $p < 0.001$), for life-stage/sex ($\chi^2_{(2)} = 122.83$, $p < 0.001$), for size ($\chi^2_{(1)} = 77.76$, $p < 0.001$). There was a significant interaction between damage to different crop types and life-stage/sex ($\chi^2_{(4)} = 49.50$, $p < 0.001$) but not size ($\chi^2_{(2)} = 3.31$, $p = 0.19$). For females, there was significantly greater damage to lupin than the other crops ($p < 0.001$ for both comparisons), with no significant difference between lucerne and canola ($p = 0.54$). For juveniles and males, there was no significant difference in damage caused between any of the crops. Large individuals caused significantly more damage than small individuals for all crops ($p < 0.001$).

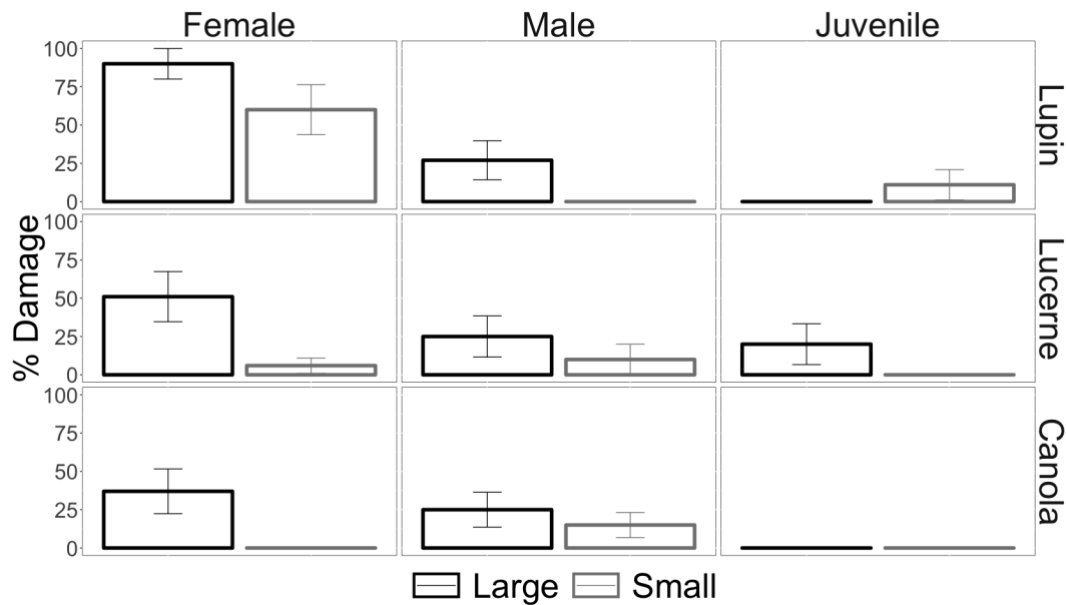


Figure 4.1. Bar graph of lupin, lucerne and canola seedlings consumed by different life-stages/sex and sizes of *O. moreleti* after 15 days. Mean values \pm standard error are provided for each treatment.

Trial 2 investigated feeding damage (leaf matter removed) three female *O. moreleti* caused to cultivated seedlings of *L. angustinus* (two varieties) and *L. albus* (one variety) and wild-type *L. angustinus* seedlings. Significant differences were found for the overall level of damage caused between varieties ($F_{3,56} = 16.69$, $p < 0.001$) and an interaction between feeding damage and variety over time ($F_{3,236} = 16.35$, $p < 0.001$). No significant differences in feeding damage was found between the two cultivated *L. angustinus* varieties ($p = 0.24$). The amount of damage to cultivated varieties of *L. angustinus* was significantly greater than *L. angustinus* wild-type and *L. albus* seedlings ($p < 0.001$ in all comparisons). By day 14, mean feeding damage on the cultivated was approximately seven times higher than on wild-type seedlings and four times higher than on *L. albus* seedlings (Figure 4.2).

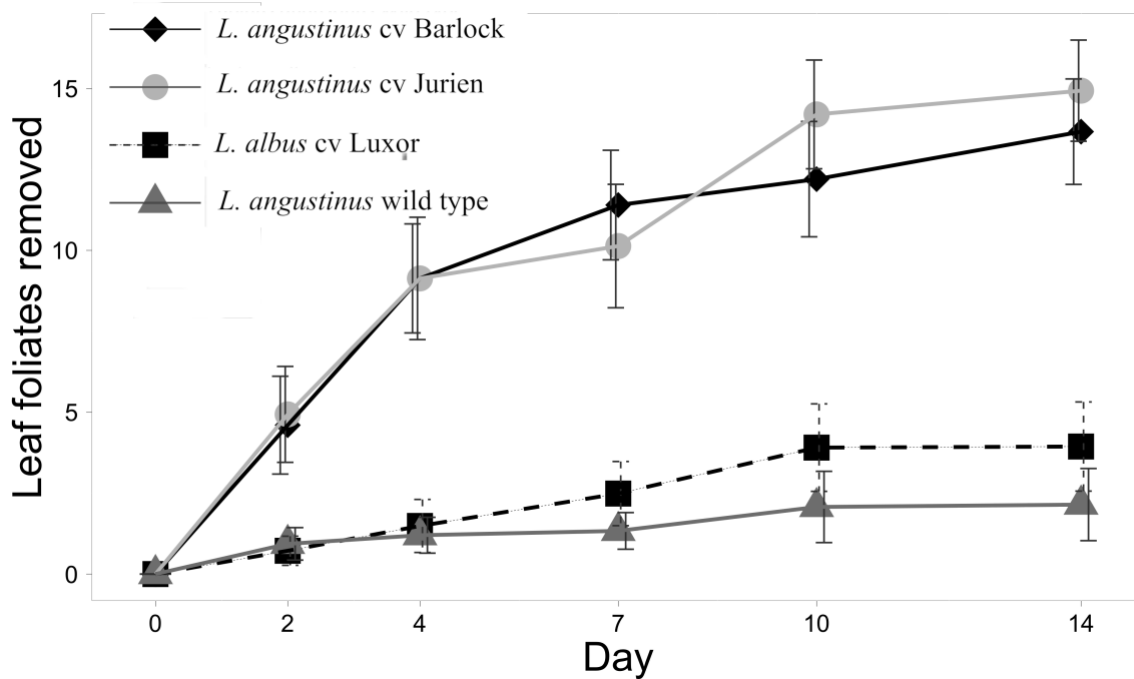


Figure 4.2. Number of lupin multifoliate leaves removed from two cultivated varieties of *L. angustinus* (cv. ‘Barlock’ and cv. ‘Jurien’), wild-type *L. angustinus* seedlings and *L. albus* seedlings over the course of 14 days. Mean values \pm standard error are provided for each treatment.

Trial 3 investigated feeding damage (leaf matter removed) *O. moreleti* caused to *L. angustinus* seedlings when placed in cups in a no-choice (bare-soil) or choice environment where they had the option of feeding on crop stubble. While we found a significant treatment effect on feeding damage ($F_{3,177} = 9.71$, $p < 0.001$), this effect was not what we expected. As expected, the lowest level of feeding damage was found in the positive control (mixed stubble), however the highest feeding damage was in the treatment where individuals were provided with canola stubble (Figure 4.3). The level of feeding damage over time in this treatment was significantly different from both the positive ($p < 0.01$) and negative ($p < 0.05$) controls. All other pairwise comparisons were non-significant.

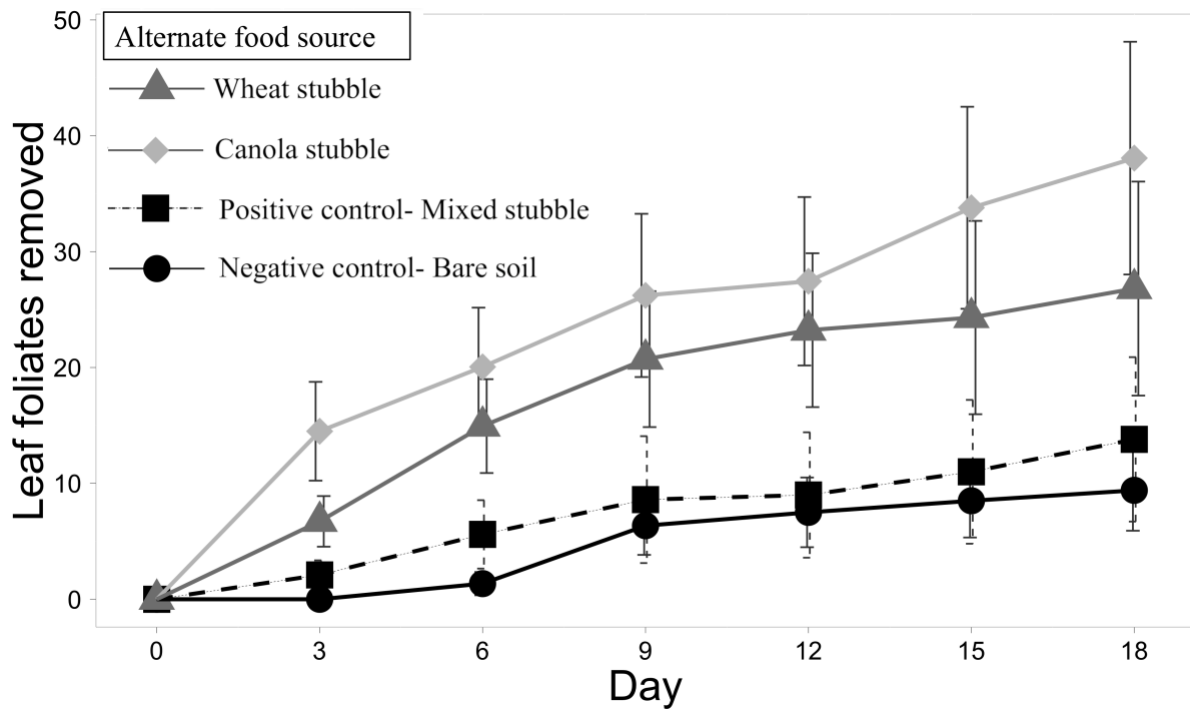


Figure 4.3. Number of *L. angustinus* multifoliolate leaves removed by *O. moreleti* when placed in one of four feeding treatments: lupin seedling plus wheat stubble; lupin seedling plus canola stubble; lupin seedling plus mixed stubble (canola and wheat), which acted as a positive control; and lupin seedling with bare soil, which acted as a negative control. Mean values \pm standard error are provided for each treatment.

Trial 4 investigated the change in weight of female *O. moreleti* over 22 days when feeding on seedlings and a variety of other known food sources. Mean weight gain was found in all treatments but varied between food sources ($F_{6,176} = 17.43$, $p < 0.001$) from 0.58 ± 1.37 mg in the negative control (bare soil) to 23.45 ± 2.76 in the positive control (seeds and potato). Post-hoc tests showed differences in the amount of weight gained could be split into two categories; millipedes in the lupin seedling, potato, seed and the positive control (potato and seed), gaining significantly more weight than individuals in the lucerne seedling, canola stubble and negative control (bare soil) treatments (Figure 4.4). We found an interaction with weight gain over time ($F_{6,176} = 2.70$, $p < 0.05$). All treatments recorded an overall mean weight gain during the first 11 days, but only the positive control treatments (potato and seed) and the lupin seedling treatment recorded further mean weight gain (Figure 4.4). For lupins, a significant correlation between the amount of leaf tissue consumed and the weight gained by individual millipedes was found at 11 days ($F_{1,45} = 14.28$, $p < 0.001$) but was weakly non-

significant at 22 days ($F_{1,43} = 3.12.28$, $p = 0.08$). For lucerne, no correlation was found at either 11 ($F_{1,45} = 0.41$, $p = 0.53$) or 22 ($F_{1,41} = 1.15$, $p = 0.28$) days.

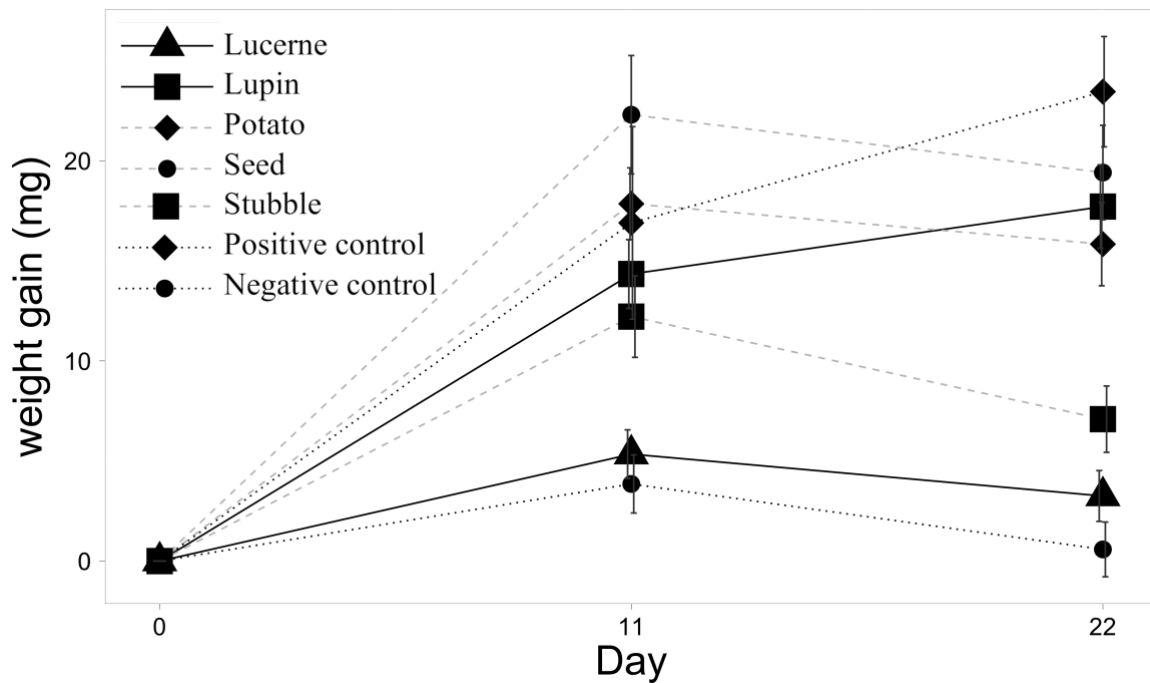


Figure 4.4. Weight gain (mg) (mean values \pm standard error) of *O. moreleti* over 22 days when provided a variety of food sources. The positive control provided individuals with two food sources (potato and seed). The negative control had millipedes in cups with bare soil.

The amount of leaf tissue consumed was significantly greater for millipedes feeding on lupin seedlings (average 25 mg, 9.6% of their body weight) than lucerne seedlings (average 4.3 mg, 2.3% of their body weight) ($F_{1,86} = 58.78$, $p < 0.001$) over the course of 22 days. The majority of millipedes substantially fed on both crops within the first 11 days (as defined in the methods, Table 4). For individuals that substantially fed on lucerne within the first 11 days, a decreased number of individuals substantially fed during the second 11 days ($\chi^2_{(1)} = 5.5$, $p = 0.02$). For lupin however, individuals that substantially fed during the first 11 days continued to substantially feed during the second 11 days ($\chi^2_{(1)} = 0.27$, $p = 0.61$) (Table 4.4).

Table 4.4. The relationship between the number of individual *O. moreleti* that ‘minimally’ and ‘substantially’ fed on lupin and lucerne in the first time period (0-11 days) with the number of those individuals that continued to ‘minimally’ and ‘substantially’ feed during the

second time period (12-22 days). ‘Substantial’ feeding on lupin and seedlings is defined as one or more multifoliate leaves. For lucerne, substantial feeding is defined as one or more lucerne seedlings.

Treatment	Number of individuals	Minimal feeding days 12-22	Substantial feeding days 12-22	Total
Lupin	Minimally feeding days 0-11	3	6	9
	Substantially feeding days 0-11	9	27	36
	Total	12	33	45
Lucerne	Minimally feeding days 0-11	8	5	13
	Substantially feeding days 0-11	17	13	30
	Total	25	18	43

4.5 Discussion

O. moreleti is known to feed on a small number of crop types, including canola, lucerne and lupin seedlings, which are quite susceptible to attack (Douglas et al. 2017). In order to help assess pest risk, this study investigates factors that influence the feeding behaviour of *O. moreleti* on seedlings under controlled conditions. The level of feeding damage varied with life-stage, sex and size *O. moreleti*, while the reduction in plant defences in commercially cultivated lupin could be linked to the susceptibility of this seedling. However, the level of feeding damage to seedlings was not reduced when crop stubbles were provided as alternate sources of food. Furthermore, despite *O. moreleti* being thought to utilise living seedlings as a food resource, we found feeding on lupin (but not lucerne) provided *O. moreleti* with fitness benefits, in terms of weight gain.

The relative susceptibility of lupin seedlings to be fed on by *O. moreleti* was likely due to differences in chemical differences and physical architecture. Cultivated varieties, which have had quinolizidine alkaloids removed (Adhikari et al. 2012), did not differ in the level of feeding damage recorded in our trials. On ‘wild type’ *L. angustinus*, which produce this compound, there was seven times less feeding, indicating this compound deters feeding. Commercial breeding programs therefore appear to have increased the ability of *O. moreleti* to be a facultative herbivore on lupin. Cultivated *L. angustinus* leaves may in fact be palatable, with millipedes substantially feeding on these leaves over an extended period. The

other cultivated species of lupin in Australia, *L. albus*, which also has had quinolizidine alkaloids removed (Chen et al. 2007), was fed on four times less than the cultivated *L. angustinus* varieties. The thicker leaf tissue of this species may limit the ability of *O. moreleti* to feed (Douglas et al. 2017), given millipedes are known to have a limited ability to consume tough detrital food sources (Bailey and de Mendonça 1990, Ashwini and Sridhar 2005).

For lupin, but not for lucerne, crop feeding provided *O. moreleti* a fitness benefit in terms of weight gain. Female *O. moreleti* gained similar weight feeding on lupin seedlings as on lupin seeds (on which they are known to readily feed) (Koprdoová et al. 2010) or potato (which has been used to culture this species) (Baker 1978). This ability to utilise and process plant material may be surprising given *O. moreleti* do not possess their own cellulolytic enzymes. However, the millipede hindgut is a site for cellulolytic microbes (Anderson & Bignell 1980, Bignell 1989, Taylor 1982b) and millipedes are known to adapt their digestive capabilities to survive in different environments. For instance, in the arid environment, *Orthoporus ornatus* is thought to have an increased ability to process cellulose due to its increased reliance on plant matter as a food source (Taylor 1982b). This may be similar for *O. moreleti* in an agricultural environment.

Feeding damage to lupin was not reduced in the presence of crop stubbles, which are alternate food sources *O. moreleti* feeds on in a field environment. If our findings are applicable to the field environment, feeding damage to lupin seedlings may not be influenced by the presence of crop stubble. In the field other alternate food sources may be important as millipedes are known to gain nutrients from a diverse range of microbial food sources (Taylor 1982a, David & Célérier 1997) and move large distances to find more suitable food (Baker 1988). Counter-intuitively, this study actually found more damage to lupin in treatments with canola stubble than in treatments with bare soil. Several hypotheses may potentially explain this result. Firstly, the alternate food sources may have improved the ability of *O. moreleti* to digest plant matter, thereby promoting further feeding. Secondly, higher moisture retention by the stubble may have led to organisms feeding on plants in search of water (eg. Mensink and Henry 2011), although we do not believe water stress was a factor in our study. Thirdly, this result suggests that *O. moreleti* may have adaptive feeding strategies in different environments and at different times of the year (Bailey and de Mendonça 1990), given this trial was conducted in early summer.

Damage to all crop seedlings was related to life-stage/sex and size of *O. moreleti*, with large females causing the most damage. This may be explained by food intake of different sizes and life-stages (Elliot 1970, Dangerfield and Telford 1989, Lawrence and Samways 2003), rather than a bias for feeding on living plants. Thus, the number of large females may be a better indicator of pest risk than the overall millipede density. Female millipedes are known to greatly increase their feeding consumption during their reproductive period (Blower 1974). This may be particularly important given *O. moreleti* feeding is largely limited to the early plant growth stages of crops (Douglas et al. 2017).

In conclusion, this study has demonstrated that while a few crop seedlings are fed on by *O. moreleti*, their susceptibility and palatability is highly variable. Feeding damage varies with different life-stages and sexes, while the presence of crop stubble as alternate food sources may not preclude feeding on seedlings. We have therefore built a basis for future research to investigate how the pest status of *O. moreleti* varies through the life-cycle and under variable environmental conditions. Long-term field studies could manipulate crop sowing date and alternate food sources to understand how environmental and agronomic factors influence both feeding rates and food choice during the autumn crop establishment period. This may be particularly important given *O. moreleti* feeding is largely limited to the early plant growth stages of crops (Douglas et al. 2017). These studies would help in predicting the pest status of *O. moreleti* in fields with variable environmental conditions and agronomic practices.

4.6 References

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Chapter 5- The influence of water stress on feeding by *Armadillidium vulgare* to canola seedlings: a preliminary investigation

5.1 Introduction

Under stressful environments, organisms must adapt their feeding behavior to fulfil their physiological requirements by feeding on sub-optimal food sources (Edney and Spencer 1955). For soil-dwelling invertebrate species that lack a waxy cuticle, maintaining water is essential for survival. Some of these species that primarily feed on decaying plant matter (i.e. species that are primarily detritivores) will turn to feed on living plants (Faberi et al. 2011, Johnson et al. 2012, Douglas et al. 2017). Because detritivores are limited in their ability to process the tissues of living plants, this switch in feeding behavior may occur when their physiological requirement for water over-rides the deterrent compounds present within plants (Mensink and Henry 2011, Johnson et al. 2012).

Isopods are crustaceans found in a range of terrestrial habitats, from the intertidal zone to the desert (Warburg 1993). While species that are found in desert environments are highly adapted to survive desiccation, even these species will die within hours under severe water stress (Linsenmair 1975). Isopods obtain the majority of their water supply from food (Spencer 1954), and do not appear to undergo a period of metabolic arrest to cope with desiccating conditions during summer periods (Sutton 1980). This suggests they are proficient at adapting their feeding behaviours in order to maintain water balance.

Armadillidium vulgare is a terrestrial isopod that is found worldwide in Mediterranean-type environments. While it was initially thought to be a beneficial, detritivorous feeding organism, in fields it is increasingly being reported as a pest of a range of establishing seedlings, such as canola, soy beans, oats, lupins and lucerne (Faberi et al. 2011, Douglas et al. 2017). The level of damage *A. vulgare* causes to crop seedlings is suggested to be correlated with dry field conditions (Johnson et al. 2012, Perry 2012). A range of mechanisms may be related to an increased level of feeding on plants under dry conditions, such as an increased level of seedling susceptibility in some plants or a decrease in the quality of alternative food sources.

Armadillidium vulgare has the ability to roll in to a ball which reduces water loss from its exposed ventral surface (Warburg 1993). In comparison to species found in more mesic regions, it also has a better ability to extract water from food (Kuenen 1959) and a lower cuticular permeability (Warburg 1993). This study focuses on understanding how the physiological stress from water deprivation impacts the level of damage *A. vulgare* causes to canola seedlings.

5.2 Methods

5.2.1 Study design

The influence of water stress on seedling damage caused by *A. vulgare* was assessed in controlled temperature (CT) cabinets. CT cabinets were maintained at 20:16°C on a 10:14 day/night cycle from 8am to 6pm.

During the day, individuals were kept in air-tight containers at different levels of ambient humidity. Different levels of water stress were created by varying the ambient humidity of air during the daytime and at night (Table 5.1). The daytime temperature was 20°C; at this temperature the rate of water loss in *A. vulgare* shows a largely linear correlation with the saturation deficit of air. Individuals exposed to 0% humidity at 20°C die within 24 hours (Edney 1954). Treatments were set-up to impose variable levels of water stress, with the driest environment causing extreme water stress to the point of mortality (see Table 5.2). I hypothesized that exposing *A. vulgare* to different levels of water stress during the day would lead to different levels of feeding on seedlings at night in order to obtain water.

During the night they were moved to cups with emerging canola seedlings as food searching primarily occurs at night (when temperatures drop and humidity increases) (Edney 1954, Dias et al. 2012). At night, the temperature was lowered to 16°C to mirror a warm night. To assess how *A. vulgare* might adapt their feeding behaviour, individuals were exposed to two distinct levels of ambient humidity during the night (Table 5.1). If individuals were placed in a dry environment it would prolong water stress but likely reduce foraging behaviour (Dias et al. 2012). Conversely an environment with humidity close to saturation would reduce water stress but provide suitable conditions for feeding. There may therefore be an interaction between the night and day treatments over the course of this study.

As *A. vulgare* will cause considerable damage to canola seedlings after a starvation period of 3-4 days (Douglas et al. 2017), I hypothesized that when individuals weren't hungry they would only feed on seedlings when searching for water. Thus individuals used in this trial weren't starved prior to introduction. The trial ran for four days in order to evaluate the cumulative effect of water stress on the propensity to feed.

Table 5.1. Trial details, showing the levels of humidity *A. vulgare* were exposed to across treatments, and the number of replicates per treatment. For the treatment where individuals were kept in a humid environment during the day only a humid environment was used at night as no water stress was imposed.

Humidity during day	90%	75%		55%		40%	
Humidity during night	Humid	Humid	Dry	Humid	Dry	Humid	Dry
Treatment no.	1	2	3	4	5	6	7

5.2.2 Trial setup

This trial was conducted in June, 2018 at the Bio21 Institute (University of Melbourne, Parkville, Victoria). Large adult *A. vulgare* (>80 mg) were collected from under logs in a nearby park. Individuals were transported back to the laboratory and placed in a plastic container with a small amount of dirt and bark. They were kept in a CT cabinet at 16°C overnight. The next morning, individuals were randomly divided into the four different levels of humidity that were maintained in the 6L air-tight containers (Seal C6, Bell Investment Co, AUS) for the day treatment (Table 5.1).

The relative humidity within the air-tight containers was maintained by a saturated solution of salts NaI, NaBr, NaCl or H₂O. Saturated solutions of these salts maintain constant relative water vapor at a given temperature, regardless of the loss of water in an organism (Winston and Bates 1960). Temperature and humidity were recorded using a Hygrochron iButton (DS1923, Thermodata Pty Ltd, AUS) throughout the course of the trial (Figure 5.1).

For the air-tight containers with the three higher levels of ambient humidity (rH levels approx. 55%, 75%, 90%, see Figure 5.1) I introduced 30 individuals. As mortality was anticipated at rH~40%, I introduced 40 individuals for this treatment. Each individual was placed in mesh-bags (approx. 5cm) to prevent them falling on to their back and being unable to get up (as they are prone to do) thus exposing their lungs to desiccation. During the first day, 10 of the 40 individuals that were kept at rH ~40% died, while unexpectedly 3 of the 30 individuals that were kept at rH ~ 75% also died. The survivors (30 for the rH~ 40%, 75% and 90% treatments and 27 for the rH~55% treatment) were divided in to individuals placed in dry an humid feeding cups that night. Each replicate consisted of a single individual, thus all treatments had 15 replicates, except for the 55% rH treatment in which there were 14 and 13 for both the humid and dry night treatments respectively.

The feeding cups in to which they were introduced at night contained 6 canola (*Brassica napus L.* cultivar Gem) seedlings grown in 200ml cups containing a mixture (1:1) of sandy loam, perlite and potting mix. A layer of vermiculite was added at the bottom to retain maintain moisture for roots to utilize. On the soil surface 20ml of a mixture (2:1) of sandy loam and potting mix was added which created a tough surface layer to prevent *A. vulgare* digging into the soil (Figure 5.3). *A. vulgare* were introduced when canola seedlings were fully unfurled cotyledons.

The feeding cups were regulated as either dry or humid environments without posing significant stress on the seedlings by altering the timing and method of watering. For humid (90% rH) night treatments, watering was conducted at night, prior to introducing *A. vulgare* individuals. On each occasion, 5ml of water was added per cup to the soil surface. Cups were then covered in cling-wrap to maintain a moisture in the upper soil and ambient humidity above 90% during the night. For dry night feeding cups, watering was conducted in the morning, immediately after individuals were removed from their cups. Watering was dispensed using a syringe needle to directly water the lower soil layers. While some water spread through the soil layer, this dried out during the day so that no surface moisture was present when individuals were placed into the cups at night. For the first two days of the trial, 5ml was added as per the wet treatment. Up to this period no seedling exhibited signs of desiccation. On day 3, seedlings in 12 cups showed signs of wilting, thus 10ml was added to these cups. On the final day, 10ml was added to all replicate cups. The success of these watering regimes in maintaining humid and dry humidity's in the respective treatments was

demonstrated by the Hygrochron monitor data (see Figure 5.2). Moisture stress on seedlings was visually assessed by careful monitoring for leaves wilting.

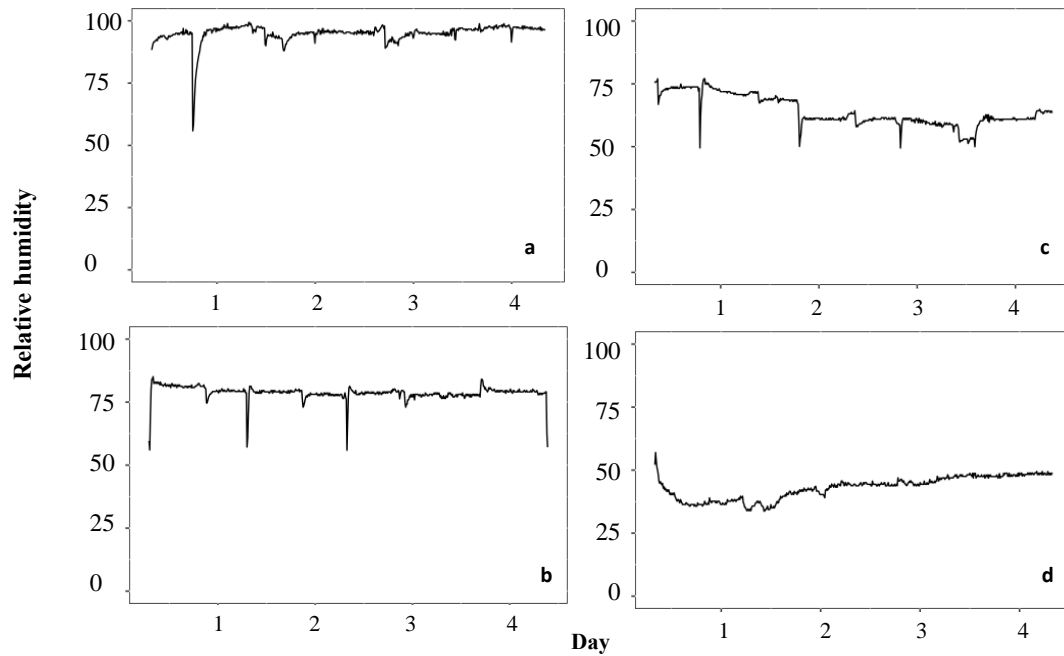


Figure 5.1. The relative humidity (rH) levels in the air-tight containers over the 4 days of the study. a) rH~ 90%, b) rH~75%, c) rH~ 60%, d) rH~ 40%. Individuals were kept within these containers from 8am to 6pm daily.

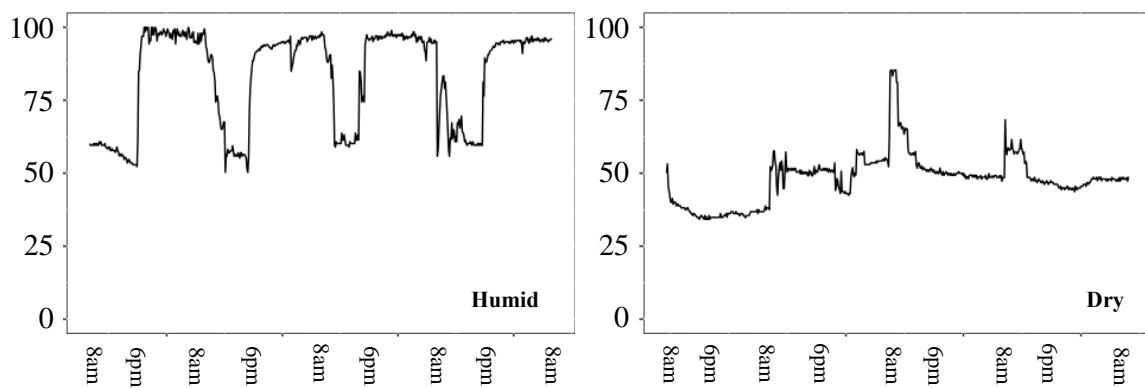


Figure 5.2. The relative humidity (rH) levels in the humid and dry environments in the feeding containers where individuals were kept 6pm to 8am daily.

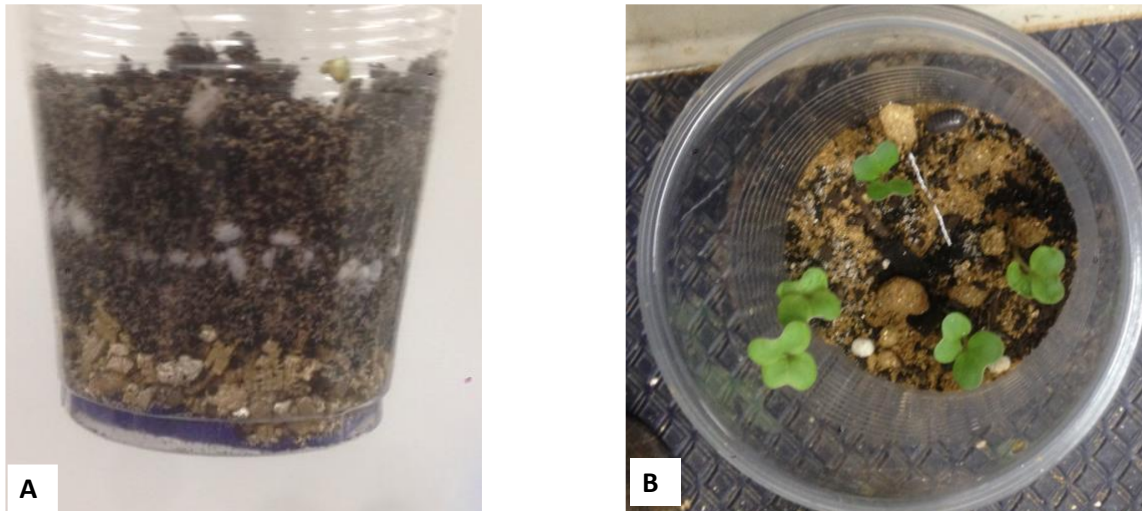


Figure 5.3. The set-up of the soil layers in microcosm cups to allow the maintenance of dry and humid environments on the soil surface without causing stress to the canola seedlings.

5.2.3 Data analysis

Individuals were assessed as being alive or dead (by the lack of antennal reflex (Edney 1951)) when they were transferred between night cups and day containers (day 1, 2, 3, 4 and night 1, 2, 3, 4). The level of damage in each cup was recorded on the morning of days 1, 2 and 4 as a cumulative number of feeding damage. Damage to each seedling was scored on a scale between 0 (undamaged) to 1 (either consumed or cut at the base), with partial damage was scored as the proportion of the leaf matter removed (as per Chapter 4). For seedling damage data, replicates where individuals died during the study were removed from all analysis to ensure the cumulative effect of humidity was consistent between treatments.

Mortality effects of day humidity and night humidity were calculated using Cox proportional hazard model (Therneau 2015). As no mortality occurred in two treatments (see Table 5.2), interactions between variables were not considered. The level of damage was determined by the amount of seedlings leaf tissue removed. Seedling damage was investigated for effects of daily and nightly ambient humidity treatments using a linear mixed effects model (Pineiro et al. 2018). The model was constructed with day humidity, night humidity, and time as fixed effects and cup as a random effect and fitted by restricted maximum likelihood estimation. Interactions were removed from the model as they were found to be non-significant. Significant effects were investigated by pairwise comparison of the slope using Tukey's HSD

post-hoc tests (Lenth 2018). Boxplots were used to visualise the relationship between the level of damage and humidity treatments (Wickham 2016). All treatment effects were considered significant if $p < 0.05$. All analysis were conducted in R Studio (RStudio Team 2015).

5.3 Results

The different levels of humidity during the day led to significantly different levels of mortality (Table 5.2). Over the course of four days, no individuals died when they were kept at high levels of ambient humidity (rH ~ 90%) during the day, but almost half the individuals died when they were kept at low levels of ambient humidity (rH ~ 40%). During the night, 3 individuals from the dry treatment died, but none from the humid treatment. As no mortality occurred in multiple treatments (see Table 5.2), interactions between variables were not considered.

The level of feeding damage to seedlings was affected by both the humidity levels individuals were kept at during the day ($F_{1,275} = 5.63$, $p < 0.01$), and the humidity level within the feeding cup at night ($F_{3,275} = 9.25$, $p < 0.001$). While I hypothesized water stress would increase the level of feeding damage, I found the opposite. The lowest levels of damage were recorded when individuals were introduced in to feeding cups with a dry environment (Figure 5.2). Individuals kept at the lowest level of ambient humidity (rH~40%) during the day caused significantly less damage than individuals kept at the most water saturated treatments ($p < 0.05$) for rH~75% and $p < 0.01$ rH~90%) (Figure 5.2). No interaction was found on the level of damage with day and night humidity ($p=0.50$), indicating these treatments were having independent effects.

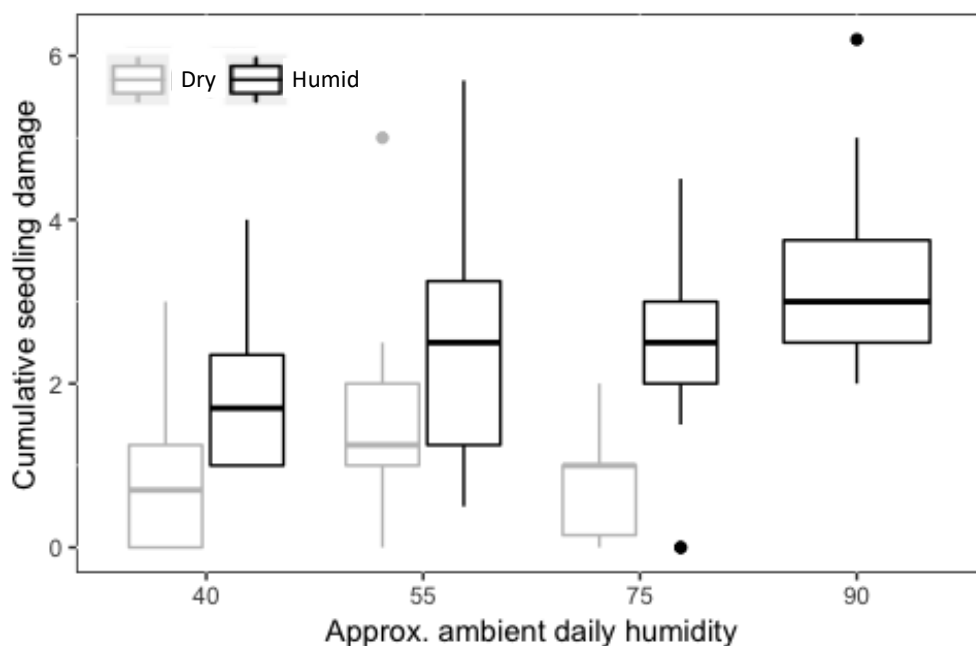


Figure 5.4. The cumulative level of seedling damage when *A. vulgare* were desiccated under four different daytime humidity treatments and then placed overnight in either humid or dry cups. The horizontal line of the boxplot shows the median value, the edges show interquartile ranges with whiskers showing the 95% confidence intervals. Closed circles show values outside of this interval.

Table 5.2. Trial details, showing the number of *A. vulgare* individuals the number alive at different levels of humidity treatments. All but 3 individuals died during the day.

Approx. humidity during day	90%	75%		55%		40%**	
Humidity during night	Humid	Humid	Dry	Humid	Dry	Humid	Dry
Day 0	15	15	15	15	15	20	20
Day 1	15	15	15	14	13	15	15
Day 2	15	15	15	14	11*	15	15
Day 3	15	14	13	14	11	15	11
Day 4	15	14	12*	14	11	11	10
% survival Day 4	100	93	80	93	74	55%	50%

* Individuals died during the night treatment. ** 10 extra individuals were initially introduced in to the rH 40% treatment day treatment.

5.4 Discussion

Armadillidium vulgare primarily feeds on decaying plant matter but has become an increasing pest of a range of crop seedlings, such as canola in Australia. This study investigated how the level of damage *A. vulgare* causes to canola seedlings would vary when individuals were kept at different level of ambient humidity. I hypothesized that *A. vulgare* would adapt to the water stress imposed by dry conditions during the day by feeding on seedlings at night in order to obtain water. Based on the results from this preliminary trial, this is not the case. Individuals that were kept at low levels of ambient humidity during the day caused less feeding damage. This effect was increased when individuals were also kept in dry conditions at night. Thus I was unable to find that the pest status of *A. vulgare* would increase during dry periods.

The reduction in feeding damage when individuals were placed in to feeding cups at night can likely be explained by the reduction in foraging behaviour under dry conditions (Dias et al. 2012). The different levels of daily ambient humidity individuals were expected to induce a range of adaptive behaviours to water stress. Despite significant water stress in some treatments, as demonstrated by mortality, this wasn't found. This suggests *A. vulgare* may shut down its feeding behaviour and instead shelter through desiccating conditions, indicating the pest status of *A. vulgare* would decrease during dry periods.

Understanding how the survival strategies of *A. vulgare* under dry conditions impacts the level of pest damage requires further research. Variable responses to water stress may occur seasonally depending on variation in their metabolic rate and osmotic pressure. Furthermore, this study didn't investigate potential interactions between body temperature and hydration state on physiological performance (Block 1996, Edney 2012). An increased level of crop feeding during dry periods (Johnson et al. 2012) may occur through an indirect effect on alternate food sources (Mensink and Henry 2011) or through variability in plant susceptibility with water stress (Cronin and Hay 1996). Future research should be conducted at a range of temperature profiles and investigate the behavioural response of *A. vulgare* when provided variability in food sources. This research is important to understand both the seasonal, and future pest risk under climate change, of *A. vulgare*.

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Chapter 6: Where, when and why is *Ommatoiulus moreleti* abundant in agricultural crop fields in Victoria and southern New South Wales?

A preliminary study

6.1 Introduction

The Portuguese millipede, *Ommatoiulus moreleti* was first found in Australia near Adelaide in 1953. From this site of introduction it spread east and west (Baker et al. 2013) at a rate of approximately 200 metres a year (Baker 1978a). A recent survey found the distribution across southern Australia had largely realised its predicted potential based on suitability of temperature and summer rainfall (Baker 1985a, Baker et al. 2013). This distribution extends throughout Victoria and south-eastern New South Wales (Baker et al. 2013) and is found in a variety of different environments from woodlands, grasslands to dunes. This broad occurrence suggests it is not particularly sensitive to variations in soil type or vegetative characteristics, although abundances can vary between different environments (Arnold et al. 2008, Baker 1978b).

Increased stubble retention practices are suggested to have led to increased presence and abundance of *O. moreleti* in broadacre agricultural fields (Douglas et al. 2017). The presence of stubble improves soil moisture retention and benefits millipedes by providing shelter and a food source (Holland 2004, Stinner and House 1990). However, a range of biotic and abiotic factors will also influence the abundance of *O. moreleti*. The distribution of *O. moreleti* in its native range of Portugal may be limited by soil hardness, litter quantity and the presence of shrubs or bushes (Bailey and de Mendonça 1990). Mortality during summer is dependent on heat (Baker 1978b) and the availability of moist habitats in the soil (Bailey and de Mendonça 1990). A range of organisms attack *O. moreleti* (Baker 1985b, McKillup and Bailey 1990, Terrace and Baker 1994) but are not thought to provide widespread regulation of abundance in Australia (Baker et al. 2013, McKillup and Bailey 1990).

This chapter aims to provide preliminary observations of regional and local variation in *O. moreleti* abundance in crop fields in Victoria and southern New South Wales to guide future research on population dynamics.

6.2 Methods and results

6.2.1 Study outline

To assist in the identification of locations where high abundances of *O. moreleti* may be present in crop fields in south-eastern Australia, we investigated *PestFacts south-eastern* bulletins from the year 2006-2016 (cesar 2017). These bulletins collate reports of pest outbreaks in broadacre fields received through a network of farmers, agronomists and extension workers. While these reports may capture a limited number of the overall incidences, they provide an indication of regional pest problems (Hoffmann et al. 2008). Individual reports can indicate different levels of abundance. I have divided them into three categories of *O. moreleti* abundance: 1) single or multiple observations of low densities, 2) single observations of high densities, 3) multiple observations of high densities. Based on these reports, *O. moreleti* appears most likely to reach high abundances in crop fields in a few areas of NSW and Victoria. In the Goulburn Valley (a farming district in central Victoria) and in the southern Riverina (near the Murray River), several fields have been reported to the *PestFacts* service with high abundances of this pest across multiple years (Figure 6.1).

Between 2014 and 2017, nineteen farms which had high field abundances of *O. moreleti* based on field reports were investigated during the crop growing season for potential sites to conduct a study of population dynamics. A limited number of these farms (six) had fields with a demonstrable high abundance. While a detailed study of the population dynamics of *O. moreleti* proved problematic, I have nevertheless summarized observations made from visual sampling as a guide for future research. Investigations were only conducted at a single time point across farms where only low abundances were found. In those farms where high field abundances were found, fields were monitored on multiple occasions (3-9 times) and observations on abundances in surrounding fields were made.

Multiple reports of high abundances
 A single report of high abundance
 + Reports of low abundances

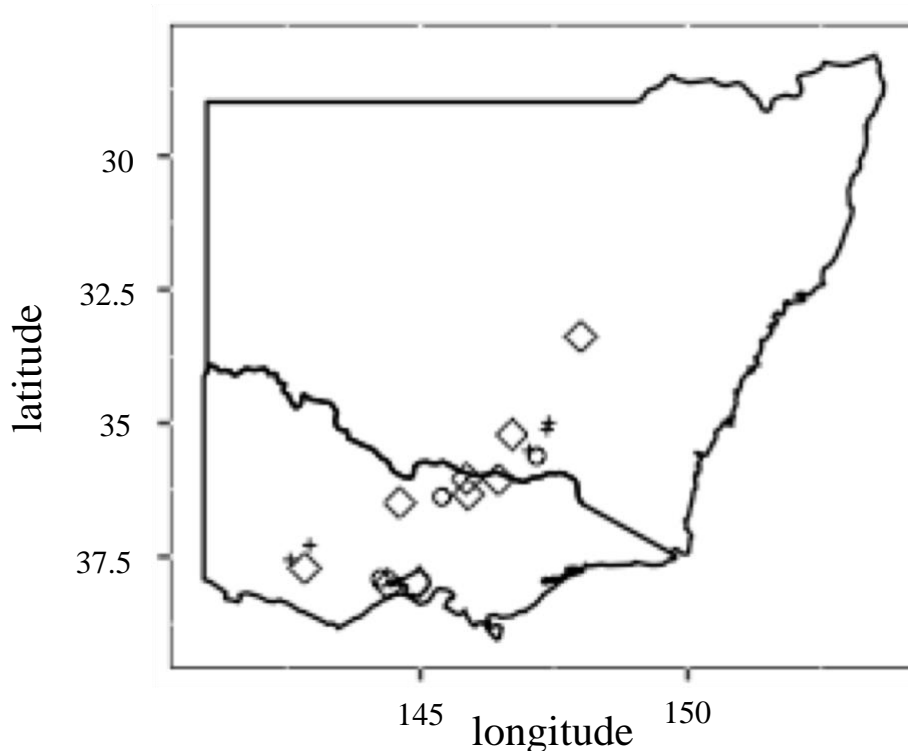


Figure 6.1. PestFacts south-eastern reports of *O. moreleti* abundance in crop fields across south-eastern Australia. Reports are divided into three categories marked by different symbols (as described in the legend).

6.2.2 Farm and field abundance observations

Of 19 farms investigated, only six farms had a crop field with a high *O. moreleti* abundance. High field abundances were observed on one farm in the Goulburn Valley in 2014, three farms in the Riverina in 2016 and two farms in Balliang East in 2017. While estimating exact densities is challenging due to the horizontal and vertical movement of *O. moreleti* in the field (Baker, 1978b), categorising abundances as high or low was straight-forward (see below). High abundances were either aggregated or spread out relatively evenly across a field. The three farms investigated in the Riverina each had a single field with high densities of *O. moreleti* aggregated in a part of the field (>20 millipedes/m², (~ 10 adults, 10 juveniles), over an area larger than 60m²) during September 2016. Similar densities were found in a single field in the Goulburn Valley during September 2014, although in this case,

abundance was high across the entire field. In two farms in Balliang East, multiple fields were found with densities estimated as > 3 millipedes/m² across the entire field during September 2017 (Figure 6.2).

Various factors may explain why high densities of *O. moreleti* were not found in crop fields in the other 13 farms where the pest was reported. Misidentification of pest problems seems to be the case in four fields, as high numbers of other soil-dwelling species were found. European earwigs, *Forficula auricularia* L., were present in high numbers in two fields, while the common pillbug, *Armadillidium vulgare*, and a polydesmid millipede species (identified by Bob Mesibov as *Cladethosoma* spp.) were found in high numbers in the other two fields. Soil dwelling organisms such as *Forficula auricularia* and *Armadillidium vulgare* cause feeding damage to crop seedlings that appears similar to *O. moreleti* feeding (Ebregt et al. 2005, Macfadyen et al. 2019), confounding in-field identification. In the other nine fields, it is possible that the abundance of *O. moreleti* changed rapidly across time, with much higher densities thought to be present in the previous year (personal conversations with farmers). As described below, *O. moreleti* abundances can be highly variable between years in crop fields.

The two farms in Balliang East were the only farms where high densities of *O. moreleti* were found in multiple fields. High *O. moreleti* abundances reportedly only occur in farms on one side of the valley in this region (Alastair Tippett, personal conversation). For the two farms investigated, 16 out of 35 fields in October 2017 had high densities of *O. moreleti* (described above). In the other 19 fields, *O. moreleti* was only found in small patches ($>10\text{m}^2$) (Figure 2). Variation in the soil profile seems to correlate with the disparity in densities as noted by both farmers and the agronomists (Tim Tjensz and Russell Griffith, personal conversations). High densities were found in fields described by farmers as crumbling red clay and cracking grey loam. Low densities were found in fields with river clay and black clay, which appear to have a harder soil layer. The impact of other variables on these patterns are unknown. The amount of crop stubble could not be considered as it was low and inconsistent across these fields (estimated by farmers as 1-1.5t/ha), although canola stubble appears biologically important as a food source and a habitat, particularly for juveniles (personal observation). Any correlation between pesticide history and *O. moreleti* densities, as is found in vineyards (Nash et al. 2010), could not be explored given pesticide applications were mostly only used to control *O. moreleti* in autumn in fields known to have high densities.

For the three farms in the Riverina (2016-17) and the farm in the Goulburn Valley, there were high abundances of *O. moreleti* in a single field (described above). Other fields in the farm were found to have either very low or no *O. moreleti*. In some cases, neighbouring fields had identical agronomic histories and very similar soil profiles, yet *O. moreleti* was largely absent. Thus the reasons why these fields had high densities are unclear.



Figure 6.2. Field abundance of *O. moreleti* in two farms in Balliang East. Fields with low abundances are marked with a white contour, fields with high abundances with a black contour.

6.2.3 Seasonal abundance sampling

From September 2016, visual assessments of abundance were made in a lucerne field near Corowa, NSW (35.93, 146.31) over the course of a year. Assessments were conducted nine times approximately every six weeks. Within this field, high abundances of *O. moreleti*

initially aggregated in a 200x60m section in the middle of the field. During spring 2016, a standardised sampling protocol was used to estimate abundances in the middle of this section (supplementary material). During summer, it was expected *O. moreleti* would burrow vertically into the soil however I could not locate any individuals despite extensive searching (supplementary material). Thus from January 2017 to September 2017, visual assessments were made both throughout the field and at the field edge to see if *O. moreleti* dispersed horizontally. Despite the large *O. moreleti* numbers in the previous spring, only a few individuals were found (Figure 6.3).

Based on monitoring during the following autumn and follow-up conversations with farmers, the other two fields in the Riverian that I sampled in 2016 were also found to have low densities in 2017. This dramatic decrease in abundance was also observed in the field from the Goulburn Valley in 2015. Farms in Balliang East, however, were not found to have visually different levels of field abundances between 2017 and 2018.

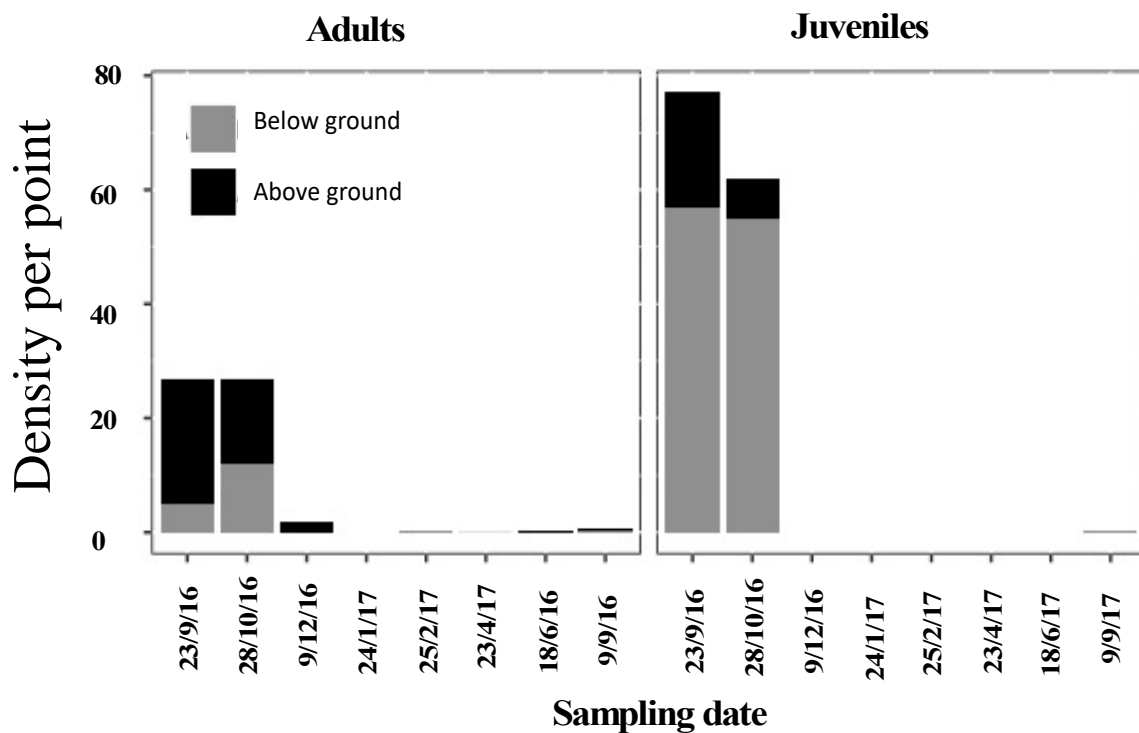


Figure 6.3. Mean numbers of adult and juvenile *O. moreleti* found at each above and below ground sampling point from September 2016 to September 2017 in a field near Corowa, NSW.

6.3 Discussion

O. moreleti has a broad occurrence in a variety of environments across Victoria and southern New South Wales. The increasing presence and higher abundance in broadacre cropping fields in southern Australia are thought to be linked to an increase in conservation farming and stubble-retention practices ((Bailey 2007, Macfayden and Nash 2018). Based on pest reports and visual sampling on farms, high abundances are found in crop fields in different farming regions in south-eastern Australia. The observations in this preliminary study suggest soil type and the presence of moist environments during summer may also be important variables leading to high abundances in crop fields.

As is commonly noted by agronomists and farmers (personal conversations), I found abundances can vary greatly between years in crop fields, unlike the case in bushlands and grasslands (Baker 1978b). I found a rapid decrease in abundances occurred with the advent of dry summer conditions. In bushlands and grasslands, *O. moreleti* is thought to migrate vertically into the soil, spending the dry period in a state of quiescence, aggregating in sites of moisture which can be present at the base of tussock grasses or in the soil shaded by trees (Bailey and de Mendonça 1990, Baker 1978b). The presence of moist environments for shelter is limited in agricultural fields, likely leading *O. moreleti* to disperse away from the field. However, a change in abundance between years was not found in fields sampled in Balliang, suggesting that consistent patterns across years can occur in some locations.

Soil type likely accounts for the variation in abundance between fields in farms near Balliang East. Fields within these farms seem to be divided into two abundance categories. Although high abundances were found in different soil-types, visually the soils with high abundances showed similarity in the softness of the topsoil. Moreover, in some fields where *O. moreleti* were aggregated in parts of the field, these parts were described by the farmer as having softer patches of soil. The property of soil hardness may define the distribution of *O. moreleti* in Portugal (Bailey and de Mendonça 1990). Oviposition sites may also be spatially structured, as suggested by the disparity between the similar distribution of adults across a field monitored in Balliang but spatial aggregation of newly emerged juveniles in this field (personal observations).

The observations in this preliminary study suggest that population abundances of *O. moreleti* vary more in crop fields than in bushland or grassland environments (see Arnold et al. 2008).

Crop stubble appears one of multiple factors that determine population abundance. Population dynamics in crop fields may vary between fields, years or in different regions. The population dynamics of this species in crop fields therefore requires multi-year studies across multiple fields and in different regions.

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6.5 Supplementary material

Seasonal sampling protocol

Year-long visual sampling of *O. moreleti* abundances was conducted in a lucerne field near Corowa, NSW (35.93, 146.31) from September 2016 to September 2017. This field had previously been grown in a canola/wheat rotation. Part of the field was burnt early May 2016 to remove crop stubble, prior to being sown to lucerne (*Medicago sativa* cv “Sardi 6”). On June 30th 2016, the farmer noticed an entire section of emerging seedlings was removed and attributed to this damage to the large proportion of millipedes present. In early July, baiting occurred in the section of the field where damage occurred, firstly with Metarex (AgNova Technologies Pty Ltd, Australia) and subsequently with Mesurol (Bayer Crop Science, Germany). Observations of field abundances were first made on September 26th 2016. At this time, I took notes on the spatial pattern of *O. moreleti* in the field. *O. moreleti* were aggregated in a patch of approximately 200x60 metres, towards the centre of the field and approximately 70-100 metres from the field boundary. This section of the field had not been burnt or baited. Few millipedes were found in other parts of the field.

From September to December 2016, sampling was visually conducted at ten points within the centre of this patch of aggregation. Sampling was conducted in an oblong pattern, with each point approximately 30 steps apart. Three observations were made at each point, assessing millipede numbers above ground and below ground. Above-ground habitats targeted included inside stubble or under stubble and weeds. Assessments of below-ground habitats were made by pulling weeds or seedlings out of the ground. At each sampling point, millipedes were counted as juveniles and adults. Juveniles had recently hatched and could be easily distinguished by size. High densities were estimated as either 20, 50 or 100. Approximate densities above and below ground, at each point, were recorded by averaging the 3 observations. Estimates of millipedes per point presented in Figure 2 are mean densities across the 10 points.

During December 2016, field monitoring remained focused on this affected areas of the field and followed the above sampling protocol, however above-ground sampling included

assessments under freshly cut and windrowed lucerne. From this date onwards, below-ground sampling was more extensively conducted at all at points of moisture where millipedes may reside during summer. These sites include the base of trees in logs or at the base of plants (Baker 1980). Below-ground sampling was conducted by digging in to the soil and pulling out plants. At the edges of the field, I dug the soil at the base of fence-posts and extensively searched in and around the few surrounding trees. Despite extensive searching, individuals could not be found and thus it was thought they had migrated horizontally either within or out of the field. Thus assessments from February 2017 were conducted as extensive searches, both across the entire field as well as at the field boundary with the farmer providing monitoring support to search for millipedes in fields.

Chapter 7: Overview and future directions

This thesis aimed to understand the likelihood of pest problems arising from four diverse pest species. Two of these are common pests and two are emerging sporadic pests of broadacre crop seedlings in southern Australia. For the two major pest mites, *Halotydeus destructor* and *Penthaleus major*, I investigated the capacity to predict the abundance of these species at a field level. The majority of this thesis focused on predicting the factors influencing the pest status of the emerging pests, *Ommatoiulus moreleti* and *Armadillidium vulgare*. This chapter is separated into sections based on these two research areas which overview the main findings and outline suggestions for future research.

7.1 Predicting damaging earth mite densities in autumn

7.1.1. Overview

The earth mites, *Halotydeus destructor* and *Penthaleus major*, are key pests of a range of establishing winter plants in fields across southern Australia (Ridsdill-Smith 1997, Robinson and Hoffmann 2001, Umina et al. Hoffmann 2004, Murray et al. 2013). The autumn generation of mites emerge from over-summering diapausing summer eggs at a similar time to emerging seedlings, and can cause severe damage to many crop and pasture species (Ridsdill-Smith 1997, Umina et al. 2004, Micic et al. 2008). A model that helps predict the abundance of these pests in a given field would allow farmers to make management decisions with greater confidence. This would be particularly useful for *H. destructor* when used in combination with life-cycle models (Ridsdill-Smith et al. 2005, McDonald et al. 2015), optimal monitoring strategies (Arthur et al. 2014a, Nansen et al. 2015) and growth-stage specific economic injury thresholds which exist for canola (Arthur 2013, Arthur et al. 2014b).

Abundance of the first generation of earth mites was assessed in 104 fields with seasonal and regional effects investigated by sampling in two geographically separate farming regions over two years. Sixty-two fields were in a pasture environment (Chapter 1) and 42 in a cropping environment (Appendix 1.3). In crop fields, I was unable to investigate associations due to low abundances in almost all fields. In pasture fields, abundances were highly variable between fields, particularly for *H. destructor*. Field abundances of *H. destructor* showed limited association with a range of common environmental and agronomic field variables

known to be of biological importance (Ridsdill-Smith 1997, Nansen et al. 2015) and thus an abundance model provided little predictive power. The ability to predict *P. major* abundances was further complicated by a regional effect.

However for *H. destructor*, when fields were grouped according to *field type* categories by common plant composition, I found abundances could be separated in to three risk categories. Based on my research, crop fields are predicted to have low numbers of *H. destructor* unless significant broadleaf weeds are present (also found in (Macfadyen 2014) and thus can be described as low risk. Pasture fields primarily composed of lucerne, grass or sub-clover were identified as medium risk, with numbers unlikely to be greater than 300mites per m². Fields that are substantially composed of a mixture of grass, clover and/or broadleaf weeds were found to be highest risk, with densities greater than 1000/m² likely to be found. Given 300 mites per m² is an approximate economic threshold for canola (Arthur et al. 2014b), this single variable appears to be an important consideration to guide farmers of different risk-tolerance in their monitoring and pest-management practices when planting susceptible crops.

7.1.2. Future directions

The data I collected in this thesis included a range of environmental and agronomic variables known to affect *H. destructor* and *P. major* abundances. The fields sampled were collected in two farming regions over the course of two years. Within each region, fields were highly diverse in rainfall, soil-type and landscape features. One of the regions had a particularly dry spring in one year, thus impacting the size of the spring generation and the number of diapause eggs produced (Ridsdill-Smith 1997). Despite these diverse conditions, my research was unable to find associations with mite numbers, principally due to the overwhelming association with the variable *field type*. For *H. destructor*, which has been shown to quickly build up to the carrying capacity of a pasture (Ridsdill-Smith and Annells 1997, Gower et al. 2008, Ridsdill-Smith et al. 2013), this suggests some fields have a limited carrying capacity for this pest mite.

H. destructor densities may be regulated by density-dependent factors through food quality and available oviposition sites (Gaull and Ridsdill-Smith 1996, Ridsdill-Smith and Annells 1997, Ridsdill-Smith and Pavri 2000, Ridsdill-Smith et al. 2013). For fields with primarily lucerne or grass, the low abundance of *H. destructor* in fields can be explained by both the

low suitability of plant host and the lack of suitable microclimates provided by the growth of these plants (Ridsdill-Smith and Annells 1997). For fields with primarily sub-clover the low abundances found was surprising given sub-clover is known to be biologically important as a primary food source and oviposition site (Gauil and Ridsdill-Smith 1996, Ridsdill-Smith and Pavri 2000). This suggests that while sub-clover may provide biological benefits in terms of food and available oviposition sites, a mix of grasses and sub-clover may be needed for high *H. destructor* densities.

The differences in vegetative structure provided by a mixture of plants may promote mite densities by providing a humid micro-environment (Ridsdill-Smith and Pavri 2000), or increase larval survival by promoting the growth of microflora (MacLennan et al. 1998). Future research should consider small-scale studies to investigate the fitness parameters of fecundity, oviposition and mite abundance using field enclosures containing only sub-clover and field enclosures containing mixed grass and sub-clover. Controlled studies could manipulate enclosures by introducing fungal food sources and artificial structures that provide a range of humid microclimates, sheltering and oviposition sites. This research would help understand the biological mechanisms for field abundance, which is particularly important given the infinite variability in fields across Australia.

For *P. major*, the variation in field abundances between different farming regions should be investigated by extending my study across a much wider part of southern Australia. Linked to this, laboratory studies that investigate fecundity and diapause strategies of mites collected from different regions, would help to understand life-strategy differences between regions. This information is important in order to better predict the abundance of this species at a field level. The mechanisms for variability in mite abundances could also be investigated by further studying the fitness parameters of the different clones of this species (see Robinson et al. 2002).

7.2 Understanding the sporadic feeding behaviour of *O. moreleti* and *A. vulgare* on crop seedlings

7.2.1. Overview

The Portuguese millipede, *Ommatoiulus moreleti* and the common pillbug, *Armadillidium*

vulgare are species that generally feed on decaying plant matter but can also feed on living plants. Over the past decade these two species have increasingly been recognised as emerging pests within the Australian grain farming context. The research conducted in Chapters 3-6 of this thesis aimed to investigate factors that influence pest risk. Here, I discuss how this research can be used to inform pest management advice and create sustainable practices, and how it can be used as a basis for future research.

In Chapter 3, I investigated the capacity of these two species to damage crop seedlings. While principally detritivorous feeders, I established that these species differ greatly in their capacity to damage crop seedlings. This finding was established using controlled studies in a shadehouse and validated by pest reports from fields across southern Australia over the past decade. *O. moreleti* has a limited ability to feed on a range of crop seedlings. *A. vulgare* can damage a wide range of crops, although some crops are only susceptible in the first days of seedling establishment. This information provides a vital first step in developing an integrated pest management program. Sowing crops with low susceptibility can prevent damage to seedlings by *O. moreleti* and minimise damage by *A. vulgare*. For both species, wheat and faba bean are good options when field abundances of these species are known to be high.

My findings suggest these species can, in certain circumstances, be solely beneficial due to their inability to damage crop seedlings; this is particularly the case for *O. moreleti*. In other fields, the level of damage to susceptible seedlings may be influenced by a range of agronomic and environmental factors that dictate the availability and quality of food resources (Bernays and Minkenberg 1997, Agrawal et al. 1999, Agrawal and Klein 2000). In Chapters 4 and 5, I investigated how a range of variables would influence pest status.

In Chapter 4, I firstly investigated the mechanisms limiting *O. moreleti* feeding on seedlings. I hypothesised that (1) lupin was particularly susceptible due to the removal of the defensive compounds, quinolizidine alkaloids, and (2) *O. moreleti* was limited in the range of seedlings on which it could feed by the relative strength of their mouthparts. Both of these hypotheses were strongly supported in Chapter 4, where I found 7x the amount of feeding damage to commercial *Lupinus angustinus* seedlings compared with wildtype *L. angustinus* seedlings that express quinolizidine alkaloids, and 4x the amount of feeding damage to the thick-leaved lupin variety, *Lupinus albus*. The second area investigated in Chapter 4 was to understand the impact of alternate food sources, and the size and sex of *O. moreleti* on the level of feeding

damage to susceptible crop seedlings. I found the level of damage is likely to be influenced by the size and sex of *O. moreleti*, with large females causing the majority of damage. In this study, the level of damage was not strongly influenced by the presence (or absence) of crop stubbles when presented as an alternate food source. However, these findings are limited by the controlled nature of this research, which was conducted using small plastic ‘microcosm’ containers in a shade-house.

Clearly, environmental factors can influence the level of damage by influencing the feeding choice and/or feeding behaviour of any organism (Bernays and Minkenberg 1997, Coll and Guershon 2002). In Chapter 5, I undertook a preliminary trial which aimed to investigate the impact of water stress on *A. vulgare* feeding damage to canola seedlings (recognising canola was found to be susceptible to *A. vulgare* in Chapter 3). I hypothesised water stress would result in individuals increasing their feeding on plants, as suggested by Johnson et al. (2012) from field results. Under controlled conditions, I found less feeding on canola when individuals were water stressed and/or exposed to a dry (low humidity) environment. This indicated *A. vulgare* were responding to water stress by reducing their feeding rate.

A crucial part of understanding and managing pest species is understanding their population dynamics across seasons and years. In Chapter 6, I undertook preliminary observations of regional and local variation in *O. moreleti* abundance in crop fields in Victoria and southern New South Wales. I found *O. moreleti* numbers varied considerably between fields and regions. Stubble is known to be important in influencing population sizes (Holland 2004), however I also found evidence that soil-type has an important effect on *O. moreleti* numbers. Interestingly, abundances were found to vary greatly between years in some crop fields. This appears to be due to seasonal population dynamics, with *O. moreleti* leaving fields and, in some cases, not returning.

7.2.2. Future directions

Research presented in Chapters 3-6 provide some important new knowledge that will assist in the development of pest management programs for *O. moreleti* and *A. vulgare* during the critical seedling establishment period. However, there remain many knowledge gaps, in part due to the minor pest status of these species and thus limited research that has been conducted previously (Macfadyen et al. 2019). There are some major limitations of the

research conducted in Chapters 3-5. For instance, the use of small contained environments does not allow the feeding behaviour observed to be translated to a field environment. The use of individuals collected from the field may have led to variability in the feeding behaviour due to differences in their life history. The lack of a behavioural analysis limits the ability to understand the mechanisms behind variability in feeding damage. Here I discuss how the research in this thesis should be used to guide future studies.

Among the first questions that should be addressed is whether to consider these organisms as detritivores that sometimes feed on plants or alternatively as generalist feeding pest organisms. A key part of this is understanding their ability to utilise seedlings as a food resource. In Chapter 4, I found a clear discrepancy between the weight gain *O. moreleti* obtained from feeding on lupin in comparison to feeding on lucerne. Future research should further this understanding by investigating other aspects of fitness advantages such as fecundity. I attempted this, but females did not produce viable eggs under shade-house conditions during May 2018 (unpublished data). As the ability to utilise crop seedlings may vary between environments (Taylor 1982, Kukor and Martin 1986), this research should ideally include individuals collected from both crop and bushland environments.

Future research should investigate how variability in different plant chemicals limit the susceptibility of different seedling cultivars (see Farmer and Dubugnon 2009), as these may vary greatly in chemical deterrents. As demonstrated by the relative level of damage *O. moreleti* caused to *L. angustinus* seedlings that express quinolizidine alkaloids, commercial breeding practices that reduce deterrent plant chemicals in agricultural crops are at least partly responsible for the pest status of these two species. In preliminary studies, I found considerable variation in the susceptibility of oat cultivars (unpublished data). Perhaps judicious selection of crop cultivar may provide a similar level of protection as chemical seed treatments in limiting the susceptibility of seedlings for these species?

In Chapter 4, I found the presence of crop stubbles, as an alternate food source, did not dictate whether *O. moreleti* individuals will feed on seedlings within a contained

environment. This research should be replicated in a field environment. For *A. vulgare*, Johnson et al. (2012) found the level of damage *A. vulgare* caused to soybean stands in the field was not influenced by the removal of crop stubbles. In South America, *A. vulgare* is clearly considered a generalist feeding pest in emerging soybean and sunflower fields, given economic injury thresholds have been created to assess the pest risk based on abundance (Saluso and Trumper 2004, Faberi et al. 2014). Perhaps this varies between species and their interaction with different crops? In Chapter 4, I found that after the introductory period, *O. moreleti* would continue to substantially feed on lupin but not on lucerne. Future research should investigate how the presence of alternate food sources affects the feeding behaviour on different crop types.

Future research should also consider how an extended period of deprivation of a specific nutrient may lead to variation in the feeding behaviour of these organism. This research should be conducted using a laboratory colony to help standardise studies on feeding behaviour. For *A. vulgare*, this research could be conducted using an artificial diet, (which have been developed for another species of terrestrial isopod (see Carefoot 1984)) as has been conducted for herbivores and generalist feeders (Behmer et al. 2003, Lee et al. 2006, Raubenheimer and Jones 2006, Raubenheimer and Simpson 2018). A behavioural analysis of movement (walking, sheltering) and feeding behaviour (tasting, feeding) would further help understand how they adapt their feeding behaviour may vary in chemically heterogenous environments under periods of nutrient deficiency.

Environmental effects can influence feeding behaviour and food choice. Understanding both short-term and long-term effects on feeding behaviour is crucial to guide monitoring activities, and to predict seasonal future risk. In Chapter 5. I investigated how short periods of water limitation would affect the feeding of *A. vulgare*. Here I set-up variable degrees of water stress and hypothesised that water stress would lead to increased feeding as *A. vulgare* searched for water. However I found the opposite. Presumably they reduced their feeding and remained in a ball during dry periods (Dias et al. 2012), however an analysis of their behaviour (walking, feeding, sheltering) would help explain the mechanism. To understand

how foraging may vary in different environments, future research should construct microcosms with variability shelters and alternate food sources in wet and dry states.

Understanding how these organism adapt their feeding behaviour to different life histories is crucial to predicting their feeding behaviour during autumn. *O. moreleti* undergoes a period of quiescent behaviour during summer from which it emerges during autumn to feed and reproduce (Baker 1978a). Autumn rainfall is thought to trigger the cessation of quiescence (Bailey and Kovaliski 1993), but the mechanisms which regulate the life-cycle events have not been researched. In another millipede species, metabolic regulation and reproduction are at least partly regulated by photoperiod (David et al. 2003). A laboratory colony could be used to investigate how factors such as rainfall, photoperiod and food availability regulate these events. The timing of this reproductive period may be important given large females cause most feeding damage (Chapter 4) and seedlings are susceptible for a very short period (Chapter 3). Furthermore, the life-cycle of *O. moreleti* has not been examined within agricultural fields (see Baker 1978b), which warrants consideration.

Pest report data was used in Chapter 3 (cesar 2018, SARDI 2015) to view the pest association with different crop seedlings. Due to limited data, and uncertainty around the data collected (see Chapter 6), this data was of limited use in understanding pest associations with environmental or agronomic field variability. Furthermore, correlative analysis of pest associations using environmental and agronomic factors in this data-set may be problematic. Factors that directly or indirectly promote population build-up or seasonal migration may have variable effects factors on the feeding behaviour of the organism (eg. food availability promotes population build-up but may influence seedling feeding behaviour). As the population dynamics of *O. moreleti* may be variable between fields and/or regions (Chapter 6), pest risk is likely associated with different factors in fields where high population densities are found sporadically, rather than annually. Sufficient, long-term data in fields where high densities are found annually may help understand which factors influence pest risk in these fields.

The population dynamics in crop fields needs further research. For *O. moreleti*, my field monitoring (Chapter 6) outlines many questions. Firstly does this species remain or leave during summer? If it does remain how does it survive? If it does leave, does it return? In bushlands and grasslands (Baker 1978b), *O. moreleti* finds refuge in moist environments which may not be present in crop fields. Seasonal migration may therefore vary depending on the environmental conditions and the sites of refuge both in and outside of the field. While this research should ideally be conducted in farms in different regions, farms in Balliang, Victoria (see Chapter 6) presents an ideal site to investigate how variability in field dynamics can be influenced by diversity in soil-profile between fields. Field sampling should consider intensive sampling and directional traps which can be created for this species using tin which *O. moreleti* are unable to climb. This research should consider monitoring the thermal conditions during summer to understand how the survival and field-dynamics of adult and juvenile millipedes are influenced by changes in environmental conditions in the field; this has been explored in other environments (Baker 1978b).

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